Faculty of Medicine • Masaryk University • Brno • Czech Republic

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DEDICATED TO THE 60th ANNIVERSARY OF PROFESSOR BOHUMIL FIŠER

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2003

The Symposium takes place under the auspices of

Prof. RNDr. Jiří Zlatuška, CSc., Rector of Masaryk University Brno

Prof. MUDr. Jan Žaloudík, CSc., Dean of Faculty of Medicine, Masaryk University Brno

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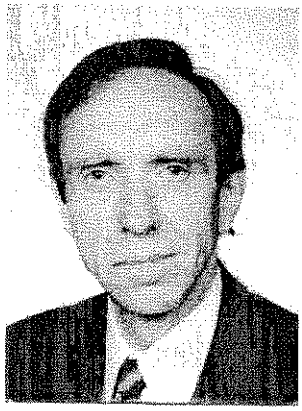

Prof. MUDr. Bohumil Fišer, CSc.
60 years of age

Prof. Bohumil Fišer, Head of the Department of Physiology, Faculty of Medicine, Masaryk University, Brno, Minister of Health in the Czech Republic in 2000-2002, is a highly regarded scientist of worldwide renown in the field of normal and pathological physiology and a successful organizer in health service. On October 22, 2003 Bohumil Fišer celebrated his sixtieth birthday, full of physical and intellectual energy.

During his studies at the Faculty of Medicine of Masaryk University he had been working in the Institute of Medical Physics and later Physiology where he extended his considerable knowledge of physics and mathematics; that became a basis for his further activities, mainly in the research of cardiovascular system. After his graduation in 1966 he acquired a valuable medical experience in surgery in the course of his military service (1967-1968). Then he continued his scientific and teaching activities in the Department of Physiology as a lecturer and since 1989 as a senior lecturer. He was appointed a professor in 1995; at that time he was already Head of the Department of Physiology at Masaryk University in Brno.

The scientific and publication activities of professor Fišer, started in 1966 in cooperation with professor Peňáz, were concentrated on frequency analysis of blood pressure oscillations in rabbits. The oscillations of cardiovascular variables were studied in man, also using direct recording of blood pressure in a. brachialis. These experiments using exact original methods of frequency analysis brought the evidence of the ten-second rhythm in heart rate in man. Together with professor Semrád he performed the analysis of pulse pressure and pulse interval in patients with atrial fibrillation, using various mathematical methods and method of Monte Carlo including the heart rate rhythm regulation of heart contractility in man. The study of heart contractility in man confirmed the findings of contractility regulation in isolated heart experiments discovered by professor Vladislav Kruta, professor Pavel Bravený and others co-authors from the same physiological department. His scientific work from the studies mentioned above was finished by CSc. thesis in 1978. He also contributed to the first clinical measurement of cardiac output and first electrophysiological examination of the human heart in St. Anna Teaching Hospital. Further studies analysed the interrelationships between heart rate fluctuations and blood pressure fluctuations using autocorrelation functions and power spectral densities. In 1978 the first description of coherence between pulse interval and

systolic blood pressure was published. Heart rate oscillations in frequency domaine 0.1 Hz were analyzed. These findings are fundamental in determination of baroreflex sensitivity in man. His studies aimed at assessing baroreflex sensitivity continued with the development of another non-invasive method of determination baroreflex sensitivity. Sudden decrease of blood pressure of about 10 to 20 mmHg, evoked by out-flow of blood into vasodilated lower extremities after 5 minutes occlusion, brought about baroreflex-mediated blood pressure changes and changes in cardiac interval. Changes of cardiac interval, monitored beat-to-beat, were used for measurement of baroreflex sensitivity. The new developed method allowed further hemodynamic studies in healthy subjects and clinical examinations, done together with prof. Siegelová, in patients with essential hypertension. The results of his scientific work professor Fišer published in numerous monographs, in experimental original papers and in several pedagogical texts both in his country and abroad. Partial results were given at many scientific meetings.

International cooperation of professor Fišer in science and research could be fully extended only after the velvet revolution in 1989. The cooperation with Medical Faculty, Lariboisiére Hospital (France) was very intensively developed and the common hemodynamic studies were provided as a non-invasive measurement of aortic compliance and blood flow regulation in cerebral arteries, both in healthy subjects and patients, and published in international journals. At the same time, the cooperation with Halberg Chronobiology Center of the University of Minnesota (USA) started. Further studies of circadian variability of cardiovascular variables and baroreflex sensitivity were published in many papers as the result of this common work. In 1995 he was invited as a distinguished scientist for a lecture to the Supercomputer Institute of University of Minnesota (USA) and presented there the paper about the use of supercomputers in prevention of stroke and cardiac death. The international cooperation continued with the Department of Physiology in University in Graz (Austria), where the original studies of heart rate variability, baroreflex sensitivity and chronobiology have been realized and included in the common international project of analysis of cardiovascular control in physiology and pathophysiology.

Scientific, medical, and organization capabilities of professor Fišer were appreciated by a number of awards, citations and memberships in scientific and health organizations and institutions. Professor Fišer is a member of a range of international scientific societies such as International Society of Physiology, French Physiological Society (Societé de Physiologie Francaise), as well as Czech Physiological Society. He participated in many international physiological congresses, symposia and workshops, some of them also organized by him. He was invited to many lectures concerning cardiovascular control mechanisms in France, United Kingdom, Austria and USA. In our republic, he was a member of International Grant Agency of Ministry of Health from 1995 to 2000 and of Grant Agency of the Czech Republic. In 2000, he was nominated in the function of Minister of Health of the Czech Republic. In this position he was very successful and everybody esteemed his activity in the government. In 2002 he returned to Masaryk University and is in the head position until today. In 2003, he became a Member of Executive Board of WHO (World Health Organization).

Noble personal qualities of professor Fišer include not only an extraordinary diligence, but also modesty and tolerance, almost permanent good mood and friendly relation to people. He is always ready to give advice and assistance to younger colleagues to whom he imparts his extensive scientific, research and pedagogical experience. His productive life is filled mainly with professional work and with work which is of benefit to the public.

Dear Bohumil, I would like to wish you for myself and on behalf of all colleagues and friends and all those to whom you have been helping and who like you, many happy years, success in your work and first of all good health.

Prof. MUDr. Jarmila Siegelová, DrSc.
Head, Dept. of Functional Diagnostics and Rehabilitation
St. Anna´s Teaching Hospital
Faculty of Medicine
Masaryk University
Pekařská 53
656 91 Brno

PROF. MUDr. BOHUMIL FIŠER, CSc. – 60th BIRTHDAY

I knew prof. Fišer from Brno since the late sixties as a pupil of very famous Czech physiologists prof. Vladislav Kruta. We met several time in Physiological Days organized by Czechoslovak Physiological Society. Our first long - lasting meeting was during our common participation on the World Congress on Physiology organized by IUPS in New Delhi. With several friends we organized the trip of Czech and Slovak Physiologistes to this Congress. One of the organizers was also prof. Jarmila Siegelová from Brno. It was one of several escapes form the "gray" of that time. We visited Lebanon (just before the unfortunate war) and than India. During this trip we had the opportunity to discuss nearly all problems of our life in that time. I recognized prof. Fišer as a very intelligent, gentle and sensitive man with a very deep interest for science and especially for physiology. We met than regulary during Physiological Days and in the international level during the meeting of Association des Physiologistes. It was the tradition of Brno' physiological school, which was very phrancophonic. Prof. Kruta was an excellent ambassador and of this tradition in the whole Czechoslovakia. Since 1947 up to his death 1979 he was the first secretary for Czechoslovakia in the executive bord of Association des Physiologists. I was elected as his successor in 1980. We organized several visits to the Reunion Annuelle of the Association des Physiologiestes and prof. Fišer was the regular participants of this trips. Since that time is dated his collaboration with french physiological laboratories, especially with that of prof. Martinaud. Prof. Fišer organized also very successful Physiological Days in Brno in 1998.

In the role of Minister of Health of Czech Republic he did not change his character. He was still very helpful, friendly and nice person.

I wish him from all my heart to keep his very high standard in all roles which he plays now and in which he will play in the future.

Prof. MUDr. Richard Rokyta, DrSc.
Dept. Physiology and Pathophysiology,
3th Faculty of Medicine,
Charles University
Ke Karlovu 2
128 00 Praha

HEALTH PROMOTION BEYOND BOHUMIL FISER'S 60TH BIRTHDAY

Salutationes ad sexagesimum diem natalem in multos annos et transannos adjunctos -- tempestive sanos, reciprocos et ergo chronomicos, resolutos quam simplicissime sed non simplicius[1]

Franz Halberg*, Germaine Cornélissen*, Othild Schwartzkopff*, George Katinas*, Zhengrong Wango•, Roberto Tarquini‡, Federico Perfetto‡, Cristina Maggioni§, Yoshihiko Watanabe¶, Earl E. BakkenΔ

*Halberg Chronobiology Center, University of Minnesota, Minneapolis, MN, USA
•West China University of Medical Sciences, Chengdu, Sichuan, China
‡University of Florence, Italy
§University of Milan, Italy
¶Tokyo Women's Medical University, Daini Hospital, Tokyo, Japan
ΔNorth Hawaii Community Hospital Inc., Kamuela, HI, USA

[1]Greetings, with (best wishes for) many added years and transyears (the latter an ~1.3-year signature of the largely invisible solar wind) -- timely, healthy, reciprocal, and hence resolved chronomically, as simply as possible, but not simpler.

Address correspondence to

Franz Halberg, MD
Director, Chronobiology Center
University of Minnesota
MMC 8609
420 Delaware St. SE
Minneapolis, MN 55455, USA
TEL +1 612.624.6976 · FAX +1 612.624.9989 · E-MAIL halbe001@umn.edu

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DEDICATION

Bohumil Fiser, Figure 1, originally head of what used to be Purkinje (now Masaryk) University's department of physiology, and later Minister of Health of the Czech Republic, now assumes an even broader responsibility in Geneva for everybody's well being, poor or rich. Bohumil set an example for his country and for the world when, many years ago, he, Jarmila Siegelova and Jiri Dusek, on arrival in Minnesota, each placed a blood pressure cuff on their arms and carried it for a week for health promotion and a chronomic evaluation of any elevated vascular disease risk, as the basis for countermeasures, when needed. Pavel Homolka came later and monitored for longer, and now the Brnoese Trio, Figure 2, has become a quartet.

Self-surveillance and self-experimentation are in the tradition associated with Jan Evangelista Purkinje's studies on digitalis and many other drugs. By contrast to Purkinje, however, the Quartet took to the clinic the specification of timing drugs and of the duration required for assessing effects (1-4), much longer than a profile of 24 or 48 hours; the background from the laboratory, demonstrated for ouabain, that timing, ignored by Purkinje but not by the Quartet, can account for the difference between life and death (5) awaits more general information transfer to the clinic.

Whereas another Czech scientist, Ivan Raska, focused on the eradication of smallpox worldwide by WHO, the eradication of an elevated vascular and other disease risk by pre-habilitation (6-8) is a current international goal, not yet implemented, yet within the state of the available technology. One of us (EEB) reminds us of Oliver Wendell Holmes' *The Deacon's Masterpiece* (a carriage which was built with no component any stronger or weaker than any other, ran for 100 years to the day and then collapsed into a pile of sawdust). As Holmes argued for antisepsis and asepsis, among other goals, so do we make the case for the recognition and treatment of an elevated vascular disease risk and thus for its eradication by now-available software, hardware and a documented set of procedures.

PROLOGUE

Chronobiology (9), the study of mechanisms underlying diversity in time, and chronomics, the mapping of chronomes (time structures), both still largely unused, could complement genetics, the study of mechanisms underlying diversity in space, and genomics, the mapping of the genomes involved. Genomics and chronomics, while complementary, Insert 1, are Bohemian frontiers in their own right, dating back to Mendel's pea patch and Purkinje's self-experimentation. We here focus on the chronobiologic-chronomic assessment of blood pressure (BP) and heart rate (HR) variability as the alternative to the spotcheck of *the* blood pressure advocated by official current guidelines (10). The conventional approach risks a diagnosis and treatment dependent on appointment time (8; cf. 11, 12), Figure 3, irrespective of the dynamics of time, gender, age, ethnicity and geographical location. Evidence here presented with outcomes supports the desirability of around-the-clock monitoring for 7 days in initial screening. We implement this goal with modern automatic hardware and software. Thereby, we emulate the lifetime self-measurements carried out around the clock on themselves, following a diagnosis of a blood pressure elevation, MESOR-hypertension, by late major advocates of a chronobiologic approach (13, 14). We make our case by documenting first that what we propose is not new: it is common sense advocated a century ago by Theodore C. Janeway (15; italics ours):

> ... *it is essential* that a record of the pressure be made at frequent intervals *at some time previous* [presumably to an examination], to establish the *normal level* and the *extent of the periodic variations*. When this is done, it may be possible to demonstrate changes of small extent, which, lacking this standard for comparison, would be considered within the limits of normal variation.

Nor is it unsupported by leaders in the field of blood pressure: Howard Levine and Frederic C. Bartter, both professors of medicine (13, 14), practiced what Bartter, as head of the Hypertension-Endocrine Branch and later of the Clinical Center at the U.S. National Institutes of Health, wrote in 1974 regarding a patient whose blood pressure status was diagnosed differently by two different physicians whom he saw at different times of day (16; italics ours):

> By conventional standards, this patient is clearly normotensive every morning. But the blood pressure determined each day at 6 in the afternoon provides especially convincing evidence that this patient is a hypertensive. ...
>
> My plea today is that information contained in [data curves compiled under differing circumstances, such as 24 hours a day/7 days a week] become a *routine minimal amount* of information accepted for the description of a patient's blood pressure. *The analysis of this information by cosinor should become a routine.* It is essential that enough information be collected to allow objective characterization of a periodic phenomenon, to wit, an estimate of M [the time structure or chronome-adjusted mean, or MESOR] ... an estimate of [the amplitude] A itself, and finally an estimate of acrophase, [a measure of timing]. In this way, a patient can be compared with himself at another time, or under another treatment, and the patient can be compared with a normal or with another patient.

Agostino Carandente, the first physician in the pharmaceutical industry to indicate timing in the name of a drug (17), published a monograph in 1984 (18) dealing with the results of the methods Bartter had advocated. He optimistically titled the monograph "Chronobiology of blood pressure in 1985". Acceptance of the approach, however, was established neither within a year, nor within a decade and more.[1]

STATUS QUO

Ninety-nine years after Janeway's recommendation, 29 years after Bartter's and 19 years after Agostino's, we can here present substantial evidence gathered in the interim and note that the suggestions made earlier can be more readily implemented (15, 16). Instrumentation for automatic measurement underwent a first precipitous price reduction, currently of 90%, through the international BIOCOS project (corne001@umn.edu) (19) and upon increasing demand, the monitors may become still more readily accessible, and, of course, much more reliable; the analytical computer procedures have also been developed for use along with the start of chronomic data bases that allow us to carry out analyses in the light of gender , age , in some cases ethnicity , geography- and social class-qualified reference values. Within the next generation, such data bases based on "clinical health" will have to be replaced by bases aligned with lifetime outcomes, an endeavor started in the perspective of nearly three decades.

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[1]Delayed acceptance of well-documented facts, however, need not wait for more than centuries, as discussed in other contexts by OS in this volume (25). The case of scrubbing before surgery constitutes another example (113; cf. 17).

At this time, analyses through BIOCOS, with the as-yet outcome-unqualified reference standards (8) are free of charge and perhaps eventually they will be provided through international organizations concerned with health care. Moreover, by automatic monitoring of BP and HR, not only the health of the individual but also that of the community is served, by the archivization of the individuals' data, their alignment with outcomes and depending on the latter, with a potential feedback to the health care of all concerned.

By a further alignment of biological monitoring outcomes with physical and other environmental factors, as a dividend, with good biomedicine, an overdue, truly transdisciplinary science could emerge (6, 19, 20), long heralded as cosmobiology but with few hard inferentially validated facts. The modern alignment, however, has already led to discoveries of signatures and mechanisms of new biospheric and new physical environmental cycles (19-21). New rules such as the complementarity of reciprocal cycles in the biosphere and its cosmos have emerged (19). These developments and others in chronomics (20-24) hold promise of further spin-offs for health care.

Awaiting implementation with existing tools

We hold the following to be self-evident:

First, that the most important evidence relating to a given individual is that on this person him/herself, starting thereby where a historical review ended (25). We paraphrase Alexander Pope's (1688-1744) "the proper study of Mankind is Man" by proposing that the critical measures of an individual are those of variability in that individual himself/herself (and not spotchecks of a roller coaster). The individual's profile has to be compared with gender , age- and, as soon as possible, otherwise-qualified chronomic reference norms instead of time- and otherwise-unqualified values or limits, such as a "target" blood pressure, still based on the fiction that there is a "true" BP if one only measured long enough. In this context, conventional evidence-based medicine, its great advantage of randomization notwithstanding, cannot be considered to be the highest level of evidence; instead, the time series collected on the given individual remain the most important requirement, so that longitudinally each monitored person's data constitute his/her main reference values to be compared as need be, also with gender, age and, when possible, ethnicity, geography, social class and chronomically qualified reference values.[2]

Second (this and all later considerations follow from the first), that the data collection in terms of density and length should be made dependent upon the time structured (chronomic) endpoints documented to be pertinent for a given purpose, such as a standard deviation of HR or a circadian and circaseptan amplitude for BP in vascular disease risk assessment (26, 27), Table 1.

The handling of a given person's data by applying the stringent inferential statistical criteria of large clinical trials to the individual must not be based on the often very flimsy methods of spotchecks in clinical trials. In clinical trials, apart from the major flaw of failing to account for inter-individual differences, as in the case of the response to sodium (28) and for any intra-individual changes as well, the large numbers of subjects involved

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[2]The Gaussian "much" *(multum)* applies to time series needed on the individual. We can paraphrase Gauss's added "but not many" *(sed non multa)* with "**and** many" *(et multa)* of clinical trials, as soon as we account in these trials for *multum*, i.e., for "intra " and "inter "individual differences (read, immediately chronomic and eventually genomic reference values; see Insert 1).

compensate for the small numbers or single measurements/subject; this is obviously not the case when an individual is being screened. Hence, the fallacy of "what is good procedure in government-sponsored costly clinical trials is good for clinical practice", notably the dangers of time-unqualified or fixed clock-hour-based spotchecks must be made obvious.

Third, that whenever feasible, conditions that have become indispensable for reliable health care research should be necessarily implemented in health care practice. This desideratum applies to hypothesis tests and an indication of the associated probability and estimates of uncertainties in endpoints underlying decisions, such as 95% confidence intervals.

Fourth, that this general goal can be applied as self-help as well as in medical practice to the universal chronomic assessment of unavoidable variability in BP and HR. The computer-savvy next generation can be taught, as part of their late primary and again their secondary education, how to collect and transmit the data electronically and cost-effectively to a center for analysis, and how to interpret the center's results. This interpretation of analyses is provided at this time by the international BIOCOS project's Minnesota center and eventually, we trust, will be within the scope of an international organization into which BIOCOS should develop and serve as a critical aspect of good general education, its "purity" to be monitored (and updated) like that of the air we breathe.

Fifth, that inferential statistical analyses, applied as n-of-1 studies required for the individual person, be based on objective dynamic reference standards that include the quantification of any periodicity, whenever the corresponding no-rhythm assumption can be rejected. Thus, in addition to valuable endpoints such as a time structure-adjusted mean (MESOR) and standard deviation and any other time-macroscopic impressions, such as non-dipping, the interval as well as point estimates of all pertinent rhythm characteristics such as any period (τ) and its amplitude (A), phase (ϕ) and waveform (A, ϕ) be assessed and interpreted in the light of objective numerical reference standards. Reference values should be from peers of the same gender, age and, as soon as possible, ethnicity, geographic location, social class and long-term outcome including life expectancy. When the data are dense and long enough, focus on chaos and trends will also be desirable. We refer to the assessment of a correlation dimension and/or an approximate entropy among other endpoints, also gauging variability, and to polynomials, fitted to account for age-related trends, in order to differentiate these from trends due to increased risks.

Sixth, that any diagnosis based on inferential criteria invariably include major focus upon all endpoints documented as harbingers of risk elevation for the eventual institution of preventive countermeasures, pre-habilitation, notably in developing areas, that can ill afford the high price of devices and surgical interventions required in developed areas for rehabilitation after the fact of disease.

Seventh, that in all areas, but again with emphasis on underdeveloped and developing ones, the diagnosis and treatment of actual validated MESOR-hypertension and other blood pressure disorders can also be based on the preceding guidelines, so that the large costs of false positive diagnoses are saved and the possible health consequences of false negative results are prevented, as severe disease risks are recognized and, when possible (as in the case of blood pressure overswinging), treated.

Eighth, that while the statistical significance of a diagnosis (sic) and of a treatment effect is a necessary condition, as such alone it is not enough. Equally important, as an

essential condition, is biological significance, a benetensive rather than trans- or maletensive effect (28), gauged first by the return of monitored endpoints into a gender , age- and eventually ethnicity- and geographic location-specified range of acceptable values and next by endpoints such as a left ventricular mass index (LVMI) or tests of kidney function when end organ damage has already occurred. This inferential statistical validation can be based on time series for the BP for an individual. For instance in the case of a statistically significant lowering of a BP-MESOR, only a return to the acceptable range of the single value into its personal as well as into a peer-established chronodesm or of a rhythm characteristic (into its personal and peer-derived parameterdesm) is benetensive. Thereby we arrive at an also-inferential validation of biological significance by no longer finding an undesirable difference between an individual's own prior and his/her clinically healthy peers' characteristics. Otherwise, the statistical significance of a change notwithstanding, an increase in BP-A and/or of a BP-MESOR can be maletensive and an insufficient decrease can be transtensive (29).

Ninth, that the effectiveness of treatment may be (when feasible, continuously) monitored and an appropriate (benetensive) effect be validated, as one goes by sequential testing, e.g., via CUSUMs (29, 30) and parameter tests (31).

Tenth, that the development of a system with tools, a data base handling center and instruction manuals in different languages, introducing chronobiology generally (32) and specifically with the foregoing points in mind, are indicated for early and adult education.

The foregoing has been summarized by one of us (GC) as follows:

> Education in chronomics, of particular interest in under-developed and developing countries, is the most immediate and fundamental channel through which universal health promotion and care, including the truths by the Janeway-Bartter criteria, can be improved and rendered cost effective. Chronobiology has such pervasive applications that it can be part of almost every topic in the curriculum (32-36). Lessons in chronobiology can be the topic of reading and writing classes, even in classics or in physical education, where instructions on how to measure different variables can be taught, while also being part of biology and health care, along with other important topics on hygiene to prevent the spread of infectious diseases. It can be part of physics and mathematics, up to calculus. In statistics, the fit of periodic functions could be instructed along with other regression problems in computer science. Emphasis on the meaningfulness of rhythm parameters belongs to engineering and other technical courses, including physics and chemistry. The building and development of known and new monitoring devices can also be an integral part of electronic and informatics classes, where the gathering and dissemination of information is most important for preparing the reference data bases to be used for detecting abnormalities in a timely fashion. The opportunity of creating such a system may even have a better chance of success in under-developed and developing countries because it should be easier to implement such an integral system of education from scratch than to modify existing programs.

For the implementation of the foregoing points and their economical and scientific (chronomic) reasons, the collection of international reference standards should have the highest priority for health promotion, with the target changed from "hypertension" to chronomic (BP, HR and other) disorders, to be evaluated for preventive and curative endeavors. The same points noted herein apply equally to all other aspects of health care, involving time-structured variables. Chronomic clinical trials and their individualized results are applicable and cost-saving for diagnosing or treating a patient and are even

more important, when a disease such as MESOR-hypertension is to be prevented since it concerns very many individuals in most populations and the condition can be treated. Most important, chronobiological trials need not be large to give consistent results (37).

Summary of chronobiologic and now chronomic, i.a., BP & HR endeavors leading to BIOCOS

An excessive variability of BP, an excessive pulse pressure (PP) and an insufficient variability of HR constitute very high risks of vascular disease (38). These risks can be detected by a time-structural (chronomic) interpretation of serial BPs and HRs. The historical development is shown in Table 1 with respect only to endeavors involving at least participation by data analysis in Minnesota, with the broad pertinent literature reviewed elsewhere (39).

Original Minnesotan work on BP variability is documented by outcomes based on 97 stroke-prone rats undergoing longitudinally measured 4-hourly-24-hour profiles at about-monthly intervals for their lifetimes. Thereafter, 144,641 measurements were analyzed from studies with partly linked cross-sectional (hybrid) designs (40) involving systematically >2,533 people, Table 2. These studies include data from manual measurements, 6-7 times per day for 2 days and from automatic quarter-hourly, half-hourly or hourly ambulatory monitoring for 2, 7 or 9 days, with follow-up for 6, 7 or 28 years in Germany, Italy, Japan and Minnesota. In their light, Table 2 documents the very high risk of BP overswinging. These results are now being complemented in a broader, albeit as yet spotty international context in Armenia, Belgium, China, the Czech Republic, India, Italy, Japan, Mexico and Norway, as well as in California, Michigan and Minnesota, USA. Complementary, not tabulated results from autorhythmometry, self-measurement and self-interpretation of BP and HR by children in secondary schools in Arkansas, Connecticut and Minnesota, USA, and Florence, Italy have served for concept formulation and experimental validation that chronobiology can be taught in schools (41). The automatic monitoring of adolescents in Russia, Spain and Japan has detected effects of exposure to betamimetics in utero (42), an iatrogenic risk elevation that calls for action in obstetrics.

Longitudinal surveillance is also ongoing with half-hourly automatic or up to ~5/day self-measurements around the clock for up to 16 or 37 years, respectively, on MESOR-normotensive (untreated) "test pilots" and now at half-hour intervals for up to 5 years under treatment with antihypertensive medication (43), extending by far the scope of the measurements made for most of their lifetime after the diagnosis of MESOR-hypertension by opinion-leading MESOR-hypertensive colleagues (13, 14; cf. 30). Table 2 extends the scope of Figure 4, wherein, in a population at large, in the absence of an over-threshold (>95% upper prediction limit of peers of the same gender and age) circadian amplitude (A) of blood pressure (CHAT, Circadian Hyper-Amplitude-Tension) and of an over-threshold pulse pressure, a risk of 5.3% increases to nearly 100% when both conditions coexist (for further details see 8).

The risk of stroke with CHAT is greater than the risk associated with an elevation of the BP-MESOR, Figure 5, and so is the risk of kidney disease, Figure 6 (38). The stroke risk of CHAT remains greater than that from hypertension, whether or not the average pressure (MESOR) is elevated, Figure 7, and is an added risk in the presence of malignant hypertension, Figure 8. CHAT and a deficient heart rate variability (DHRV) are missed by a conventional interpretation but are detected by a chronomically analyzed 24-hour record from ambulatory BP and HR monitoring. We recommend at least 7-day monitoring

at first to rule out transient CHAT. Against the background of the foregoing trials, notably those in Table 2, it seems appropriate also to present illustrative records.

Case reports

The subject of Figure 9 is a mayor, lawyer and father of a youth ice-hockey player, who, for one day, had a statistically significantly higher than normal BP-A under the pressure of threats from husky hockey players, whose tournament game he had to monitor on an unfamiliar computer. During six other days, his values were acceptable (44).

By contrast, Figure 10 presents the BP profile of a woman whose obstetrician did not heed the written warning of her CHAT (45). This is readily understood if one asks only whether the average BP is or is not acceptable. She swung too much, however, as seen from Figure 10, which also shows the acceptable limits of variation as the horizontal upper and lower limits. Hence, bed rest, further monitoring and if the condition persisted, treatment, in this case only of overswinging, were recommended in writing, but were ignored. Her record barely covers 2 days of monitoring and her MESOR of 115 mm Hg is perfectly acceptable for her systolic (S) BP. The same applied to a diastolic BP MESOR of 64 mm Hg. It can be readily understood that with exclusive concern about how high or low the BP may be, a systolic average of 115 mm Hg and a diastolic average of 64 mm Hg seem perfectly acceptable. Eight weeks later, an ensuing eclampsia led to delivery in the 27th gestational week, requiring hospitalization of the baby boy for 26 months, partly cost-accounted. The total expense in this one case was ~ U.S. $1 million (45). Such a large expense was avoided in other cases when chronomic advice was heeded. This case remains anecdotal (45, 46); Table 2 is not anecdotal.

Figure 11 introduces records of a woman who had conflict or grief during 5 days with alterations of BP characteristics, but not during the following weeks of monitoring. Her history over 8 years testifies to mostly MESOR-normotension with occasional BP disorder, including overswinging and/or high BP (47).

Another woman, TS, 27 years of age, had intermittent MESOR-hypertension and intermittent overswinging (CHAT) (48), the conditions introduced in Figure 12. On two office visits by two university physicians, she was described as healthy, even though she showed the record of her ambulatory monitoring. In 2003, the measurement in a physician's office can override the prior record, simply because Janeway (15) and Bartter (16) are not read. The alternative approach of long-term monitoring practiced by Bartter and others on himself (13, 14) is recommended. Figure 13 in turn shows the record of a colleague taking antihypertensive treatment who had these conditions under treatment (43). Such cases, notably the record in Figure 12, indicate the need in some patients for continuous monitoring in order to treat when needed and not to treat when not needed (48).

The even broader scope of chronomic analyses in BIOCOS is to cost-effectively reduce misdiagnoses of a MESOR-hyper-, hypo- or normo-tension. A spurious diagnosis of hypertension is associated with an unnecessary expense, stigma and possible side effects, sometimes for a lifetime. The misdiagnosis of MESOR-normotension and/or the failure to diagnose CHAT, an excessive pulse pressure, and/or DHRV may lull one into a false sense of security. If correctly recognized, however, CHAT can be treated and the risk of massive stroke, nephropathy, myocardial infarction and blindness can be greatly reduced (49). Rendering this treatment timed and timely is within the scope of BIOCOS.

HISTORY OF BIOCOS

The international project on The BIOsphere and the COSmos originated on June 30, 1997, when the Russian Academy of Medical Sciences convened a special session at its headquarters in Moscow to discuss and, at the end of this meeting, to unanimously endorse a project on "The BIOsphere and the COSmos" (BIOCOS), as a follow-up on various international resolutions reviewed elsewhere (38, 45). BIOCOS recommends the introduction of the science of the body's time structure, chronobiology (9), not only into basic science where it belongs, but also in the form of the cartography of chronomes (time structures) as chronomics, Insert 1, into everyday health and environmental care via nationally organized and internationally aligned physiological and physical monitoring and educational endeavors. More specifically, BIOCOS aims at the collection and archivization for basic and applied purposes at different latitudes and longitudes of physical and physiological chronomes. The first step of BIOCOS is the systematic mapping of variation in human BP and HR from womb to tomb, the alignment of these results with centuries-long physical environmental monitoring of terrestrial and atmospheric physical environmental variables and decades-long monitoring in satellites. The opportunistic mapping of many other variables in human and other life forms with particular emphasis on neuroendocrine and vascular mechanisms underlying environmental signal reception such as melatonin and other neuroendocrines are all actively implemented within the scope of BIOCOS, with special focus on feedsideward mechanisms (50-52), as a pertinent resolution (53), backed by the bibliography on the senior author's website, documents.

On July 1, 1997, BIOCOS was introduced at the XXXIII International Congress of the International Union for Physiological Sciences in St. Petersburg, in the context of a symposium on "Adaptation to the Environment" (54). Thereafter, BIOCOS was presented in a course on chronobiology in Mexico City, August 27-30, 1997, in lectures at your Masaryk University, Brno, Czech Republic (September 1, 1997); at a meeting on "Chronobiology with roots in the cosmos", September 2-6, 1997, in Stara Lesna, Slovakia, under the auspices of the Slovak Medical Society; at Safarik University in Kosice (September 8) and the Institute of Clinical Endocrinology in Lubochna (September 9), both in Slovakia and at the International Conference on the Pineal Gland and Cancer (October 2-5, 1997) in Blaubeuren, Germany (55, 56), with follow-ups, such as at an international conference on neuroimmunomodulation in Lugano, Switzerland, September 29-October 2, 1999 (57) and at conferences specifically devoted to chronomics, the mapping of chronomes, i.e., time structures in us, communicating as open systems with chronomes around us (20-22), the last one on chronomes in child development in Munich, Germany, November 29 & 30, 2002 (58).

In 2003, chronomics was the topic of an opening presentation at an international conference on free radicals (February 11-13, 2003) (59), and of an address at BPCON 2003, a national conference on blood pressure in Lucknow, India (February 15-16, 2003) (60, 61; cf. 62), and in other locations in India and Hong Kong, advocating self-help in health care. It is particularly noteworthy that Prof. R.B. Singh, executive director of the International College of Nutrition, is now routinely monitoring notably lowest-class South Asian Indian patients who are at high risk of vascular disease. BIOCOS is associated with a similar endeavor in Amritsar by Prof. Adarsh Kumar, while Prof. R.K. Singh, with the full backing of Shri Vishnu Kant Shastri, the governor of the state of Uttar Pradesh and ex officio the University's chancellor, wishes to introduce chronomic BP monitoring in Lucknow and eventually in Uttar Pradesh more broadly. Accumulating evidence showing

that Southwest Indians are at a special vascular disease risk prompts focus on BP in that region (60-62).

Chronomics (for origin see reference 28) preceded a current functional genomics now based on spotchecks and interpretations of normal ranges; chronomics is a time series (chronome)-based functional genomics, aiming to uncover internal-external interaction among cycles both in the BIOsphere and its COSmos.

BIOCOS and health promotion targeting Non-Communicable Diseases and Mental Health

In the case of communicable conditions, pre-disease, as rhythm alteration within the physiological range, precedes fever in the nosocomial infection along the scale of hours (63). Incubation spans in a number of infections are found along the scale of at least days. The recognition of early vascular disease risk also precedes MESOR-hypertension, albeit a longer span of months is involved in Okamoto's stroke-prone hypertensive rat and probably along still longer scales in humans, Table 1. There is more blood pressure overswinging (CHAT, circadian hyper-amplitude-tension) in conditions of both pre-hypertension, more with BP MESORs between 130 and 140 mm Hg than at lower and higher BP MESORs (64), and there is more CHAT in pre-diabetes (65), i.e., more in glucose sensitivity than either in normoglycemia or in overt diabetes.

Since BP and HR monitoring pick up early risk elevations, we propose the integration of the BIOCOS project for "prehabilitation" by "chronomics" for "chronobiologic health promotion", Figure 4 (cf. 8). An extended BIOCOS, the study of broad time structures (chronomes) in organisms and their environments focuses on health promotion, with emphasis on Non-communicable Diseases and Mental Health, along the following lines:

1. There is growing evidence suggesting that time structures in us and around us are intricately interwoven. Most if not all components of variation found in biota are also found in the environment, and vice versa. For instance, about daily changes are seen in almost every biological variable under 24-hour synchronized conditions. It has also been shown that the phase of circadian rhythms can be manipulated by changing the phase of the environmental cycles. At least for the case of circadian rhythms, their genetic inheritance has been demonstrated on a molecular basis, suggesting that the influence from the environment has been acquired genetically during the course of evolution (66-68).
2. The mapping of chronomes generally should benefit our understanding of human health and disease in several ways. The study of human chronomes contributes refined reference values to better define health and to identify pre-disease, so that prophylactic interventions can be instituted as early as possible, preferably before disease sets in. The focus is thus put on pre-habilitation, in the hope that the need for re-habilitation will thereby be reduced. The study of chronomes of other organisms such as bacteria is also meritorious so that actions can be taken to protect humans and other animals from possible infections, and to apply any eradicating methods at the most opportune time so as to achieve highest efficacy with least side effects. The study of time structures in the environment in turn may help safeguard the integrity of the environment while also gaining a better understanding of the relations between biota and their environment.
3. The Minnesota chronomics center has accumulated archives with reference values, notably in the fields of BP and HR monitoring and of marker rhythmometry for the purposes of screening, diagnosis, treatment and prognosis. Information gained from this

work suggests a close link between mental health and cardiovascular health (7, 69, 70), also apart from chronomics (71-79), but with the documentation by chronomics of BP and HR rhythm alteration during episodes of depression (7).

Within the context of the ambulatory monitoring of BP and HR, the goals of chronomics are to:

a. Implement (manual) home measurement, so that in the next WHO guidelines, the recommendation of home monitoring of blood pressure once or twice a day in specific cases is extended to screening everybody rather than only special cases, with more than 1 or 2 (preferably 6-8) measurements a day, more or less equally spaced for 2 days. This approach detects the risk of overswinging, CHAT, in relation to life expectancy by outcomes in the perspective of 28 years (69, 70; cf. 8). Reasons for the proposed extensions of self-measurements are that the error associated with single measurements is great, that BP is characterized by a circadian variation of large extent, so that one or two measurements a day, always at awakening and/or at bedtime may fail to reveal abnormalities seen only at other times of day, and that some major abnormalities apply to the dynamics of BP rather than affecting only the single or mean value.

b. Document the need for extending automatic ambulatory monitoring from the current 24-hour records for questionable cases to routine 7-day/24-hour screening of everybody. Abnormalities in BP variability, as implied, will be necessarily missed in single measurements. Moreover, there is mounting evidence showing that a non-negligible fraction of the population has a circadian variation in blood pressure that can change drastically from one day to another.

c. Provide evidence for the need of a chronobiologic approach to interpret records of BP and HR. By extending the monitoring to the population at large, clinically healthy individuals can provide the needed reference values to be further refined as a function of gender, age, ethnicity, geography, social class, and long-term risk. Whereas the presence of a prominent circadian rhythm in BP is no longer contested, this knowledge has not been applied to time-specify reference values, so that the diagnosis does not depend on the clock hour of the clinical examination (8, 11, 12, 80, 81).

d. Introduce a double-barreled approach to the interpretation of BP and HR records, consisting of a parametric and a non-parametric analysis of the data, each comparing results from the individual subject being monitored with reference values in health both for the rhythm parameters and for time-specified values. Reference values for parameters have led to the identification of new disease risk syndromes, such as CHAT, BP-ecphasia and DHRV, among others (8, 80, 81).

e. Organize databases of all records, preferably with regular follow-ups of the subjects who provided the records, in order to build chronome evidence-based archives, which could serve for amending guidelines regarding the identification of pre-disease conditions and the treatment optimization by timing and the validation of this endeavor. In addition to the data, outcomes and pertinent clinical data are to be added to the existing databases. Information from both prospective trials and from retrospective analyses are to add to the current knowledge, notably regarding the identification of new disease risk syndromes and any appropriate interventions, so that a change in morbidity/mortality can be associated with changes in BP and/or HR characteristics occurring naturally or intentionally induced by a given treatment or by environmental features including magnetic storms (cf. 19, 24, 82, 83).

f. Broaden the databases to include the special analysis of records provided by test pilots to be recruited with incentives yet to be developed for lifetime monitoring. Such records

are also invaluable to basic science to further the understanding of the influence of environmental factors near and far on human physiology and pathology. This aspect would focus on problems such as the detection of new components of variation, such as an about 1.3-year change just observed in BP and HR, resembling by its length an about 1.3-year variation in the velocity of the solar wind. Investigations of problems related to the recent increase in mortality from stroke, which is found to be characterized by an about 50-year cycle (19, 24, 84), or to the about 10.5-year cycle in mortality from myocardial infarctions observed in Minnesota (7, 24) are also on the agenda.

The role of chronomics within the context of Non-communicable Diseases and Mental Health is not limited to the cardiovascular system. The same methodology remains applicable to a wide range of problems. Cancer prevention and optimization by the scheduling of treatment administration is another important problem. BIOCOS is now following up on the earlier demonstration of doubling the 2-year survival rate of oral cancers by the timing of radiotherapy by a marker rhythm (at the circadian peak of tumor temperature [85-87]). New trials have just begun in China that are coordinated by BIOCOS. But the main focus of BIOCOS in health promotion upon the circulation by prehabilitation, to reduce the cost of rehabilitation, notably in un- or under-developed areas, by education.

The challenges of instrumentation and new data

It seems fitting that in Brno, the home of Mendel, who originated the study of genetics -- biological diversity in space -- chronomics, the cartography of diversity in time chronomes (structures in time) is well on its way by the 7-day/24-hour monitoring done as a model for the world. The baby monitoring also done in Brno places Jarmila Siegelova's team further into the forefront of chronomics, so that, with the broader BIOCOS' evidence in hand, new, even less obtrusive instrumentation will be developed and used in the home city of Jan Penaz who started it all with dense monitoring. The instrumentation from A&D that we now use was a dream half a century ago; but implantable prototypes are already available for humans. With the current monitors, BIOCOS is ongoing, beyond the Czech Republic, in pregnant women in Armenia and Italy, in general practice in Belgium, in the elderly in China and Japan, on diabetics in Mexico (88), and in cardiology in India and Japan, among other themes and locations. At a meeting on chronomes in Munich, the detection of links of biological variables in the human circulation with the cosmos was reported by several speakers from different geographic locations. Claims of long standing (89-91; cf. 19, 24, 92-94) by the Soviets and currently from Russia are now being documented by inferential statistics with the added major point that some of these nonphotic effects are cyclic and, perhaps, just like the circadians, are built into us. Cases in point are the built-in week demonstrated in babies in Brno by Jarmila Siegelova (95), the ~50-year cycle in strokes (19, 24, 84), which is also reflected in 2,556 years of international battles (96), the half-year of epilepsy (97) and of several conditions of the oral mucosa and skin (98) and the most recent discovery of a 1.3-year component in human blood pressure, heart rate and myocardial infarction corresponding in period to a similar component in the solar wind (23, 99; cf. 6), all centering on the prediction made by BIOCOS (19) of the reciprocity of the spectral components in the environment and in the biosphere. We are open systems to our cosmoi, exhibiting their signatures in morphology, physiology and pathology.

At one time in the past, all circadian rhythms we tested could be switched by manipulating lighting (100, 101). Thereafter, under conditions of a diet restricted in

calories, the in-retrospect obvious primacy of feeding came to the fore half a century ago (67). The extent to which a broad photoperiodism has to be complemented by a magnetoperiodism has to be scrutinized further for likely dividends in health care, since these cycles affect the health of individuals in dealing with cancer as well as with the circulation, among many other conditions that may relate, directly or indirectly, to the heart and the brain. In turning to the brain and its cycles, with a relatively low frequency, we focus, as yet in a most rudimentary way, upon diseases of society, such as crime and wars (102), and further upon spirituality (103).

Epilogue

There is a collateral hierarchy in research designs, as in an organism considering designs (104). Randomized controlled trials continuing to be discussed (105-112), considered to be the highest level of evidence, are important but the very process of randomization may obscure intra-individual changes when spotchecks based on a few or single time-unspecified samples are used. This approach will certainly obscure possibly great inter-individual differences. Case reports of chronomic n-of-1 studies, notably as they accumulate systematically and are complemented by relatively small chronomic trials, can compensate for the shortcomings of the large clinical trials. Accounting for gender, age and other considerations, in dealing with periodic variables using parameters of rhythms, characterizing what goes into a chronomic trial is best done cost-effectively again by educated self-help. Individuals can learn to help themselves by contributing to their own health care and to good biomedicine and good science as well. Only thus, new fundamental discoveries such as those in Figure 14 came about. When starting self-measurements about 5 times each day, now for 37 years, Robert B. Sothern was motivated to do good science. He did not think about the largely unseen part of the cosmos, such as the ionized gas that streams continuously into space from what E.N. Parker, just a few years earlier in 1958, theorized was a solar wind, with an about 1.3-yearly change that can beat with effects of changes from winter to summer. Such information sooner or later will yield dividends for a cost-effective health care, whether we call it chronomic, chronobiologic or any other name. A health care based on single time-unqualified spotchecks sooner or later will be replaced by one based on time series. Just as microscopy, then electron microscopy, and eventually molecular biology in space are obviously needed, so is microscopy in time, to resolve earliest risk elevations for cost-effective health promotion, Figure 15.

REFERENCES

1. Siegelová J, Cornélissen G, Halberg F, Fiser B, Dusek J, Al-Kubati M, Machat R, Kumagai Y, Watanabe Y, Otsuka K. Self-starting cumulative sums for the individualized assessment of antihypertensive response and optimization by timing. Keynote, 2nd Int. Symp. of Chronobiology and Chronomedicine, Shenyang, China, Sept. 28-Oct. 2, 1996. p. 48.
2. Siegelova J, Fiser B, Dusek J, Halberg F, Cornélissen G. 24-h blood pressure profile in essential hypertension after verapamil, nitrendipine and enalapril treatment. Scripta medica (Brno) 1997; 70: 373-374.

3. Siegelova J, Cornélissen G, Dusek J, Fiser B, Watanabe Y, Otsuka K, Halberg F. Diagnosis and assessment of treatment effects: a single 24-hour blood pressure monitoring profile. Scripta medica (Brno) 1998; 71: 209-214.
4. Siegelova J, Fiser B, Dusek J, Placheta Z, Cornélissen G, Halberg F. Circadian variability of rate-pressure product in essential hypertension with enalapril therapy. Scripta medica (Brno) 2000; 73: 67-75.
5. Halberg F, Stephens AN. Susceptibility to ouabain and physiologic circadian periodicity. Proc Minn Acad Sci 1959; 27, 139-143.
6. Halberg F, Cornélissen G, Schack B. Self-experimentation on chronomes, time structures; chronomics for health surveillance and science: also transdisciplinary civic duty? Behavioral and Brain Sciences, http://www.bbsonline.org/Preprints/Roberts/Commentators/Halberg.html
7. Cornélissen G, Halberg F, Schwartzkopff O, Delmore P, Katinas G, Hunter D, Tarquini B, Tarquini R, Perfetto F, Watanabe Y, Otsuka K. Chronomes, time structures, for chronobioengineering for "a full life". Biomed Instrum Technol 1999; 33: 152-187.
8. Cornélissen G, Halberg F, Bakken EE, Wang ZR, Tarquini R, Perfetto F, Laffi G, Maggioni C, Kumagai Y, Prikryl P, Homolka P, Siegelova J, Dusek J, Fiser B. Can society afford not to follow a chronobiological approach to blood pressure screening, diagnosis and treatment? This volume.
9. Halberg F. Chronobiology. Annu Rev Physiol 1969; 31: 675-725.
10. JNC 7 Express: The Seventh Report of the Joint National Committee on Detection, Evaluation, and Treatment of High Blood Pressure. Bethesda, MD: US Department of Health and Human Services, National Institutes of Health, National Heart, Lung, and Blood Institute, National High Blood Pressure Education Program. NIH Publ. #03-5233, May 2003. 34 + xiii pp.
11. Cornélissen G, Halberg F. Impeachment of casual blood pressure measurements and the fixed limits for their interpretation and chronobiologic recommendations. In: Portaluppi F, Smolensky MH, eds. Time-dependent Structure and Control of Arterial Blood Pressure. Ann NY Acad Sci 1996; 783: 24-46.
12. Watanabe Y, Cornélissen G, Halberg F. Thousands of blood pressure and heart rate measurements at fixed clock hours may mislead. Neuroendocrinol Lett, in press.
13. Levine H, Halberg F. Circadian rhythms of the circulatory system. Literature review. Computerized case study of transmeridian flight and medication effects on a mildly hypertensive subject. U.S. Air Force Report SAM-TR-72-3. Brooks AFB, Texas: USAF School of Aerospace Medicine, Aerospace Medical Division (AFSC); April 1972. 64 pp.
14. Bartter FC, Delea CS, Baker W, Halberg F, Lee JK. Chronobiology in the diagnosis and treatment of mesor-hypertension. Chronobiologia 1976; 3: 199-213.
15. Janeway TC. The clinical study of blood pressure. New York: D. Appleton & Co.; 1904. 300 pp.
16. Bartter FC. Periodicity and medicine. In: Scheving LE, Halberg F, Pauly JE, eds. Chronobiology. Tokyo: Igaku Shoin Ltd., 1974: 6-13.
17. Halberg F. Protection by timing treatment according to bodily rhythms: an analogy to protection by scrubbing before surgery. Chronobiologia 1974; 1 (Suppl. 1): 27-68.
18. Carandente F, Halberg F, editors. Chronobiology of blood pressure in 1985. Chronobiologia 1984; 11: #3. p. 189-341.
19. Halberg F, Cornélissen G, Otsuka K, Watanabe Y, Katinas GS, Burioka N, Delyukov A, Gorgo Y, Zhao ZY, Weydahl A, Sothern RB, Siegelova J, Fiser B, Dusek J, Syutkina EV, Perfetto F, Tarquini R, Singh RB, Rhees B, Lofstrom D, Lofstrom P, Johnson PWC, Schwartzkopff O, International BIOCOS Study Group. Cross-spectrally coherent ~10.5- and 21-year biological and physical cycles, magnetic storms and myocardial infarctions. Neuroendocrinol Lett 2000; 21: 233-258.
20. Cornélissen G, Halberg F, Breus T, Syutkina EV, Baevsky R, Weydahl A, Watanabe Y, Otsuka K, Siegelova J, Fiser B, Bakken EE. Non-photic solar associations of heart rate variability and myocardial infarction. J Atmos Solar-Terr Phys 2002; 64: 707-720.

21. Cornélissen G, Masalov A, Halberg F, Richardson JD, Katinas GS, Sothern RB, Watanabe Y, Syutkina EV, Wendt HW, Bakken EE, Romanov Y. Multiple resonances among time structures, chronomes, around and in us. Is an about 1.3-year periodicity in solar wind built into the human cardiovascular chronome? Human Physiology, in press.
22. Otsuka K (editor). Proceedings, 1st International Symposium Workshop on Circadian Rhythms and Clinical Chronobiology, 11 Nov 2000, Tokyo, Japan. Biomedicine & Pharmacotherapy 2001; 55 (Suppl 1): 7s-190s.
23. Otsuka K (editor). Proceedings, 2nd International Symposium Workshop on Circadian Rhythms and Clinical Chronobiology, 17 Nov 2001, Tokyo, Japan. Biomedicine & Pharmacotherapy 2001; 56 (Suppl 2): 231s-382s.
24. Otsuka K (editor). Proceedings, 3rd International Symposium: Workshop on Chronoastrobiology and Chronotherapy, 9 Nov 2002, Tokyo, Japan. Biomedicine & Pharmacotherapy, in press.
25. Schwartzkopff O, Halberg F, Cornelissen G, Katinas G, Wang ZR, Kröz M, Hecht K, Tarquini R, Perfetto F, Maggioni C, Siegelova J, Dusek J, Fiser B, Singh RB, Bakken EE. Self-experimentation -- dangerous when first done by a few, now safe for everybody's health? Symposium for 60th Birthday of Bohumil Fiser, Brno, CR, November 3, 2003, 91-104..
26. Otsuka K (organizer). Proceedings, 1st International Symposium, Workshop on Chronoastrobiology & Chronotherapy (Satellite Symposium, 7th Annual Meeting, Japanese Society for Chronobiology), Kudan, Chiyodaku, Tokyo, 11 Nov 2000, 170 pp.
27. Otsuka K, Cornélissen G, Halberg F. Circadian rhythmic fractal scaling of heart rate variability in health and coronary artery disease. Clinical Cardiology 1997; 20: 631-638.
28. Halberg F, Cornélissen G, Otsuka K, Schwartzkopff O, Halberg J, Bakken EE. Chronomics. Biomedicine and Pharmacotherapy 2001; 55 (Suppl 1): 153-190.
29. Halberg F, Cornélissen G, Spector NH, Sonkowsky RP, Otsuka K, Baciu I. Stress/strain revisited. Quantification by blood pressure chronomics: benetensive, transtensive or maletensive chrono-vasculo-neuro-immuno-modulation. Biomed Pharmacol, in press.
30. Cornélissen G, Halberg F, Hawkins D, Otsuka K, Henke W. Individual assessment of antihypertensive response by self-starting cumulative sums. J Medical Engineering & Technology 1997; 21: 111-120.
31. Bingham C, Arbogast B, Cornélissen Guillaume G, Lee JK, Halberg F. Inferential statistical methods for estimating and comparing cosinor parameters. Chronobiologia 1982; 9: 397-439.
32. Halberg F, Cornélissen G, Carandente A, Bakken E, Young E. Chronobiologic perspectives of international health care reform for the future of children. Chronobiologia 1993; 20: 269-275.
33. Halberg J, Sonkowsky P, Sonkowsky R, Halberg F. Autorhythmometry, time and the humanities. In: Halberg F, editor. Proc. XII Int. Conf. International Society for Chronobiology, Washington, D.C., 1977. Milan: Il Ponte, 1977. p. 167-179.
34. LaSalle D, Sothern RB, Halberg F. Sampling requirements for description of circadian blood pressure (BP) amplitude (A). Chronobiologia 1983; 10: 138.
35. Halberg F, Johnson EA, Nelson W, Runge W, Sothern R. Autorhythmometry-procedures for physiologic self-measurements and their analysis. Physiol Tchr 1972; 1: 1-11.
36. Ahlgren A, Halberg F. Cycles of Nature: An Introduction to Biological Rhythms. Washington DC: National Science Teachers Association; 1990. 87 pp.
37. Halberg F, Bingham C, Cornélissen G. Clinical trials: the larger the better ? Chronobiologia 1993; 20: 193-212.
38. Halberg F, Cornélissen G, International Womb-to-Tomb Chronome Initiative Group: Resolution from a meeting of the International Society for Research on Civilization Diseases and the Environment (New SIRMCE Confederation), Brussels, Belgium, March 17-18, 1995: Fairy tale or reality ? Medtronic Chronobiology Seminar #8, April 1995, 12 pp. text, 18 figures. URL [accessed 1994]

http://www.msi.umn.edu/~halberg/, AND Halberg F, Cornélissen G, Siegelová J, Fiser B. Rezoluce S.I.R.M.C.E. Amireport 1995; 2: 105-106. [In Czech.]

39. Singh RB, Cornélissen G, Weydahl A, Schwartzkopff O, Katinas G, Otsuka K, Watanabe Y, Yano S, Mori H, Ichimaru Y, Mitsutake G, Pella D, Fanghong L, Zhao Z, Rao RS, Gvozdjakova A, Halberg F. Circadian heart rate and blood pressure variability considered for research and patient care. Int J Cardiol 2003; 87: 9-28.
40. Halberg F, Nelson W, Runge WJ, Schmitt OH, Pitts GC, Tremor J, Reynolds OE. Plans for orbital study of rat biorhythms. Results of interest beyond the Biosatellite program. Space Life Sci 1971; 2: 437-471.
41. Halberg F, Smith HN, Cornélissen G, Delmore P, Schwartzkopff O, International BIOCOS Group. Hurdles to asepsis, universal literacy, and chronobiology-all to be overcome. Neuroendocrinol Lett 2000; 21: 145-160.
42. Syutkina EV, Cornélissen G, Halberg F, Grigoriev AE, Abramian AS, Yatsyk GV, Morozova NA, Ivanov AP, Shevchenko PV, Polyakov YA, Bunin AT, Safin SR, Maggioni C, Alvarez M, Fernandez O, Tarquini B, Mainardi G, Bingham C, Kopher R, Vernier R, Rigatuso J, Johnson D. Effects lasting into adolescence of exposure to betamimetics in utero. Clinical Drug Investigation 1995; 9: 354-362.
43. Katinas G, Halberg F, Cornélissen G, Otsuka K, Tarquini R, Perfetto F, Maggioni C, Schwartzkopff O, Bakken E. Transient circadian hyper-amplitude-tension (CHAT) may be intermittent: case reports illustrating gliding spectral windows. Biomed Pharmacol, in press.
44. Cornélissen G, Halberg F, Wall D, Siegelova J, Zaslavskaya RM. How long to screen: ice hockey game and transient circadian hyperamplitudetension, CHAT. Scripta medica (Brno) 1997; 70: 189-198.
45. Halberg F, Cornélissen G, Carandente F. Chronobiology leads toward preventive health care for all: cost reduction with quality improvement. A challenge to education and technology via chronobiology. Chronobiologia 1991; 18: 187-193.
46. Cornélissen G, Rigatuso J, Syutkina EV, Schwartzkopff O, Johnson DE, Halberg F, International Womb-to-Tomb Chronome Group: Case report of an acceptable average but overswinging blood pressure in Circadian Hyper-Amplitude-Tension, CHAT. Neuroendocrinol Lett, in press.
47. Halberg F, Cornélissen G, Otsuka K, Katinas GS, Schwartzkopff O, Halpin C, Mikulecky M, Revilla M, Siegelova J, Homolka P, Dusek J, Fiser B, Singh RB. Chronomics* (*the study of time structures, chronomes) detects altered vascular variabilities constituting risks greater than hypertension: with an illustrative case report. In: Mitro P, Pella D, Rybar R, Valocik G, editors. Proceedings, 2nd Congress on Cardiovascular Diseases, Kosice, Slovakia, 25-27 April 2002. Bologna: Monduzzi Editore; 2002. p. 223-258.
48. Sarabandi T, Cornélissen G, Katinas G, Halberg F, Holte J. Chronomically interpreted q30-minute 7-month record of intermittently high, gradually increasing and more and more overswinging blood pressure (BP) and mood. Abstract, 4th International Symposium, Workshop on Chronoastrobiology and Chronotherapy, Tokyo, Nov. 2003, in press.
49. Shinagawa M, Kubo Y, Otsuka K, Ohkawa S, Cornélissen G, Halberg F. Impact of circadian amplitude and chronotherapy: relevance to prevention and treatment of stroke. Biomedicine and Pharmacotherapy 2001; 55 (Suppl 1): 125-132.
50. Halberg F. Quo vadis basic and clinical chronobiology: promise for health maintenance. Am J Anat 1983; 168: 543-594.
51. Sanchez de la Pena S. The feedsideward of cephalo-adrenal immune interactions. Chronobiologia 1993; 20: 1-52.
52. Halberg F, Sanchez de la Pena S, Wetterberg L, Halberg J, Halberg Francine, Wrba H, Dutter A, Hermida Dominguez RC. Chronobiology as a tool for research, notably on melatonin and tumor development. In: Pancheri P, Zichella L, editors. Biorhythms and Stress in the Physiopathology of Reproduction. New York: Hemisphere; 1988. p. 131-175.
53. Halberg F, Syutkina EV, Cornélissen G. Chronomes render predictable the otherwise-neglected human "physiological range": position paper of the BIOCOS project. Human Physiology 1998; 24: 14-21.

54. Halberg F. Chronobiology and adaptations to environments near and far: the Cornélissen-series, Chizhevsky's legacy in 1997. Proc. XXXIII Int. Cong. International Union of Physiological Sciences, St. Petersburg, Russia, June 30-July 5, 1997, L041.02.
55. Halberg F, Cornélissen G, Conti C, Maestroni G, Maggioni C, Perfetto F, Salti R, Tarquini R, Katinas GS, Schwartzkopff O. The pineal gland and chronobiologic history: mind and spirit as feedsidewards in time structures for prehabilitation. In: Bartsch C, Bartsch H, Blask DE, Cardinali DP, Hrushesky WJM, Mecke W, editors. The Pineal Gland and Cancer: Neuroimmunoendocrine Mechanisms in Malignancy. Heidelberg: Springer; 2000. p. 66-116.
56. Cornélissen G, Halberg F, Perfetto F, Tarquini R, Maggioni C, Wetterberg L. Melatonin involvement in cancer: methodological considerations. In: Bartsch C, Bartsch H, Blask DE, Cardinali DP, Hrushesky WJM, Mecke W, editors. The Pineal Gland and Cancer: Neuroimmunoendocrine Mechanisms in Malignancy. Heidelberg: Springer; 2000. p. 117-149.
57. Halberg F, Cornélissen G, Katinas GS, Watanabe Y, Otsuka K, Maggioni C, Perfetto F, Tarquini R, Schwartzkopff O, Bakken EE. Feedsidewards: intermodulation (strictly) among time structures, chronomes, in and around us, and cosmo-vasculo-neuroimmunity. About ten-yearly changes: what Galileo missed and Schwabe found. In: Conti A, Maestroni GJM, McCann SM, Sternberg EM, Lipton JM, Smith CC (eds.), Neuroimmunomodulation (Proc. 4th Int. Cong. International Society for Neuroimmunomodulation, Lugano, Switzerland, September 29-October 2, 1999). Ann NY Acad Sci 2000; 917: 348-376.
58. Cornélissen G, Schwartzkopff O, Niemeyer-Hellbrügge P, Halberg F, editors. Proceedings, International Interdisciplinary Conference "Time structures -- chronomes -- in child development", Munich, Germany, Nov. 29-30, 2002. Neuroendocrinol Lett, in press.
59. Halberg F, Cornélissen G, Wang ZR, Wan C, Ulmer W, Katinas G, Singh Ranjana, Singh RK, Singh Rajesh, Gupta BD, Singh RB, Kumar A, Kanabrocki E, Sothern RB, Rao G, Bhatt MLBD, Srivastava M, Rai G, Singh S, Pati AK, Nath P, Halberg Francine, Halberg J, Schwartzkopff O, Bakken E, Shastri VK. Chronomics: circadian and circaseptan timing of radiotherapy, drugs, calories, perhaps nutriceuticals and beyond. J Exp Therapeutics Oncol, in press.
60. Halberg F, Cornélissen G, Katinas G, Otsuka K, Watanabe Y, Singh RB, Sundaram B, Holley DC, Aslanian N, Mikulecky M, Schwartzkopff O, Bakken E. The practicability and cost-effectiveness of blood pressure and heart rate chronomics. In: Proc BPCON 2003, Lucknow, India, 15-16 February 2003, in press.
61. Sundaram B, Holley DC, Cornélissen G, Naik D, Singh RB, Halberg F. Circadian and circaseptan (about-weekly) aspects of immigrant Indians' blood pressure and heart rate in California, USA. Proc BPCON 2003, Lucknow, India, 15-16 February 2003, in press.
62. Halberg F, Cornélissen G, Singh RB, Schwartzkopff O. The challenge and opportunity of blood pressure chronomics in India. In: Rao GHR, editor. Coronary Artery Disease in Indians: Risk Factors, Pathophysiology and Prevention. New Delhi: Jaypee Medical Publishers, in press.
63. Halberg E, Fanning R, Halberg F, Cornélissen G, Wilson D, Griffiths K, Simpson H. Toward a chronopsy: Part III. Automatic monitoring of rectal, axillary and breast surface temperature and of wrist activity; effects of age and of ambulatory surgery followed by nosocomial infection. Chronobiologia 1981; 8: 253-271.
64. Watanabe Y, Cornélissen G, Halberg F, Bingham C, Siegelova J, Otsuka K, Kikuchi T. Incidence pattern and treatment of a clinical entity, overswinging or circadian hyperamplitudetension (CHAT). Scripta medica (Brno) 1997; 70: 245-261.
65. Sanchez de la Pena S, Gonzalez C, Cornélissen G, Halberg F. Chronomics: time structure (chronome) of blood pressure and heart rate in health and diabetes. Abstract 26, 2nd International Symposium: Workshop on Chronoastrobiology & Chronotherapy, Tokyo Kasei University, Tokyo, Japan, November 2001, unpaginated (2 pp).
66. Halberg F, Visscher MB. Regular diurnal physiological variation in eosinophil levels in five stocks of mice. Proc Soc exp Biol (N.Y.) 1950; 75: 846-847.

67. Halberg Franz, Cornélissen G, Katinas G, Syutkina EV, Sothern RB, Zaslavskaya R, Halberg J, Halberg Francine, Watanabe Y, Schwartzkopff O. Transdisciplinary unifying implications of circadian findings in the 1950s. J Circadian Rhythms, in press.http://www.jcircadianrhythms.com/default/default.asp?preview=true
68. Refinetti R. Circadian Physiology. Boca Raton, Florida: CRC Press; 1999. 184 pp + 1 computer disk (3.5", Windows 3.0 or higher, 8 MB RAM, 2 MB free disk space, minimum 640 x 480, 256 colors).
69. Halberg F, Bakken E, Cornélissen G, Halberg J, Halberg E, Delmore P. Blood pressure assessment with a cardiovascular summary, the sphygmochron, in broad chronobiologic perspective. In: Refsum H, Sulg JA, Rasmussen K, editors. Heart & Brain, Brain & Heart. Berlin: Springer-Verlag; 1989. p. 142-162.
70. Halberg F, Cornélissen G, Schwartzkopff O, Hardeland R, Ulmer W. Chronobiologische Überwachung von Blutdruck und Herzfrequenz für die Praxis. Proc Leibniz Soz 2003; 54: 127-156.
71. Joynt KE, Whellan DJ, O'Connor CM. Depression and cardiovascular disease: mechanisms of interaction. Biological Psychiatry 2003; 54: 248-261.
72. Miller GE, Freedland KE, Carney RM, Stetler CA, Banks WA. Pathways linking depression, adiposity, and inflammatory markers in healthy young adults. Brain, Behavior, and Immunity 2003; 17: 276-285.
73. Bondy B, Baghai TC, Zill P, Bottlender R, Jaeger M, Minov C, Schule C, Zwanzger P, Rupprecht R, Engel RR. Combined action of the ACE D- and the G-protein beta3 T-allele in major depression: a possible link to cardiovascular disease? Molecular Psychiatry 2002; 7: 1120-1126.
74. Jones DJ, Bromberger JT, Sutton-Tyrrell K, Matthews KA. Lifetime history of depression and carotid atherosclerosis in middle-aged women. Archives of General Psychiatry 2003; 60: 153-160.
75. Roose SP. Depression, anxiety, and the cardiovascular system: the psychiatrist's perspective. J Clinical Psychiatry 2001; 62 (Suppl 8): 19-22, discussion 23.
76. Schultz R. Cardiovascular disease & depression. Australian Family Physician 2001; 30: 210-223.
77. Schulz R, Beach SR, Ives DG, Martire LM, Ariyo AA, Kop WJ. Association between depression and mortality in older adults: the Cardiovascular Health Study. Arch Int Med 2000; 160: 1761-1768.
78. Takemura Y, Kikuchi S, Takagi H, Inaba Y, Nakagawa K. A cross-sectional study on the relationship between depression and left ventricular hypertrophy. Preventive Medicine 1998; 27: 787-791.
79. Wassertheil-Smoller S, Applegate WB, Berge K, Chang CJ, Davis BR, Grimm R Jr, Kostis J, Pressel S, Schron E. Change in depression as a precursor of cardiovascular events. SHEP Cooperative Research Group (Systolic Hypertension in the Elderly). Arch Int Med 1996; 156: 553-561.
80. Halberg F, Cornélissen G, Wall D, Otsuka K, Halberg J, Katinas G, Watanabe Y, Halhuber M, Mueller-Bohn T, Delmore P, Siegelova J, Homolka P, Fiser B, Dusek J, Sanchez de la Pena S, Maggioni C, Delyukov A, Gorgo Y, Gubin D, Carandente F, Schaffer E, Rhodus N, Borer K, Sonkowsky RP, Schwartzkopff O. Engineering and governmental challenge: 7-day/24-hour chronobiologic blood pressure and heart rate screening: Part I. Biomedical Instrumentation & Technology 2002; 36: 89-122.
81. Halberg F, Cornélissen G, Wall D, Otsuka K, Halberg J, Katinas G, Watanabe Y, Halhuber M, Mueller-Bohn T, Delmore P, Siegelova J, Homolka P, Fiser B, Dusek J, Sanchez de la Pena S, Maggioni C, Delyukov A, Gorgo Y, Gubin D, Carandente F, Schaffer E, Rhodus N, Borer K, Sonkowsky RP, Schwartzkopff O. Engineering and governmental challenge: 7-day/24-hour chronobiologic blood pressure and heart rate screening: Part II. Biomedical Instrumentation & Technology 2002; 36: 183-197.
82. Breus TK, Pimenov KYu, Cornélissen G, Halberg F, Syutkina EV, Baevsky RM, Petrov VM, Orth-Gomer K, Akerstedt T, Otsuka K, Watanabe Y, Chibisov SM. The biological effects of solar activity. Biomed Pharmacother 2002; 56 (Suppl. 2): 273s-283s.
83. Masalov AV, Syutkina EV. Magnetic storms and neonatal blood pressure and heart rate chronomes. Neuroendocrinol Lett, in press.
84. Fiser B, Cornélissen G, Siegelova J, Dusek J, Homolka P, Mazankova V, Halberg F. Increase in stroke deaths after 1997 in the Czech Republic. Scripta medica (Brno) 2002; 75: 95-100.

85. Halberg F, Gupta BD, Haus E, Halberg E, Deka AC, Nelson W, Sothern RB, Cornélissen G, Lee JK, Lakatua DJ, Scheving LE, Burns ER. Steps toward a cancer chronopolytherapy. In: Proc. XIV International Congress of Therapeutics. Montpellier, France: L'Expansion Scientifique Française; 1977. p. 151-196.
86. Halberg Francine, Halberg J, Halberg E, Halberg Franz. Chronobiology, radiobiology and steps toward the timing of cancer radiotherapy. In: Goldson AL, volume editor. Cancer Growth and Progression, vol. 9, ch. 19, Kaiser H, series editor. Dordrecht: Kluwer Academic Publ.; 1989. p. 227-253.
87. Cornélissen G, Halberg F. The chronobiologic pilot study with special reference to cancer research: Is chronobiology or, rather, its neglect wasteful? In: Goldson AL, volume ed. Cancer Growth and Progression, vol. 9, ch. 9, Kaiser H series ed. Dordrecht: Kluwer Academic Publ.; 1989. p. 103-133.
88. Gonzalez V. RC, Sanchez de la Pena S, Siegelova J, Svacinova H, Cornélissen G, Zhou S, Holte J, Katinas GS, Halberg F. Putative blood pressure overswinging, circadian hyper-amplitude-tension (CHAT), in type 2 diabetes. Scripta medica (Brno) 2002; 75: 121-124.
89. Chizhevsky AL. Effect of the periodic solar activity on social phenomena. Traité de climatologie, biologique et médicale, Vol. 1, Paris (in Russian), 1934: 376-385.
90. Chizhevsky AL. Cosmobiologie et Rythme du Milieu extérieur. Verhandlungen, Zweiten Konferenz der Internationalen Gesellschaft für Biologische Rhythmusforschung, am 25. und 26. August 1939, Utrecht, Holland, Holmgren Hj. (ed.). Acta med scand 1940; 108 (Suppl.): 211-226.
91. Chizhevsky AL (Fedynsky VV, ed). The Earth in the Universe. Translated from Russian [and edited by IRST staff]. NASA TT F-345. Jerusalem: Israel Program for Scientific Translations [available from US Dept of Commerce, Clearinghouse for Federal Scientific and Technical Information, Springfield, Virginia]; 1968. 280 pp.
92. Mikulecky M (ed.) The Moon and Living Matter. Kosice, Slovakia, September 23-25, 1993. Bratislava: Slovak Medical Society; 1993. 97 pp.
93. Mikulecky M (ed.) Sun, Moon and Living Matter. Bratislava, Slovakia, June 28-July 1, 1994. Bratislava: Slovak Medical Society; 1994. 159 pp.
94. Mikulecky M (ed.). Chronobiology & Its Roots in the Cosmos. High Tatras, Slovakia, September 2-6, 1997. Bratislava: Slovak Medical Society; 1997. 287 pp.
95. Siegelova J, Dusek J, Fiser B, Nekvasil R, Muchova M, Cornélissen G, Halberg F. Circaseptan rhythm in blood pressure and heart rate in newborns. Scripta medica (Brno) 1996; 67 (Suppl. 2): 63-70.
96. Halberg F, Cornélissen G, Katinas G, Appenzeller O, Otsuka K, Sothern RB, Tarquini R, Perfetto F, Maggioni C, Wilson D, Schröder W, Schwartzkopff O, Kessler T, Wang Z, Burioka N, Watanabe Y, Bakken E. System times and time horizons for biospheric near-matches of primarily non-photic environmental cycles. Biomed Pharmacother 2002; 56 (Suppl. 2): 266s-272s.
97. Halberg F, Breus TK, Cornélissen G, Bingham C, Hillman DC, Rigatuso J, Delmore P, Bakken E, International Womb-to-Tomb Chronome Initiative Group: Chronobiology in space. Keynote, 37th Ann. Mtg. Japan Soc. for Aerospace and Environmental Medicine, Nagoya, Japan, November 8-9, 1991. University of Minnesota/Medtronic Chronobiology Seminar Series, #1, December 1991, 21 pp. of text, 70 figures.
98. Cornélissen G, Halberg F, Katinas GS, Minne H, Breus T, Sothern RB, Watanabe Y, Tarquini R, Perfetto F, Maggioni C, Wilson D, Gubin D, Otsuka K, Bakken EE. Circasemiannual chronomics: half-yearly biospheric changes in their own right and as a circannual waveform. Biomed Pharmacol, in press.
99. Cornélissen G, Halberg F, Wendt HW, Bingham C, Sothern RB, Haus E, Kleitman E, Kleitman N, Revilla MA, Revilla M Jr, Breus TK, Pimenov K, Grigoriev AE, Mitish MD, Yatsyk GV, Syutkina EV. Resonance of about-weekly human heart rate rhythm with solar activity change. Biologia (Bratislava) 1996; 51: 749-756.
100. Halberg F, Halberg E, Barnum CP, Bittner JJ. Physiologic 24-hour periodicity in human beings and mice, the lighting regimen and daily routine. In: Withrow RB, editor. Photoperiodism and Related Phenomena in Plants and Animals. Ed. Publ. No. 55. Washington DC: AAAS; 1959. p. 803-878.

101. Halberg F, Barnum CP, Silber RH, Bittner JJ. 24-hour rhythms at several levels of integration in mice on different lighting regimens. Proc Soc exp Biol (NY) 1958; 97, 897-900.
102. Halberg F, Cornélissen G, Otsuka K, Katinas G, Schwartzkopff O. Essays on chronomics spawned by transdisciplinary chronobiology: Witness in time: Earl Elmer Bakken. Neuroendocrinol Lett 2001; 22: 359-384.
103. Starbuck S, Cornélissen G, Halberg F. Is motivation influenced by geomagnetic activity? Biomed Pharmacother 2002; 56 (Suppl 2): 289s-297s.
104. Halberg F, Halberg E, Halberg J. Collateral-interacting hierarchy of rhythm coordination at different organization levels, changing schedules and aging. In: Suda M, Hayaishi O, Nakagawa H, editors. Biological Rhythms and Their Central Mechanisms. Naito Foundation. Amsterdam: Elsevier North-Holland Biomedical Press; 1979. p. 421-434 (see also discussion p. 435-438).
105. Brighton B, Bhandari M, Tornetta P III, Felson DT. Hierarchy of evidence: from case reports to randomized controlled trials. Clin Orthopaed Rel Res 2003; (413): 19-24.
106. Vos R, Houtepen R, Horstman K. Evidence-based medicine and power shifts in health care systems. Health Care Analysis 2002; 10: 319-328.
107. Vliet Vlieland TP. Managing chronic disease: evidence-based medicine or patient centred medicine? Health Care Analysis 2002; 10: 289-298.
108. Dickenson D, Vineis P. Evidence-based medicine and quality of care. Health Care Analysis 2002; 10: 243-259.
109. Kubler W. Quo vadis cardiologia? Or: An nescis, mi fili, quantilla prudentia mundus regatur? (Pope Julius III). Zeitschrift für Kardiologie 2003; 92: 113-121.
110. Hunt DP, Haidet P, Coverdale JH, Richards B. The effect of using team learning in an evidence-based medicine course for medical students. Teaching & Learning in Medicine 2003; 15: 131-139.
111. Niedzwiedzka BM. Barriers to evidence-based decision making among Polish healthcare managers. Health Services Management Research 2003; 16: 106-115.
112. Horvath AR. Evidence-based laboratory medicine [Hungarian]. Orvosi Hetilap 2003; 144: 869-876.
113. Halberg F, Cornélissen G, Schwartzkopff O, International Womb-to-Tomb Chronome Group: The costs of ignoring a long-overdue chronomedicine: the chronome initiative emerging from the catacombs. Neuroendocrinol Lett, in press.

Figure 1. Bohumil Fiser.

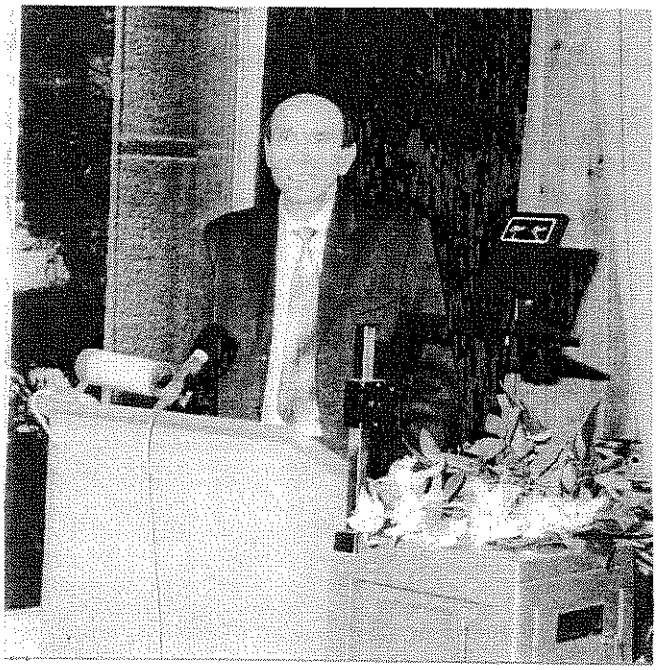


Figure 2. From left to right, Jiri Dusek, Jarmila Siegelova and Bohumil Fiser.

Figure 3. Fallacy of "controlling" any effects of rhythms by fixing the time of day of measurements. Opposite conclusions concerning hypertension may be drawn from long-term consistent spotchecks, even in a 15-year time series at a fixed clock-hour on the same person. From (12). Data of Y. Watanabe. © Halberg.

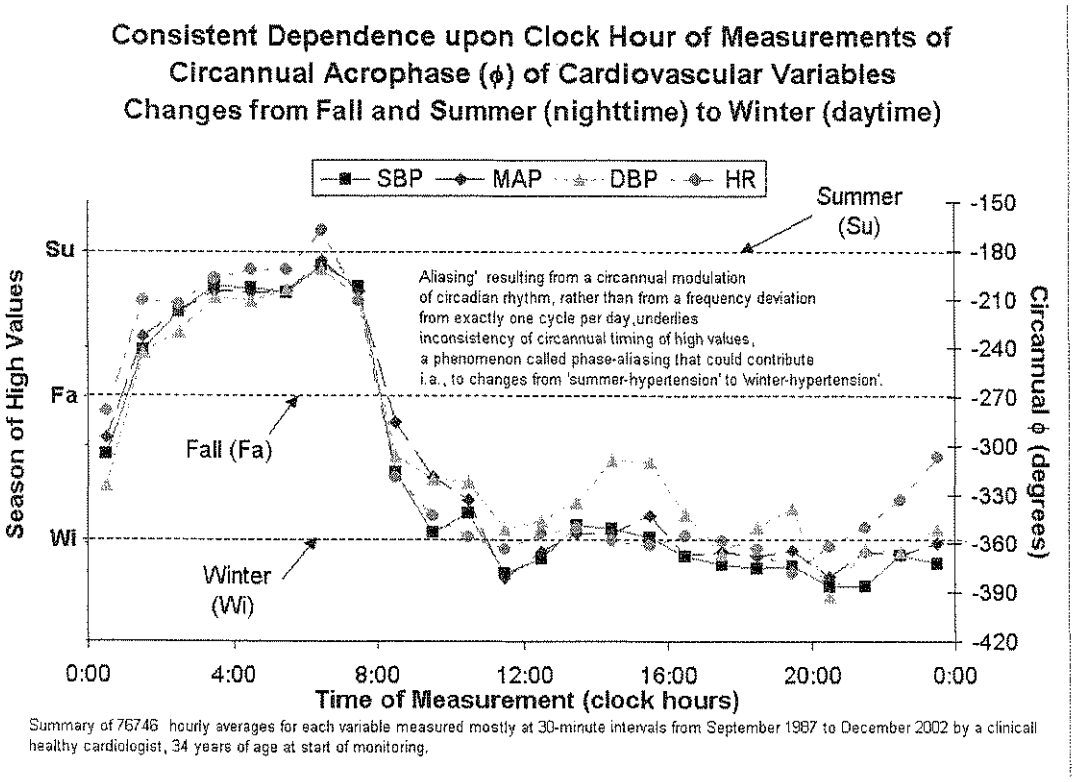


Figure 4. Altered blood pressure dynamics (chronomics) raise the risk of cardiovascular morbidity from 5.3% to 100%. Results from a 6-year prospective study on 297 patients. Data of K. Otsuka. © Halberg.

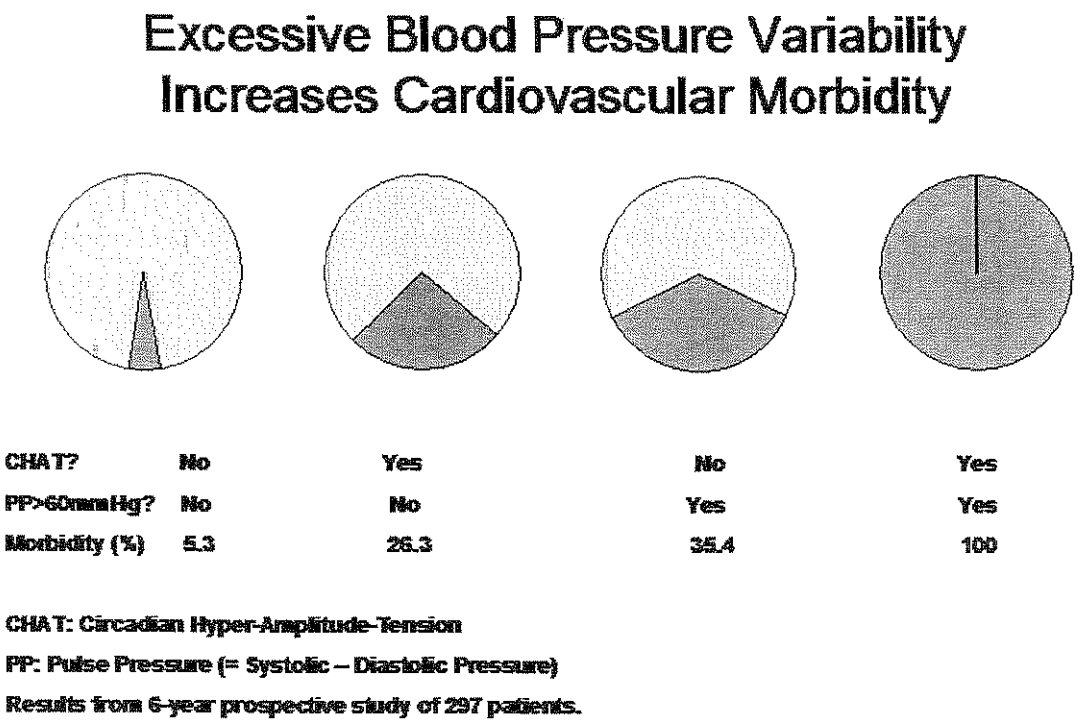


Figure 5. Relative risk of cerebral ischemic events for various risk factors, computed as the ratio of the incidence of morbidity that occurred in patients presenting with the tested risk factor by comparison with that in patients not presenting with the tested risk factor. Results of a 6-year prospective study on 297 patients indicate that the risk associated with CHAT (circadian hyper-amplitude-tension, a condition characterized by an excessive circadian amplitude of blood pressure) is larger than that of all other risk factors considered herein. As compared with an acceptable circadian blood pressure amplitude, patients with diastolic CHAT have a risk of cerebral ischemic event 8.2 times larger, representing a 720% increase in risk. Data of K. Otsuka. ©Halberg

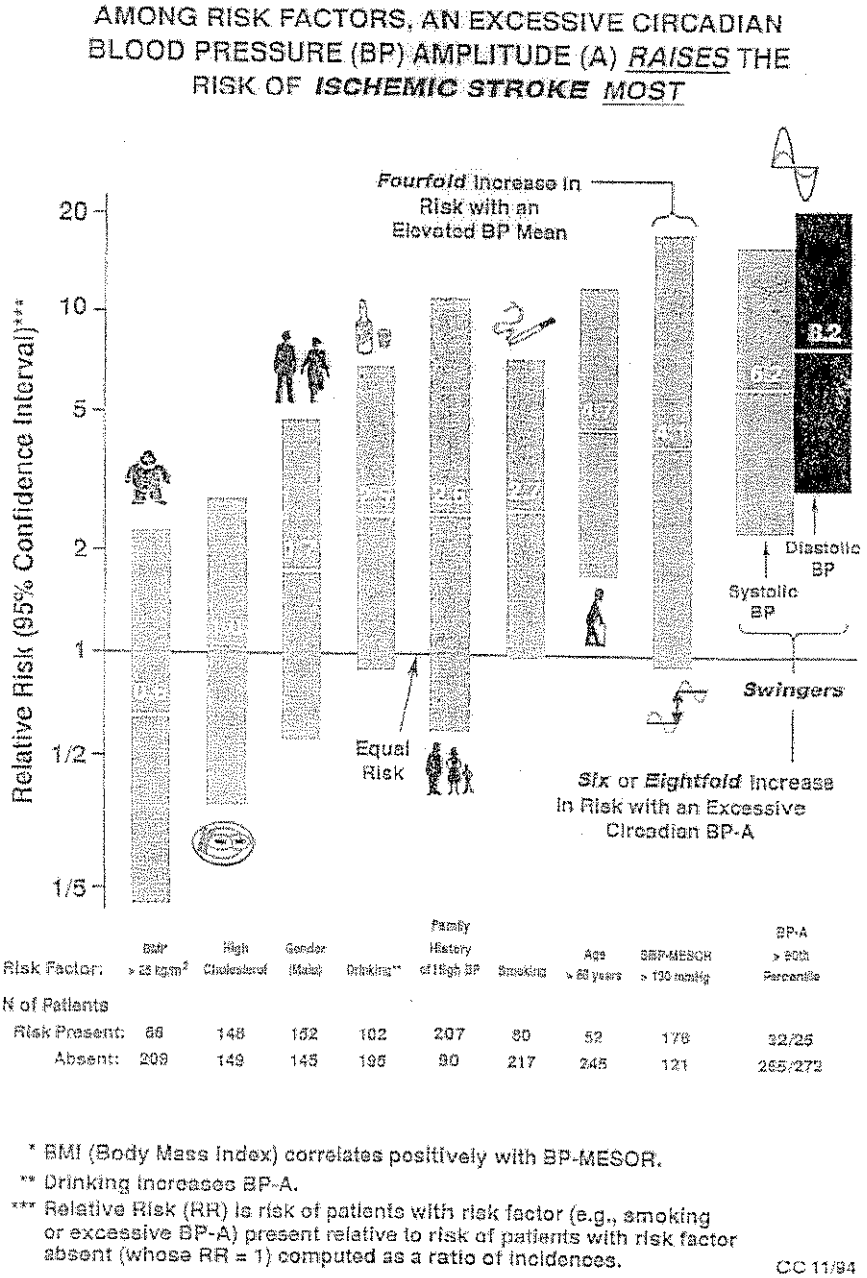


Figure 6. Of all risks considered herein, CHAT is also associated with the largest relative risk of nephropathy. Data of K. Otsuka. © Halberg.

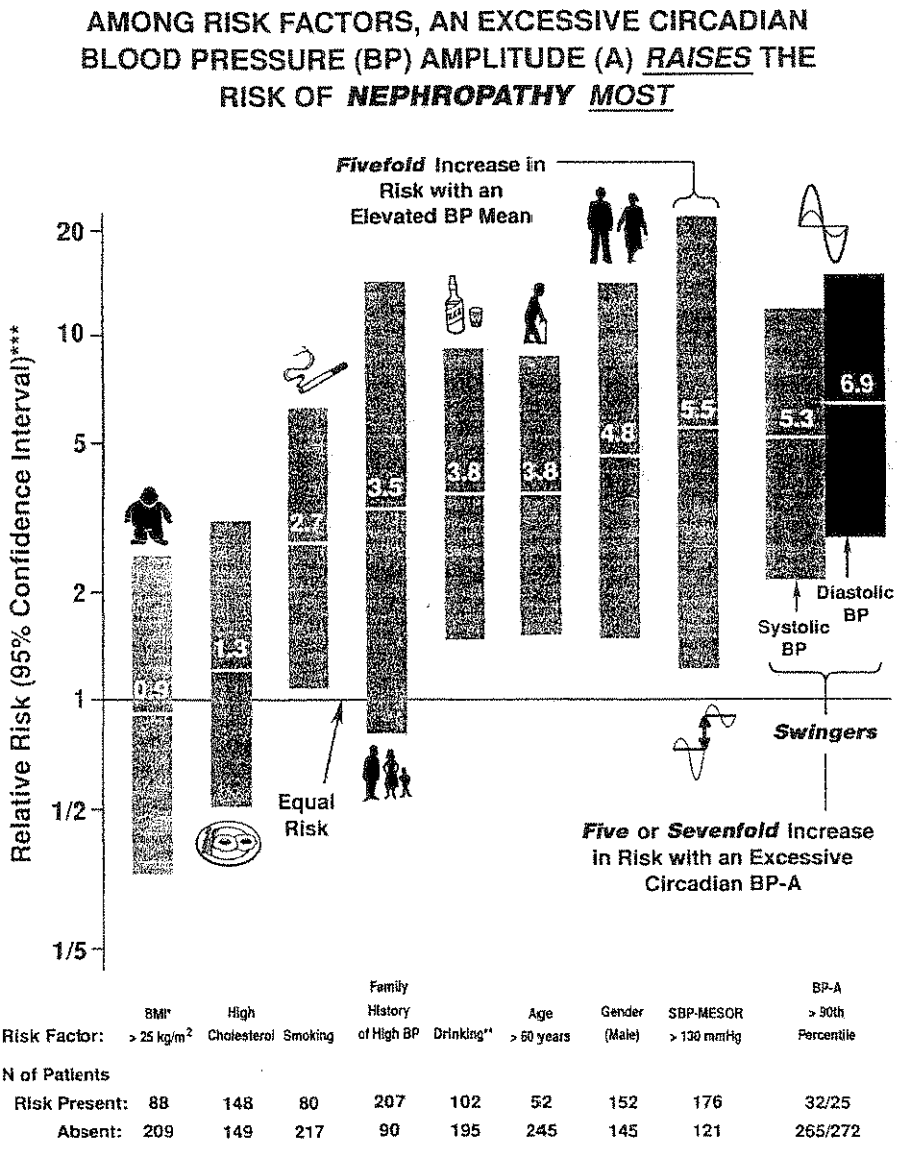


Figure 7. The increase in risk of cerebral ischemic events associated with CHAT applies to patients with different blood pressure averages, suggesting that the presence of CHAT should be a concern not only in MESOR-hypertensive patients but also in MESOR-normotensive patients. Data of K. Otsuka. © Halberg.

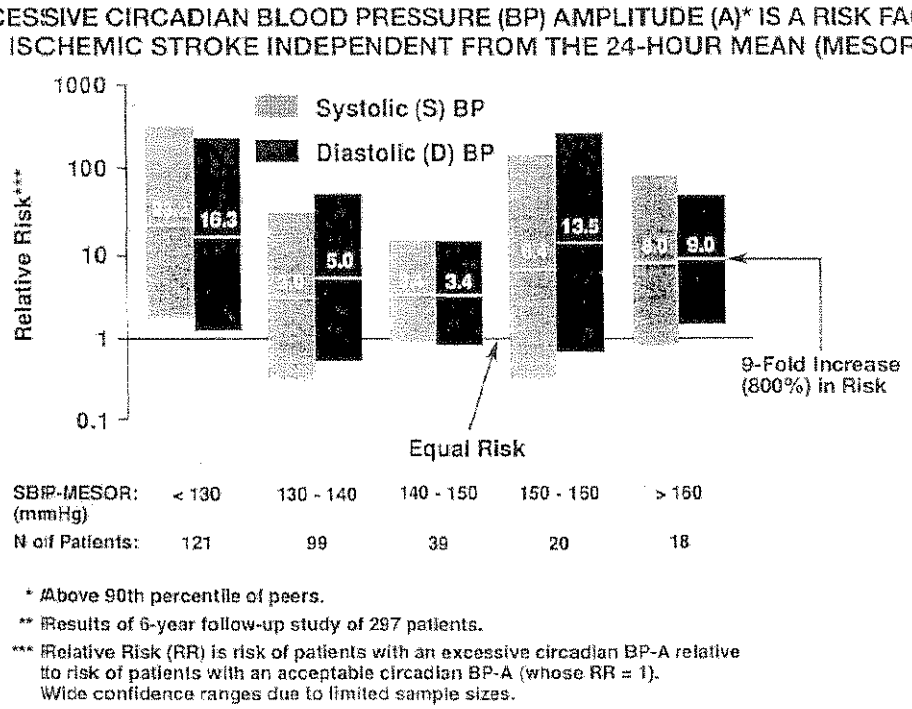
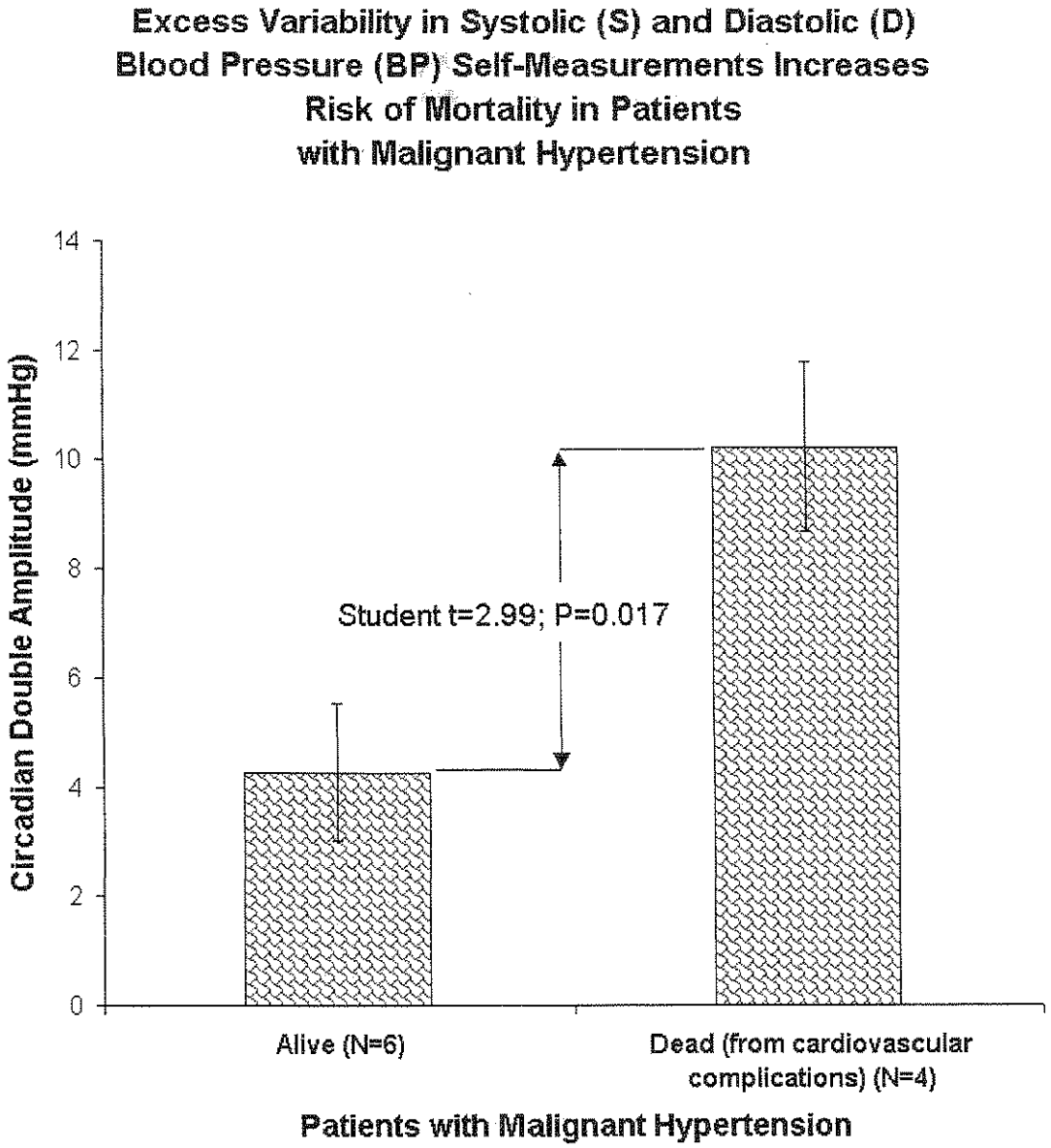


Figure 8. Patients with malignant hypertension self-measured their blood pressure several times each day. At follow-up, those who had died had a larger circadian blood pressure amplitude than patients who were still alive. This result is in keeping with the increase in vascular disease risk associated with CHAT. Data of P.T. Scarpelli. © Halberg.



From: Scarpelli PT et al. in Chronobiotechnology and Chronobiological Engineering, NATO ASI Series E No 120, pp 304-309, 1987.

Figure 9. CHAT may be transient, occurring only in response to some unusual event, as in this case of a 47-year old man who had difficulty keeping scores on the computer at his son's hockey game and was under pressure from both teams, the coaches, the children's parents and the fans in the audience. © Halberg.

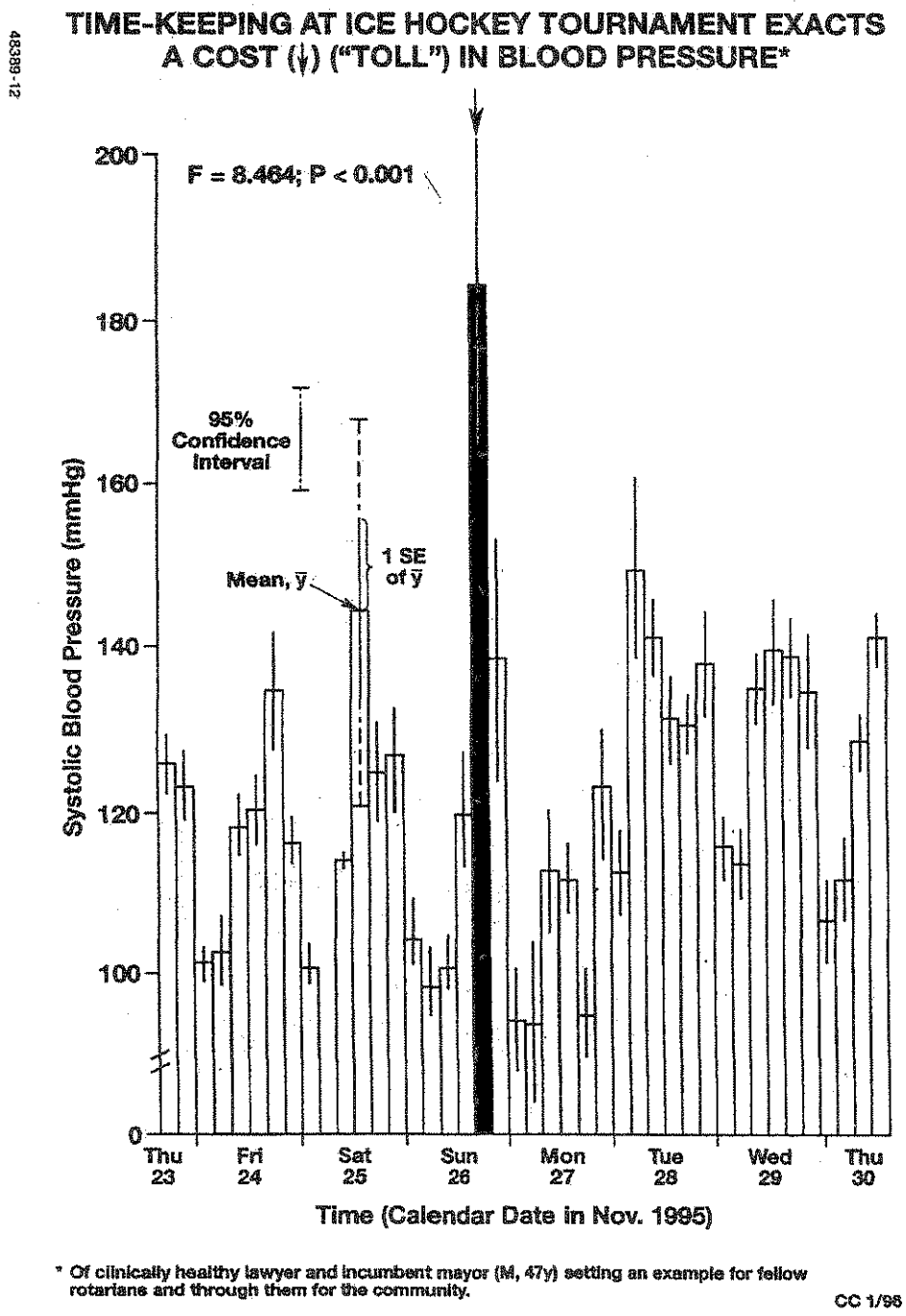


Figure 10. CHAT diagnosed in this woman in her second trimester of pregnancy was followed 8 weeks later by severe pre-eclampsia and premature delivery of a boy who needed hospitalization for the first 26 months of his life at an estimated cost exceeding 1 million US dollars, notwithstanding the acceptable MESOR of systolic and diastolic blood pressure of 115 and 64 mmHg, respectively (diastolic value not shown). © Halberg.

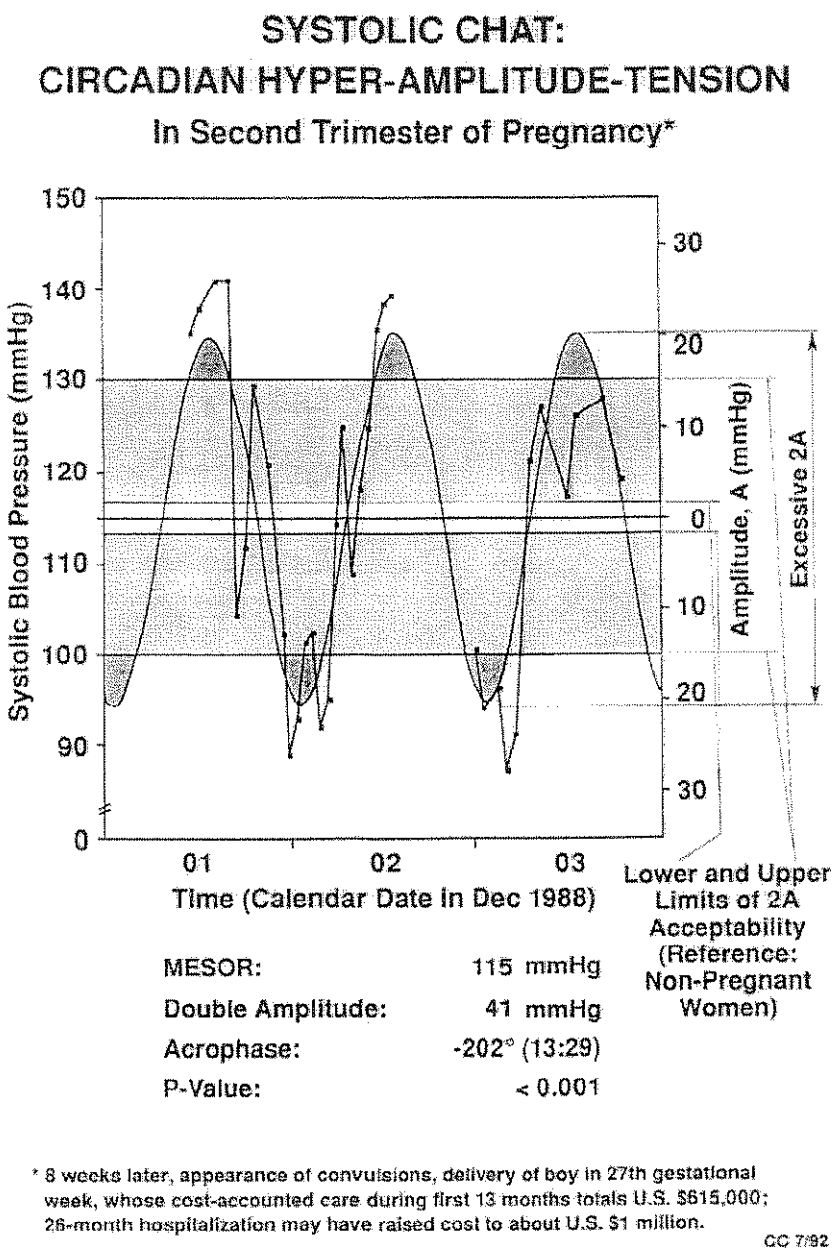
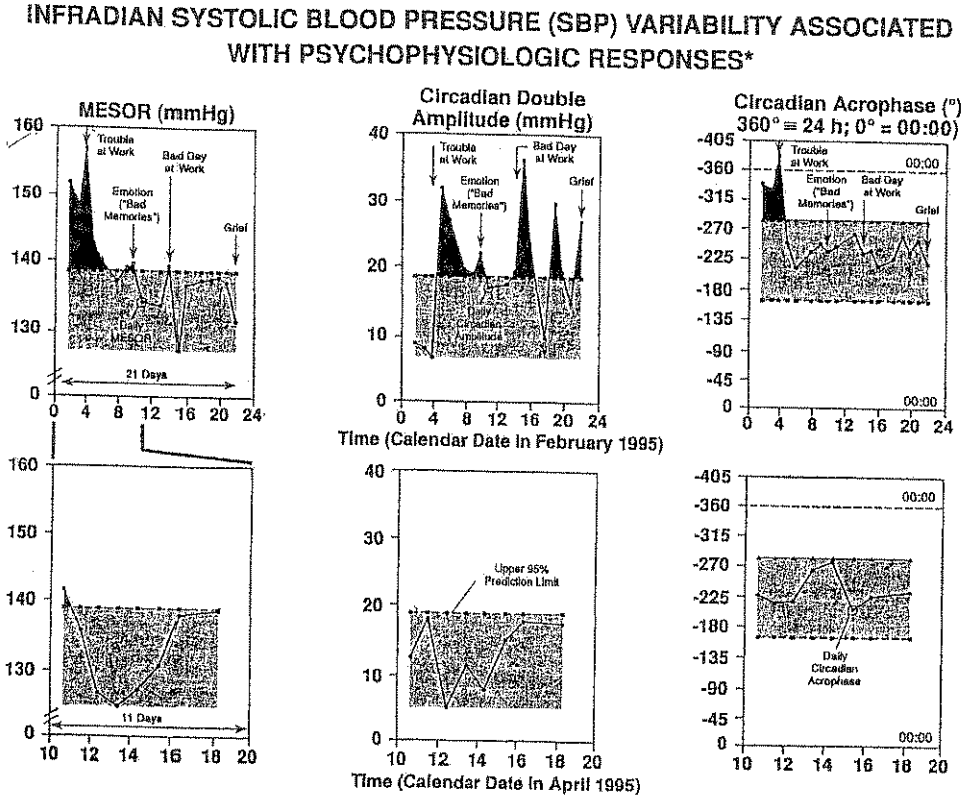


Figure 11. **a.** First two profiles of systolic blood pressure of 60-year old woman (CH) reveals large day to day variability in circadian characteristics, with occasional occurrence of MESOR-hypertension, CHAT and/or ecphasic (odd timing of the circadian acrophase). Data collected around the clock at 30-min intervals with some interruptions were analyzed for each day separately. Each dot thus represents results from up to 48 measurements. The horizontal lines represent the upper 95% prediction limit for the MESOR and the circadian double amplitude and the 90% prediction limits for the acrophase, established for clinically healthy peers matched by gender and age. MESOR-hypertension is diagnosed during the first 5 days but not during the next 16 days of the first profile (top), and practically (with one minor exception) not during the second 11-day monitoring session (bottom). CHAT is diagnosed intermittently, and was related to trouble at work, grief and conflict on the basis of a detailed diary kept during the monitoring profiles. **b.** CH monitored herself repeatedly for the ensuing 6 years. Each dot represents the MESOR of a given profile. There is a slight tendency of an increase in blood pressure over 6 years, with outlying values corresponding to an episode of worry about her daughter's health. **c.** Plot of the double circadian amplitude of each profile collected by CH over 6 years shows the great variability in the circadian pattern from one monitoring session to another, suggesting the need for continuous surveillance. **d.** Daily MESORs around the time of worry about her daughter's health indicate that large deviations from norm can last several days, suggesting the need for medications adjusted in dose and timing to the individual needs over time. **e.** During the same span as in Figure 11f, the original measurements of systolic blood pressure are shown. They span a range wider than 150 mmHg! **f.** Intermittent diagnosis of CHAT during another monitoring span in 2001, lending support to the need for long-term monitoring. **g.** Surface chart of blood pressure (and heart rate) excess suggests that in the case of CH, a diagnosis based on single measurements differs depending on the clock hour of measurement. It is also noteworthy that excess can occur in CH at most clock hours, except around the time of awakening, the precise time targeted by some anti-hypertensive medications. © Halberg.

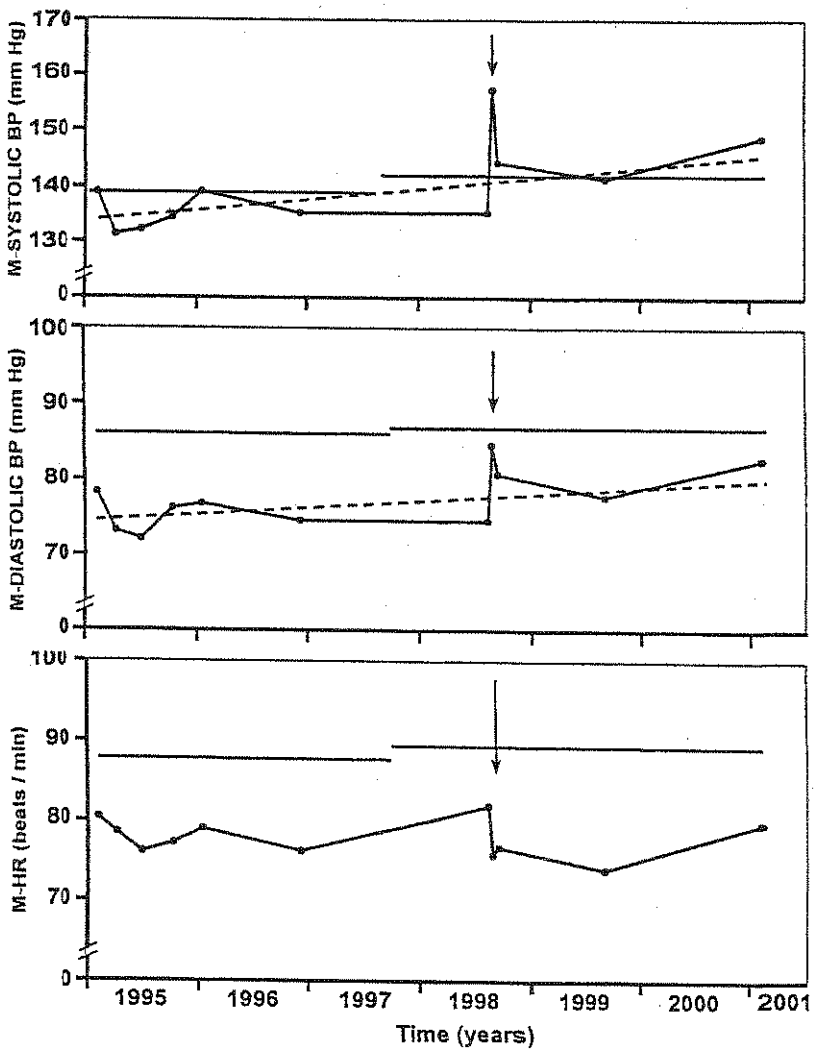
a)



* Day-to-day variability of CH (F, 60y) may mislead diagnostic and treatment decisions. Another 11-day monitoring in July 1995 found no blood pressure deviation.

b)

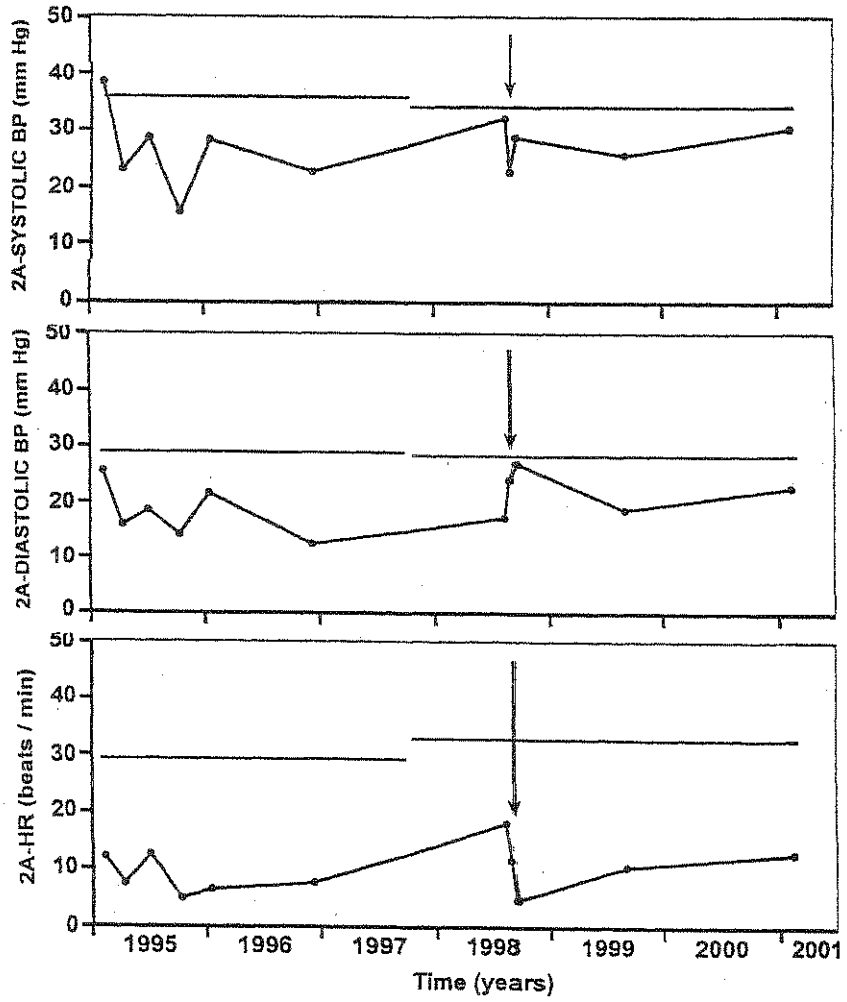
DEVELOPMENT of MESOR (M)-HYPERTENSION* over 6 YEARS and BEHAVIOR ASSOCIATED with DAUGHTER's OVARIAN CANCER



* CH, 60 years of age at start. Each dot represents a profile of measurements at 30-minute intervals for at least a week, usually for a longer span (N = 340 to 855 / profile, total: 6115). Arrow: learning about diagnosis. Sequential test (by CUSUM) does not detect statistically significant change but fit of a linear trend does so: for systolic BP without outlier P=0.010 [dashed line], (with outlier P=0.022 not shown); for diastolic BP without outlier P=0.045 (with outlier P=0.063). Horizontal lines: upper 95% prediction limits adjusted for gender in different age categories.

c)

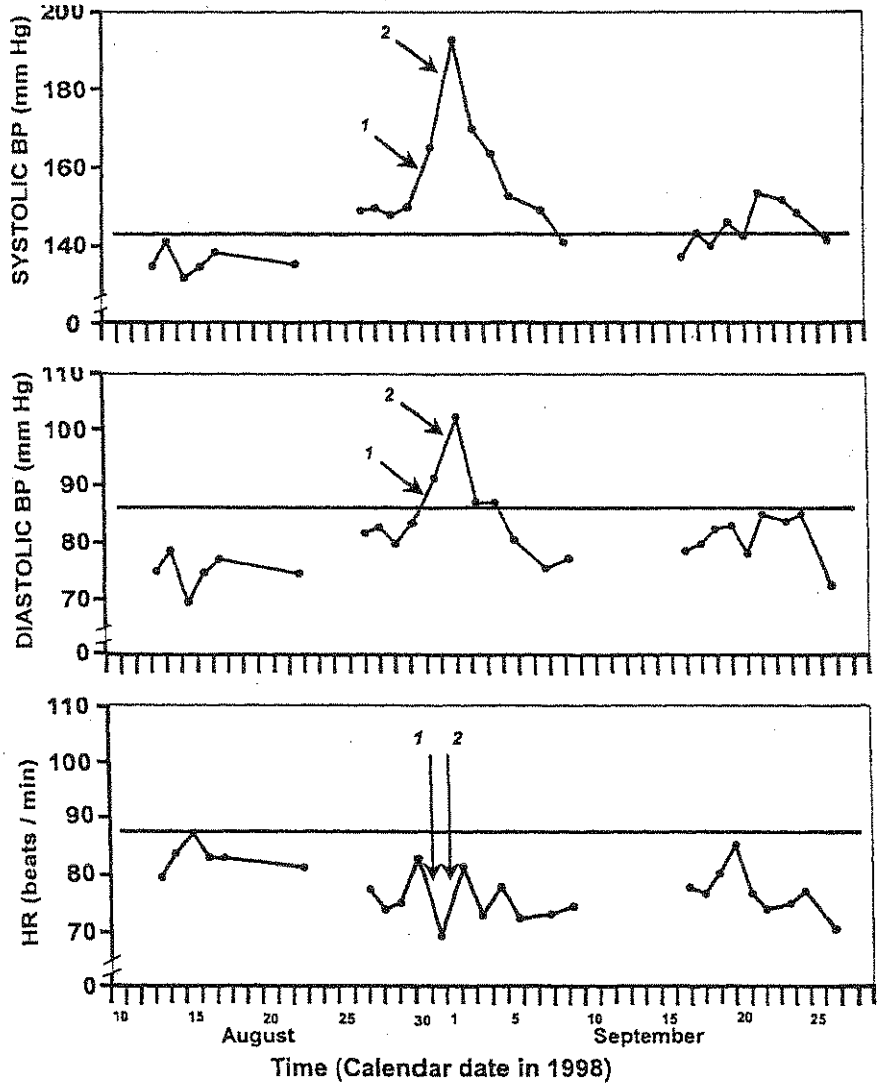
SIX-YEAR COURSE of CIRCADIAN DOUBLE AMPLITUDE (2A)* of MATERNAL BLOOD PRESSURE (BP) and HEART RATE (HR)



* CH, 60 years of age at start. Each dot represents a profile of measurements at 30-minute intervals for at least a week, usually for a longer span (N = 340 to 855 / profile, total: 6115). Arrow: learning about diagnosis of ovarian cancer. In no case is sequential test (by CUSUM) or the fit of a linear trend statistically significant. Except for first dot there is no further "profile-CHAT", occasional "~24-h-CHAT", shown elsewhere, notwithstanding. The findings of "~24-h-CHAT" with or without "profile-CHAT" are another reason for preparing about 24-hour in addition to at least week long (profile) summaries. Horizontal lines: upper 95% prediction limit adjusted for gender in different age categories.

d)

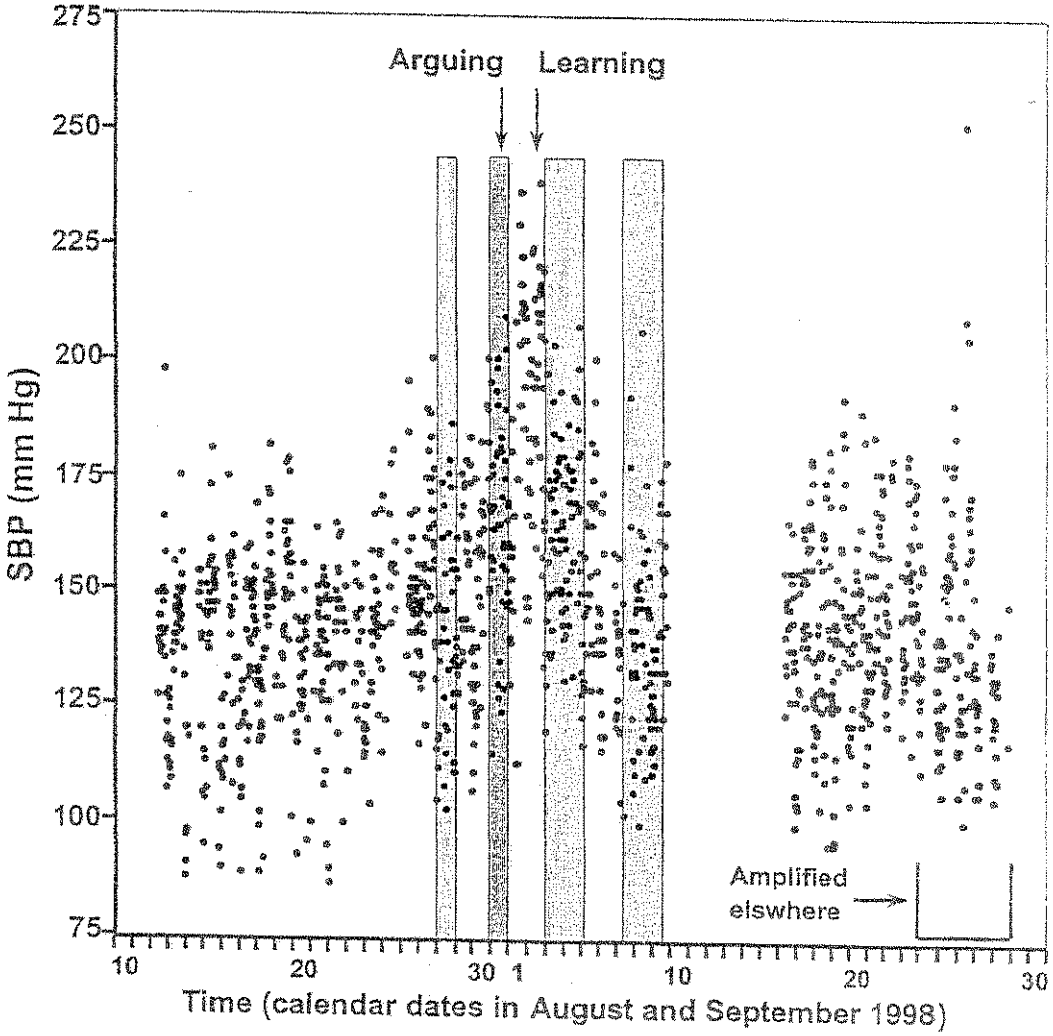
MESOR RESPONSE of MATERNAL BLOOD PRESSURE (BP) but not of HEART RATE (HR) * ASSOCIATED with LEARNING about DAUGHTER's CANCER



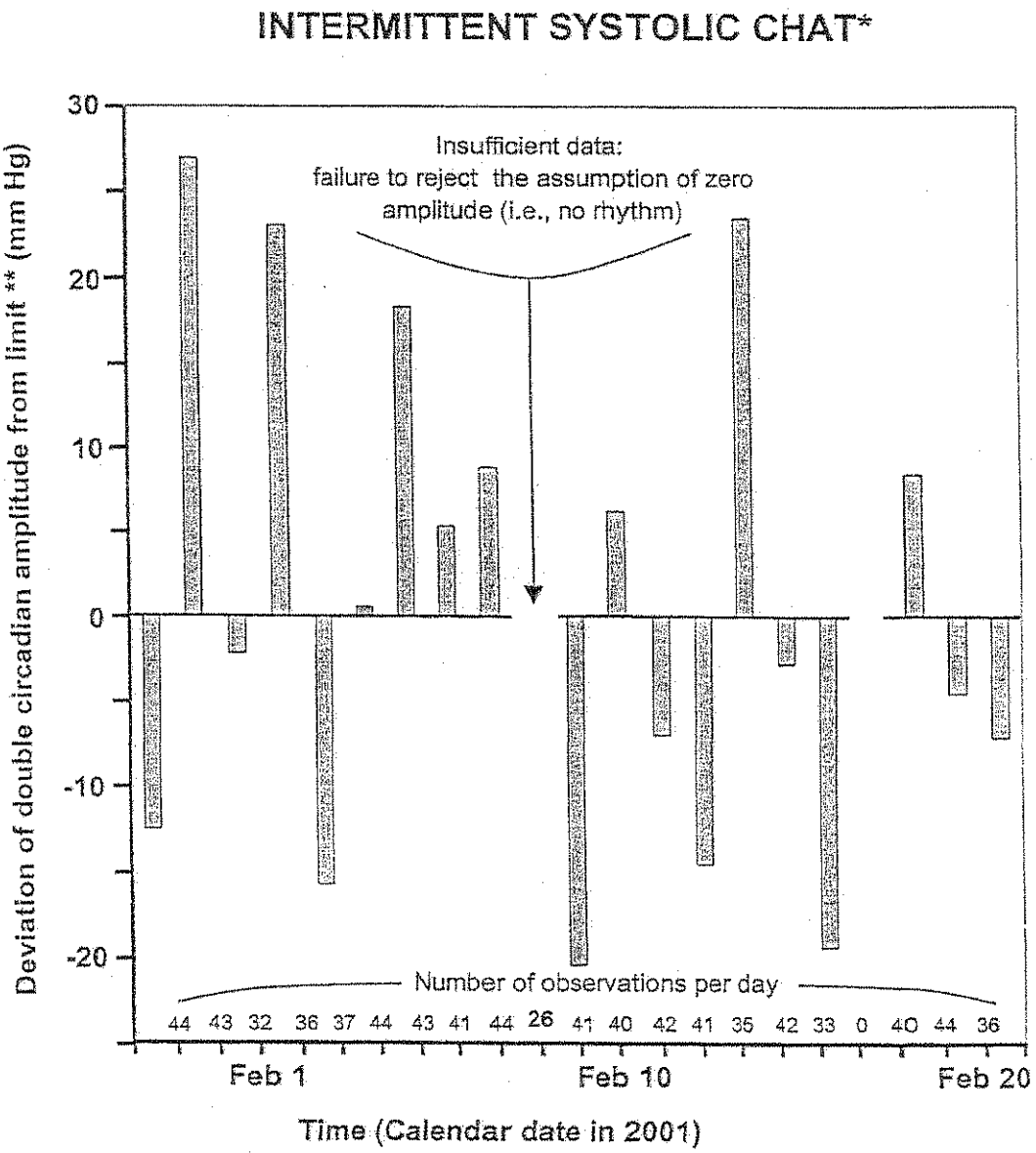
* CH, 64 years of age. Each dot is an about-daily summary. F=25.6 (P < 0.001) by 1-way ANOVA comparing deviations from upper 95% prediction limit (expressed as % of this limit) for span covering Aug 26 – Sep 9; F= 11.1 (P=0.003) for comparison between systolic and diastolic BP. N of measurements = 34 to 48 / day (1402 total). Horizontal lines: age- and gender-adjusted upper 95% prediction limits.
1 – "Arguing" about daughter's health. *2* – Diagnosis of daughter's disease.

e)

MATERNAL* SYSTOLIC BLOOD PRESSURE (SBP) BEFORE and AFTER ARGUING and THEN LEARNING of DAUGHTER's CANCER



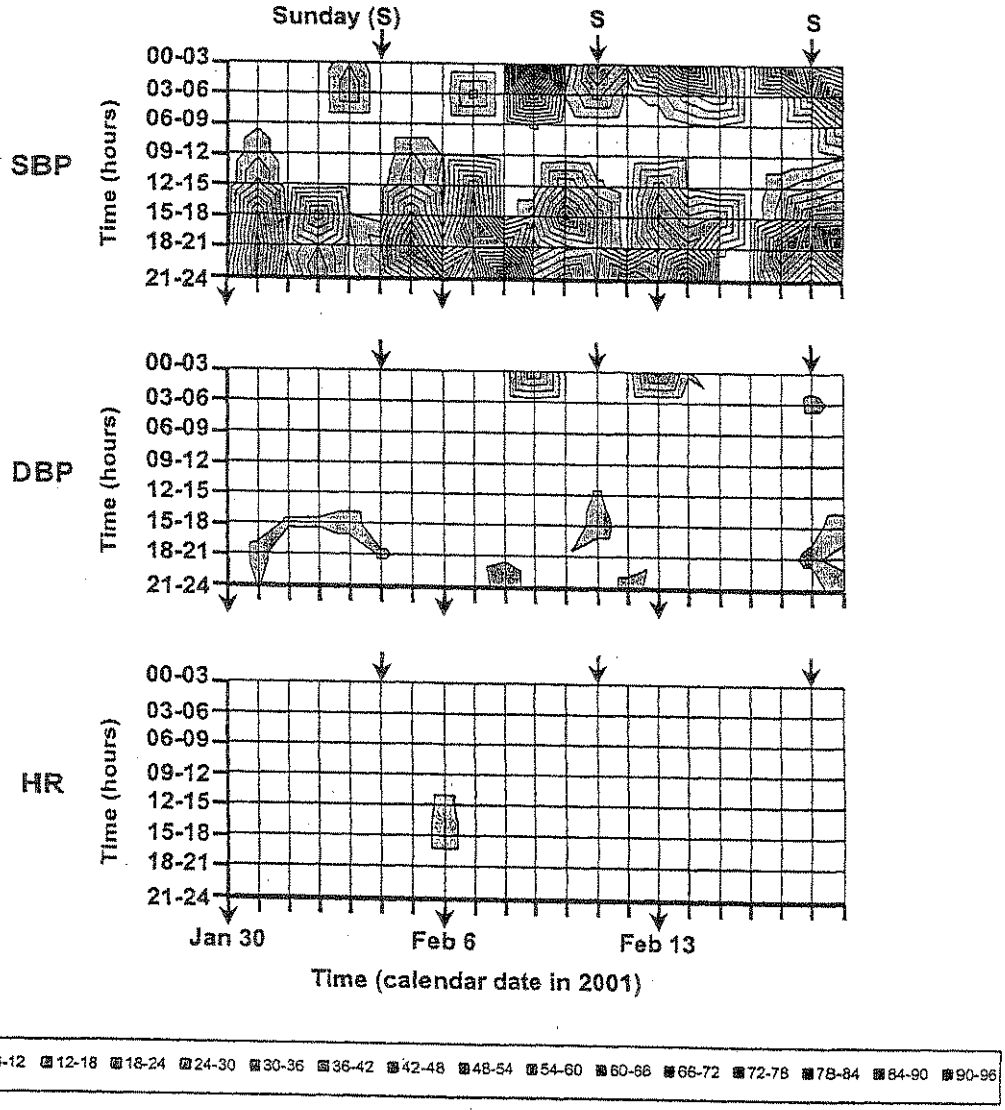
* CH, 64 years of age; shaded areas show the spans for which a zero-amplitude test of circadian rhythm is not statistically significant, as it is during 6 days out of 13 (46%) around the time of diagnosis [but only 13 times in a total of 159 days of monitoring during 6 years, excluding the time of diagnosis (<9%), not shown]. N of measurements 34 to 48 / day (graphed subtotal: 1368; overall total 6115, not shown).



* CHAT - Circadian HyperAmplitudeTension. ** Upper 95% prediction limit of circadian amplitude derived from gender- and age-matched peers; in CH, F, 66 years of age, revealed by 21-day monitoring at 30-minute intervals.

g)

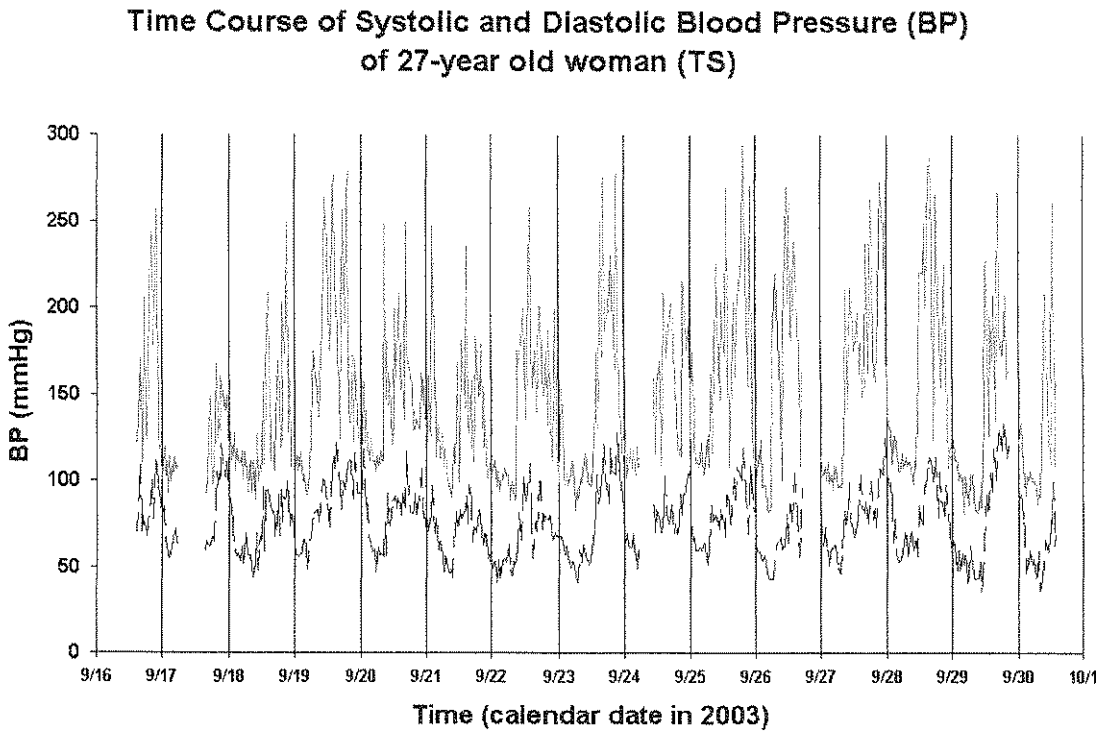
TEMPORAL PATTERN of ANY EXCESSIVE BLOOD PRESSURE (BP) and HEART RATE (HR) *



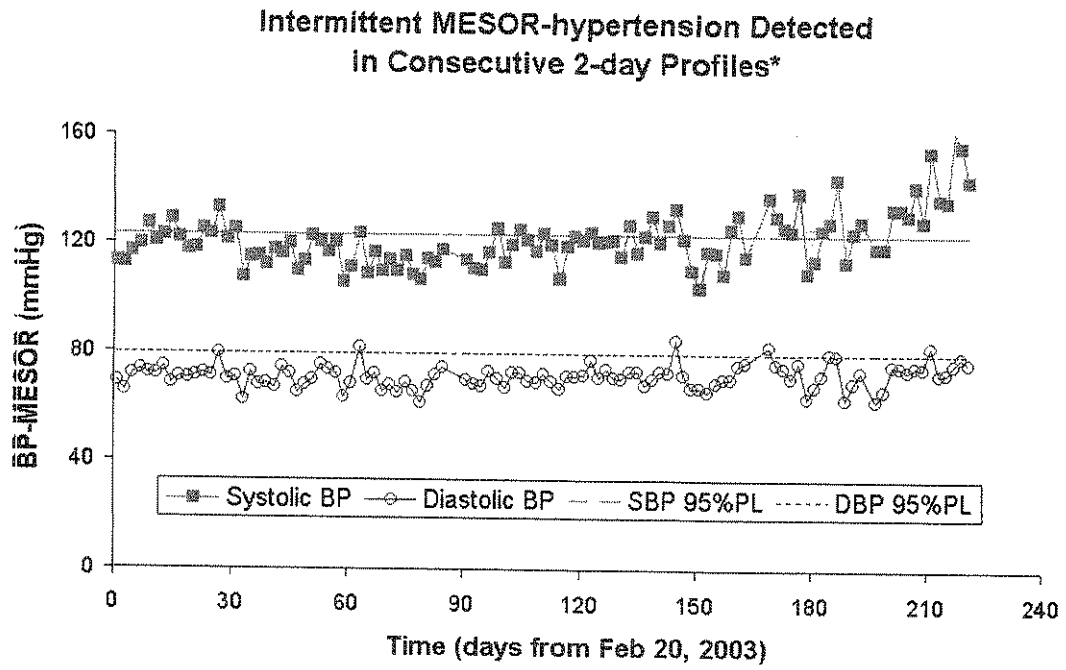
*** CH, F, 66 years of age, monitored at 30-minute intervals for 21 consecutive days; S = systolic; D = diastolic; excess: numerically integrated area between the data when they exceed the upper 95% prediction limit and this limit itself, expressed in mm Hg (BP) or beats/min (HR) x hours during 3 hours. The darker the shading the more excess; note least BP excess between 06:00 and 12:00 in CH, i.e., little or no shading during the time span at which generalized "chronotherapy" is aimed, ignoring the individual's pattern.**

Figure 12. **a.** Large moment to moment and day to day variability in systolic and diastolic blood pressure of an untreated asymptomatic 27-year old woman (TS). The high values are not likely to be outliers or erroneous readings since they occur repeatedly over several consecutive hours, and do so not only during this span but also during other spans when TS was wearing a different cuff and/or a different TM-2421 monitor from the A&D company (Tokyo, Japan). **b.** TS' data collected over 222 days were analyzed over consecutive 2-day spans. The MESORs of systolic and diastolic blood pressure, displayed as a function of time, show that MESOR-hypertension is diagnosed during some but not all spans. When the current gold standard is to monitor for only a single 24-hour span, these results suggest that whether a patient is prescribed anti-hypertensive medication may largely depend on which day the monitoring took place. **c.** There is also a large variability in the circadian double amplitude of systolic and diastolic blood pressure. CHAT is diagnosed on most but not all 48-hour spans. These results interpreted in the light of group results with outcomes suggest that TS may be at an elevated risk of an adverse cardiovascular event. Nevertheless, several physicians TS consulted are of the opinion that she does not need any treatment. **d.** TS's data analyzed over consecutive 7-day spans, according to the BIOCOS recommendation of monitoring for 7 days at the outset, also show great variability. Second-order polynomials fitted to the MESOR, hyperbaric index, and double circadian amplitude of systolic and diastolic blood pressure invariably detect an increase over time. © Halberg.

a)

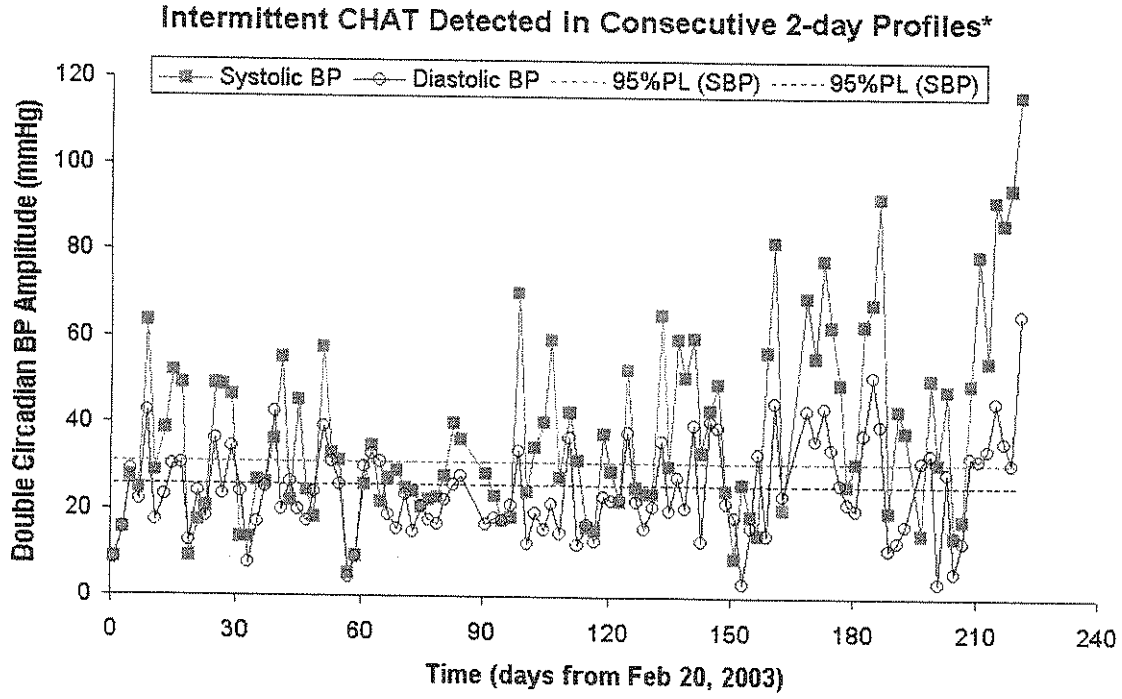


b)



*Data from 27-year old woman (TS) collected around the clock at ~30-min intervals.

c)



*Data from 27-year old woman (TS) collected around the clock at ~30-min intervals.

d)

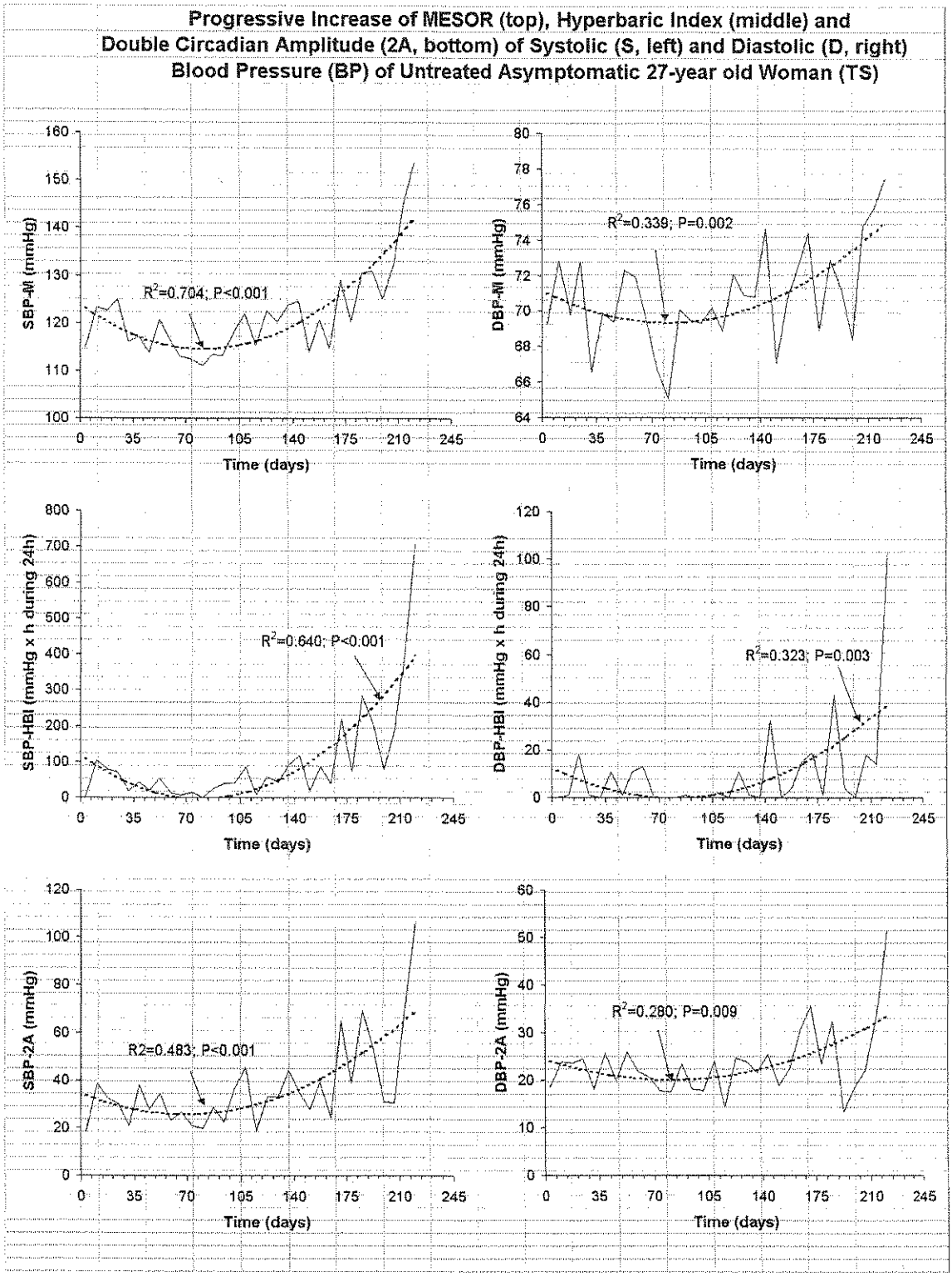
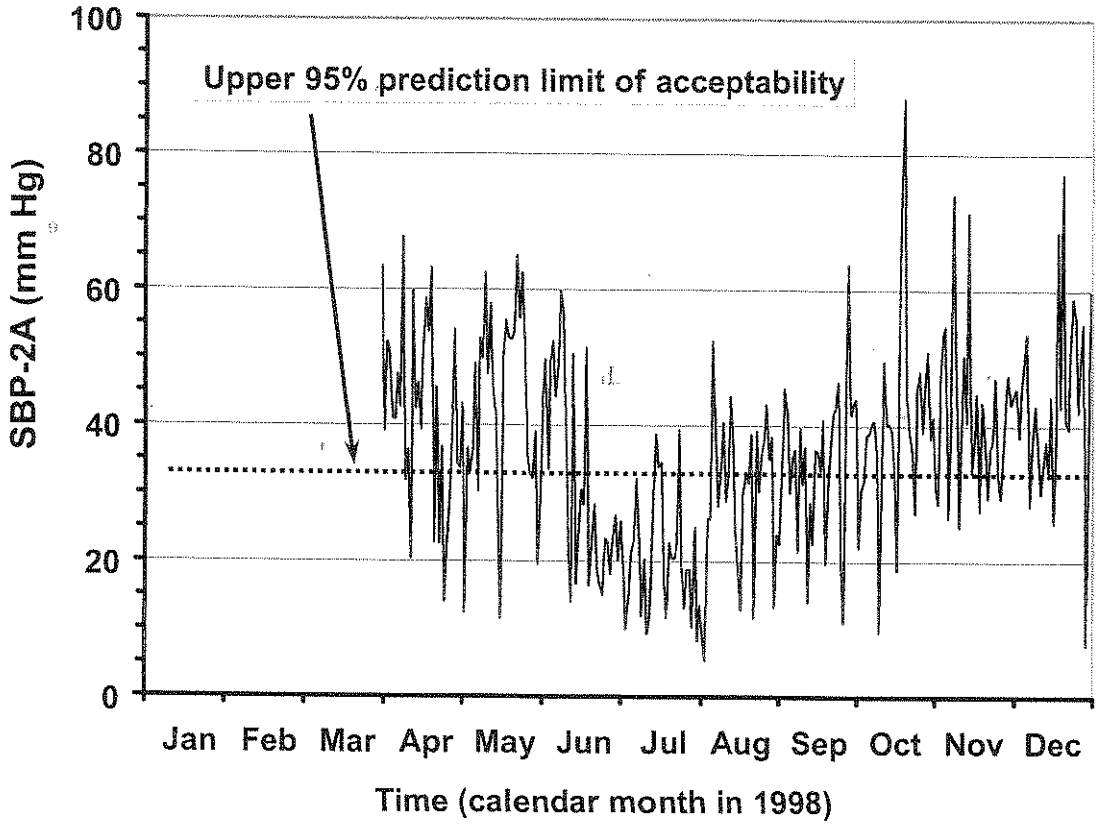

Progressive Increase of MESOR (top), Hyperbaric Index (middle) and
Double Circadian Amplitude (2A, bottom) of Systolic (S, left) and Diastolic (D, right)
Blood Pressure (BP) of Untreated Asymptomatic 27-year old Woman (TS)
SBP-MI (mmHg)
R²=0.704; P<0.001
DBP-MI (mmHg)
R²=0.339; P=0.002
SBP-HBI (mmHg x h during 24h)
R²=0.640; P<0.001
DBP-HBI (mmHg x h during 24h)
R²=0.323; P=0.003
SBP-2A (mmHg)
R2=0.483; P<0.001
DBP-2A (mmHg)
R²=0.280; P=0.009
Time (days)

Figure 13. **a.** Another long-term series of blood pressure of a treated MESOR-hypertensive man (GSK), 72 years of age at the start of measurements here recorded, illustrates the merit of longitudinal monitoring from both a basic and applied viewpoint. **a-e.** Plots during each of five calendar years (1998-2002) of the circadian double amplitude of systolic blood pressure assessed daily from around the clock measurements at 30-minute intervals illustrate further the large day to day variability in the circadian characteristics of blood pressure. The variability is so large that on some days the circadian double amplitude is within acceptable limits but on other days CHAT is diagnosed. Examination of the results during a year such as 1998 suggests that part of the variability may follow a predictable circannual pattern, but similar plots during other years do not corroborate this impression. **f.** Part of the variability in the circadian double amplitude of blood pressure may relate to changes in the treatment plan. An abrupt and drastic decrease in the circadian double amplitude of systolic blood pressure is observed after a switch from nifedipine to diltiazem treatment, but CHAT is again diagnosed on diltiazem treatment after a few days. **g.** Analysis of the whole 5-year record of systolic blood pressure by least squares spectrum reveals, among others, the presence of a double peak with periods of about 1 and 1.2 year, corresponding respectively to the season-associated circannual variation and to the non-photic trans-year observed prominently in the velocity of the solar wind. **h.** The two periods being relatively close with respect to the total observation span of 5 years, the two components were suspected to beat. A model based on the least squares spectral results was constructed to estimate the data collected during the 5-year span and to extend the prediction for the next 7 years so as to cover two beats. The simulated data (left) were then analyzed by gliding spectra (right), showing the spectral location of each component (top and middle) and the resulting beat when both components are present concomitantly (bottom). The results served as a reference for those of the actual data available during 5 years only. **i.** Results from global (right) and gliding (left) spectra of systolic blood pressure data collected over 5 years. In the surface chart, the interval used for analysis is too short to resolve both components. Only a single component is detected with a period only slightly longer than 1 year; it is visible macroscopically at the beginning of the series when both components are in phase and reinforce each other. During the end of the 5-year monitoring span, no about-yearly component is detected, likely because the two components are then out of phase and tend to cancel each other out. Results resemble those of the simulated data, lending further support to the proposition that both a photic circannual and a non-photic trans-year may characterize human blood pressure (and heart rate), as also documented in longitudinal series from others, Figure 14. © Halberg.

a)

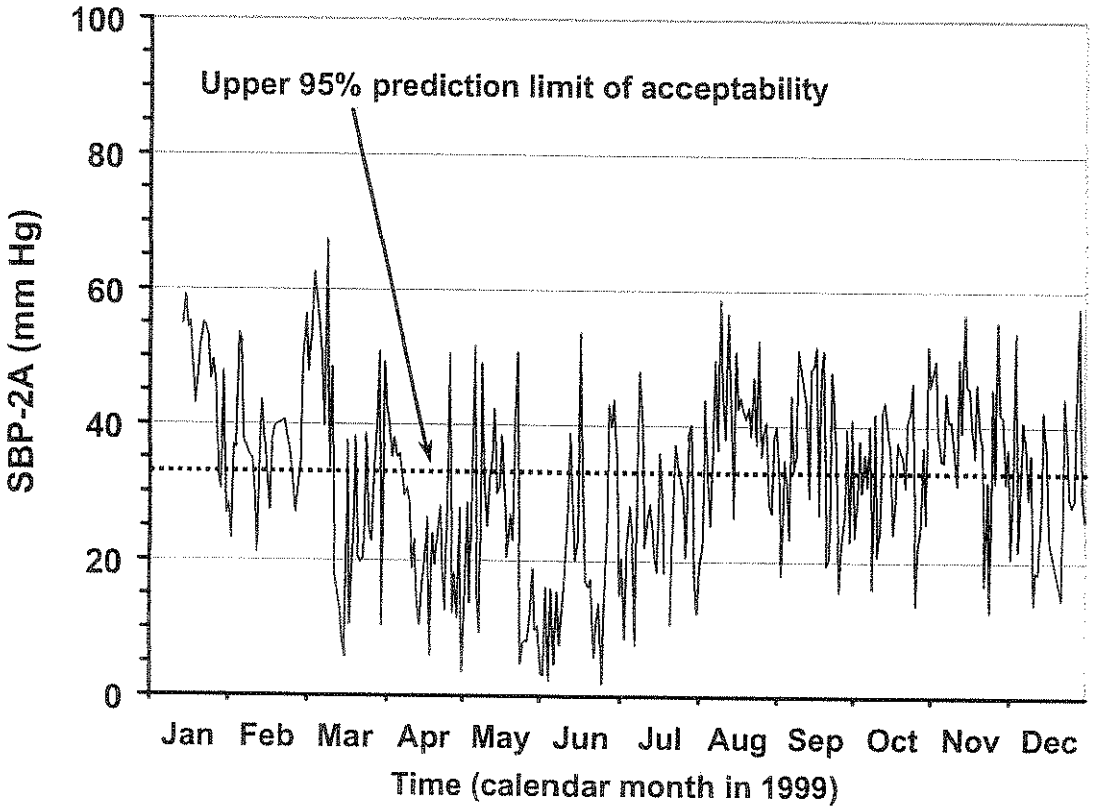
INTERMITTENT CHAT*
SHOWN BY DAILY DOUBLE CIRCADIAN SYSTOLIC (S) BLOOD PRESSURE (BP) AMPLITUDE (2A)



* Circadian Hyper-Amplitude-Tension of GSK, a treated MESOR-hypertensive man, 72 years of age at the beginning of half-hourly measurements on March 30, 1998; section of longer record (> 5 years, not shown).

b)

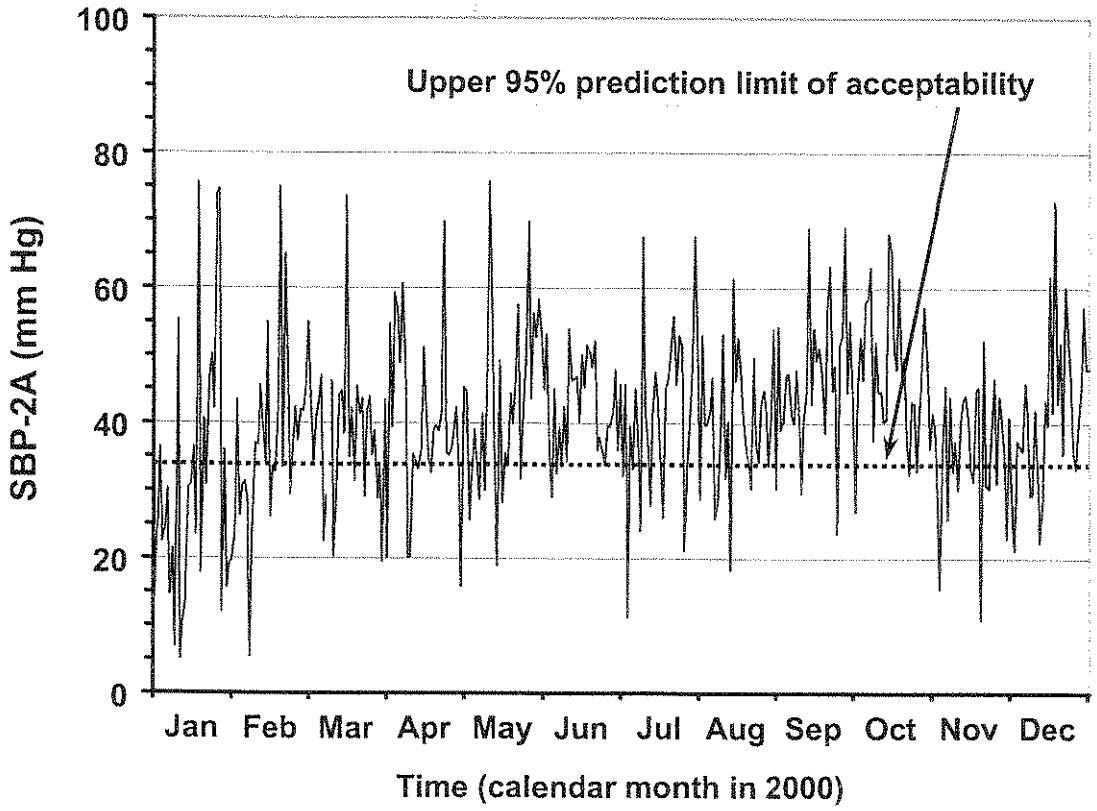
INTERMITTENT CHAT*
SHOWN BY DAILY DOUBLE CIRCADIAN SYSTOLIC (S) BLOOD PRESSURE (BP) AMPLITUDE (2A)



* Circadian Hyper-Amplitude-Tension of GSK, a treated MESOR-hypertensive man, 72 years of age at the beginning of half-hourly measurements on March 30, 1998; section of longer record (> 5 years, not shown).

c)

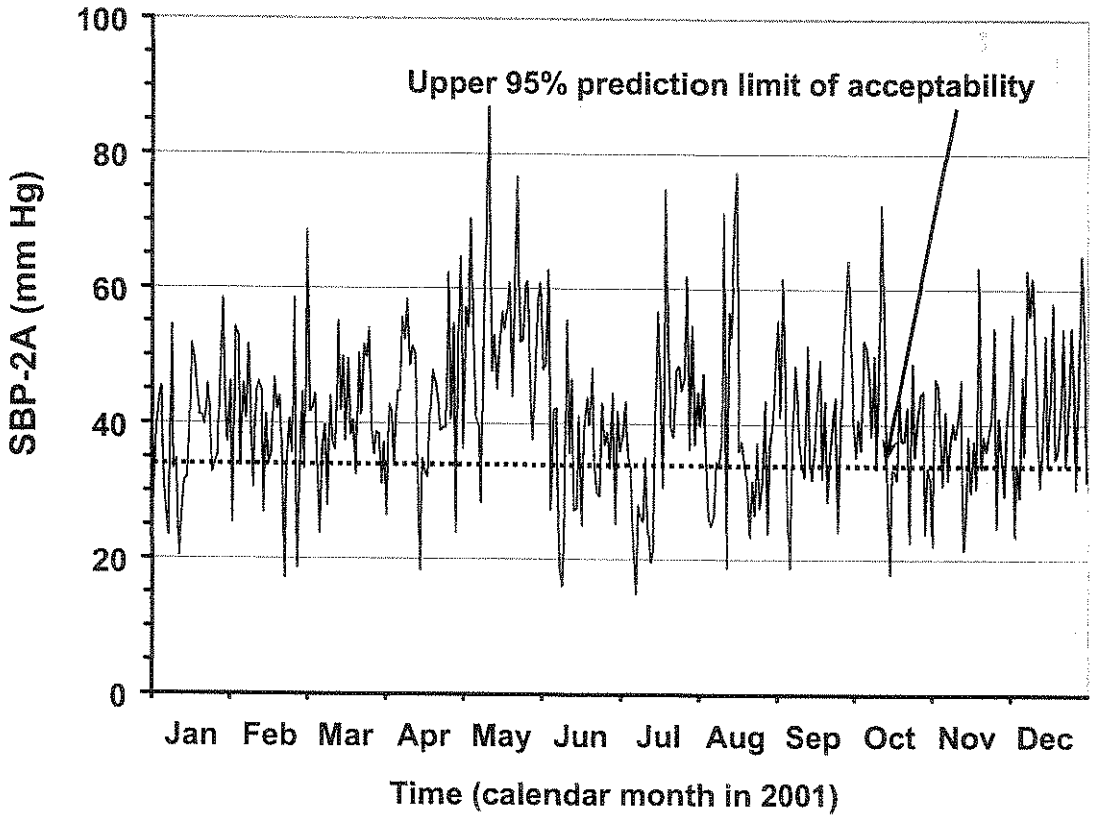
INTERMITTENT CHAT*
SHOWN BY DAILY DOUBLE CIRCADIAN SYSTOLIC (S) BLOOD PRESSURE (BP) AMPLITUDE (2A)



* Circadian Hyper-Amplitude-Tension of GSK, a treated MESOR-hypertensive man, 72 years of age at the beginning of half-hourly measurements on March 30, 1998; section of longer record (> 5 years, not shown).

d)

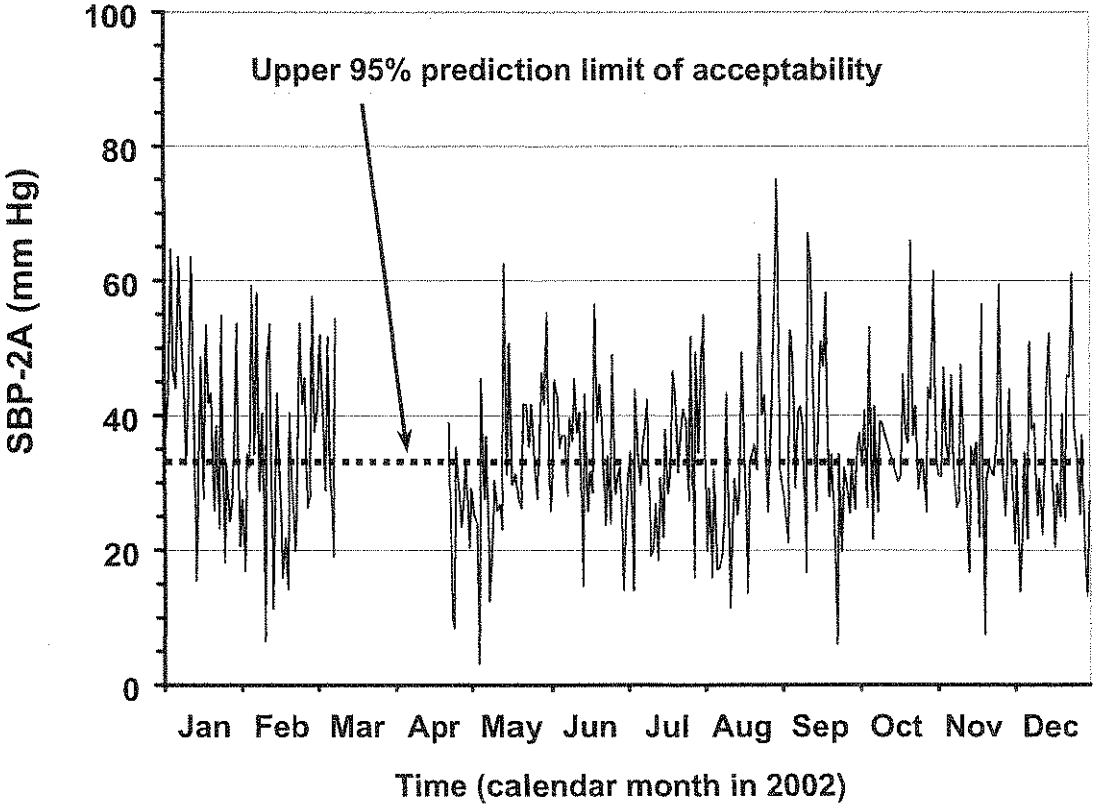
INTERMITTENT CHAT*
SHOWN BY DAILY DOUBLE CIRCADIAN SYSTOLIC (S) BLOOD PRESSURE (BP) AMPLITUDE (2A)



* Circadian Hyper-Amplitude-Tension of GSK, a treated MESOR-hypertensive man, 72 years of age at the beginning of half-hourly measurements on March 30, 1998; section of longer record (> 5 years, not shown).

e)

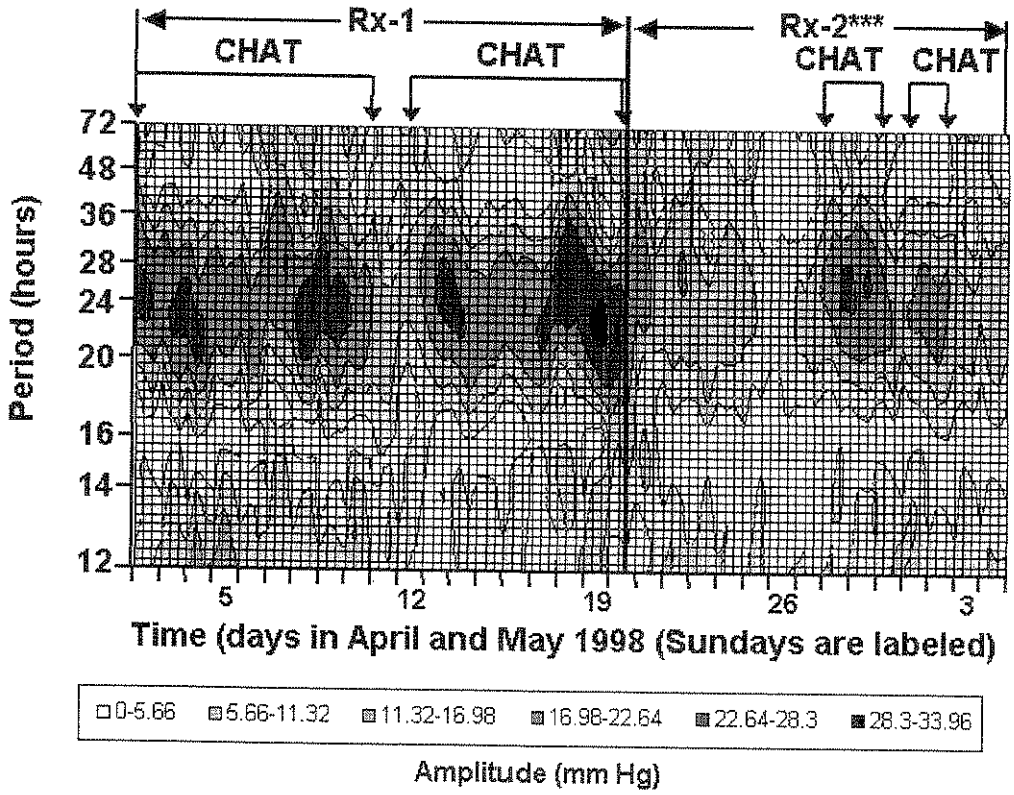
INTERMITTENT CHAT*
SHOWN BY DAILY DOUBLE CIRCADIAN SYSTOLIC (S) BLOOD PRESSURE (BP) AMPLITUDE (2A)



* Circadian Hyper-Amplitude-Tension of GSK, a treated MESOR-hypertensive man, 72 years of age at the beginning of half-hourly measurements on March 30, 1998; section of longer record (> 5 years, not shown).

NEED FOR LONG-TERM SURVEILLANCE:

TREATMENT CHANGES SYSTOLIC (S) CHAT* INTO TRANSIENT S-CHAT** WHICH LATTER APPEARS AFTER A WEEK



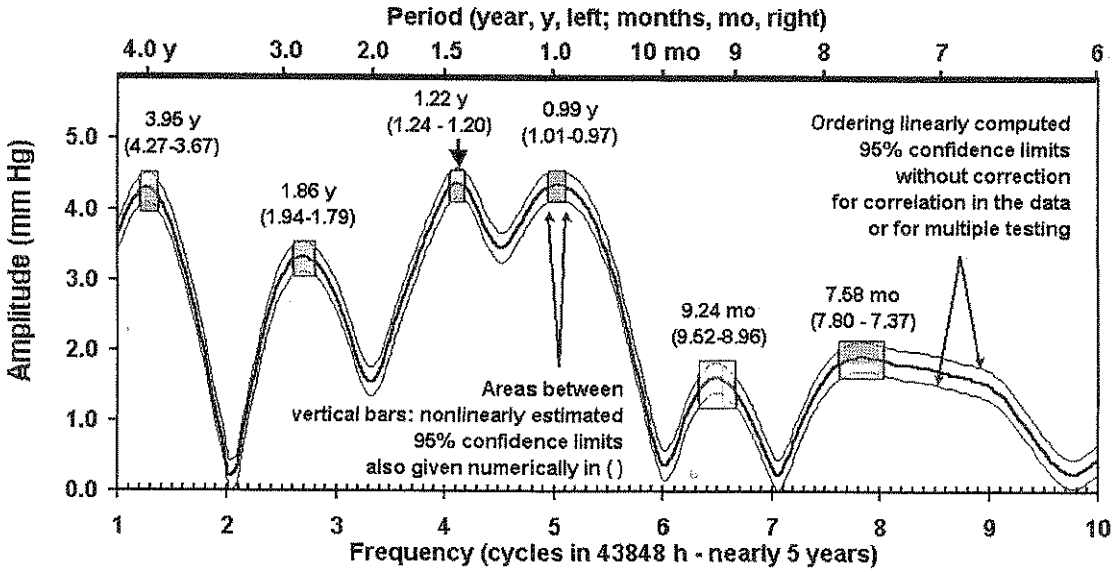
* CHAT: Circadian Hyper-Amplitude Tension.

** In GK, 72- year old man, whose blood pressure was measured at mostly 30-minute intervals, and analyzed as a moving spectrum in separete 48-hour intervals, displaced in 8-hour increments through the data set.

***Rx-1: Nifedipine 2 x 10 mg at 8 a.m. and 8 p.m.; Rx-2: Diltiazem 3 x 90 mg at 10 a.m, 3 p.m and 8 p.m.

g)

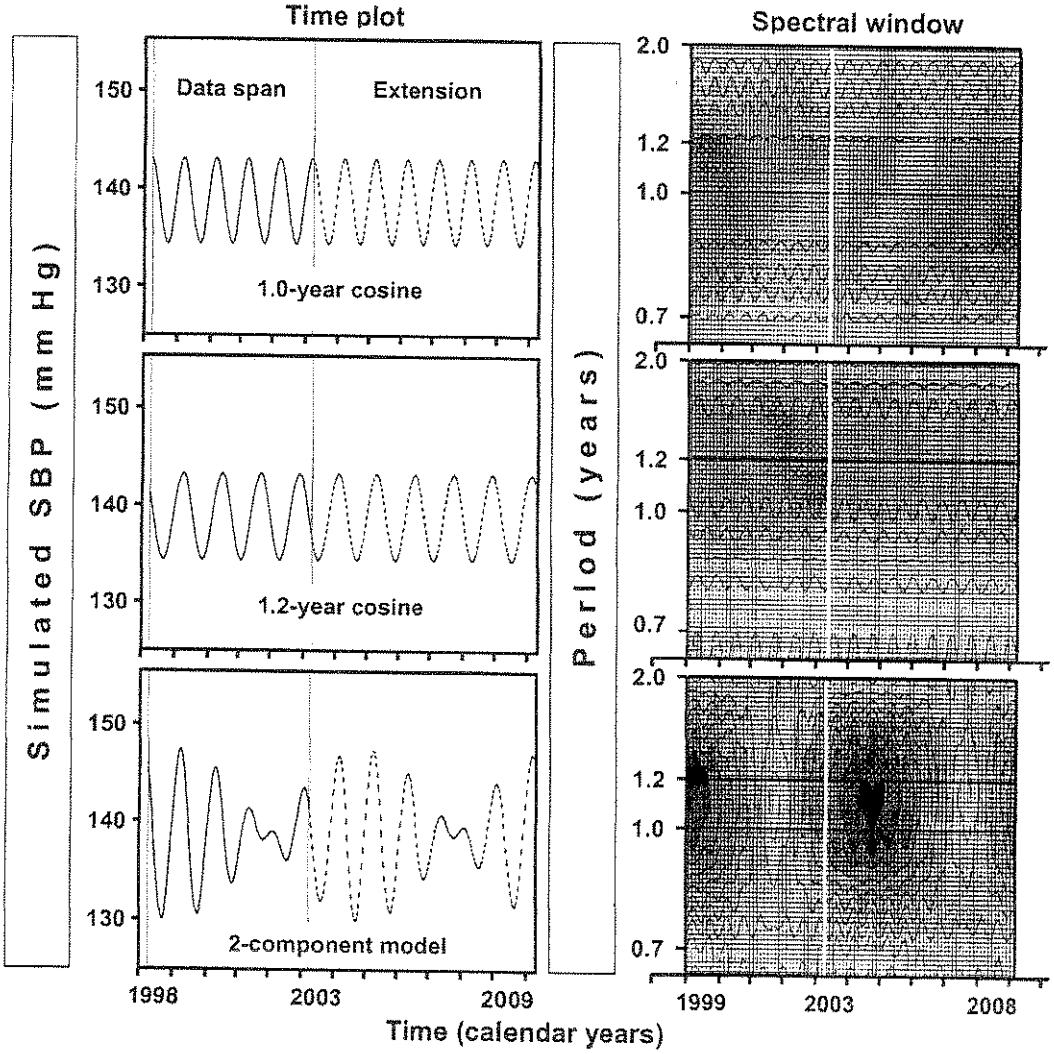
SPECTRAL WINDOW OF SYSTOLIC BLOOD PRESSURE* SHOWING BIGGEST AMPLITUDE (arrow) NEAR PERIOD OF RICHARDSON's VARIATION IN SOLAR WIND SPEED



* of a 72-year old man at the start of the 5-year record of mostly 0.5-hourly automatic "ambulatory" measurements with an A&D-monitor (except for an ~ 2-month break). Uncertainty regions serve only for ordering.

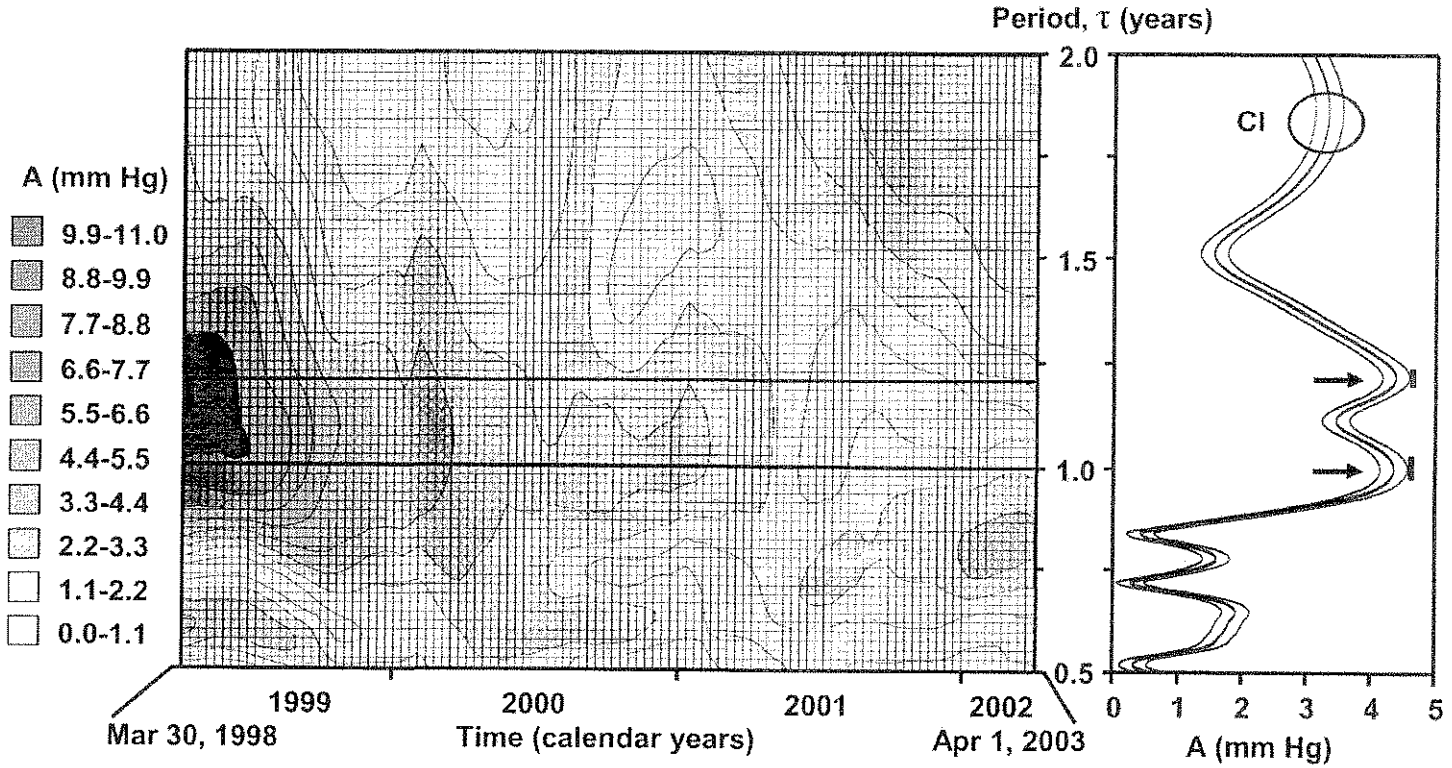
h)

SIMULATION OF BEATING (bottom row) OF 1.0-YEAR (top) & 1.2- YEAR (middle) COMPONENTS IN SYSTOLIC BLOOD PRESSURE (SBP)* SHOWS NEED FOR A LONGER THAN 5-YEAR SPAN (up to vertical line) TO RESOLVE BEAT (RATHER THAN LOSS OF RHYTHM)



* by treated MESOR-hypertensive man, 72-year old at start of monitoring for 5 years q 30 minutes. In gliding spectral windows (right): interval = 2 y, increment = 1.333 mo, longest trial period = 2 y, harmonic increment = 0.03125. Darker shading corresponds to higher amplitude (within limits from 0 to 9 mm Hg). Departure from sharp horizontal line of one cycle per 1.0 or 1.2 year and oscillations delineating different shaded areas relate to fractional harmonic increment and lack of tapering.

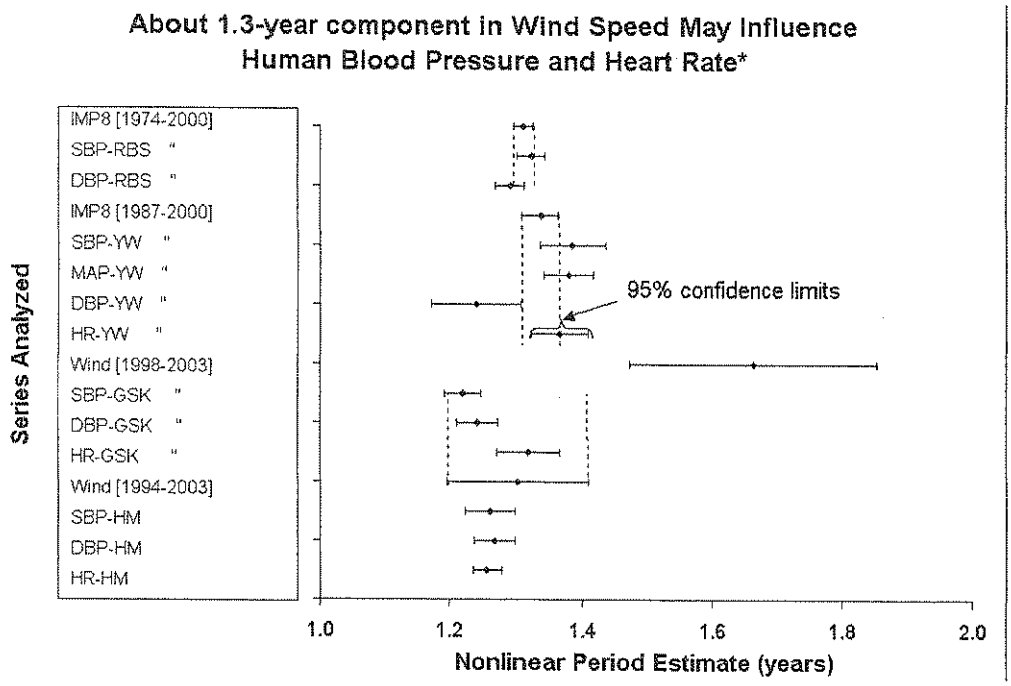
BEATING ~1.2- AND ~1.0-YEAR COMPONENTS ARE SEPARATED IN GLOBAL (right)* BUT NOT IN GLIDING (left)** SPECTRAL WINDOW OF SYSTOLIC BLOOD PRESSURE***



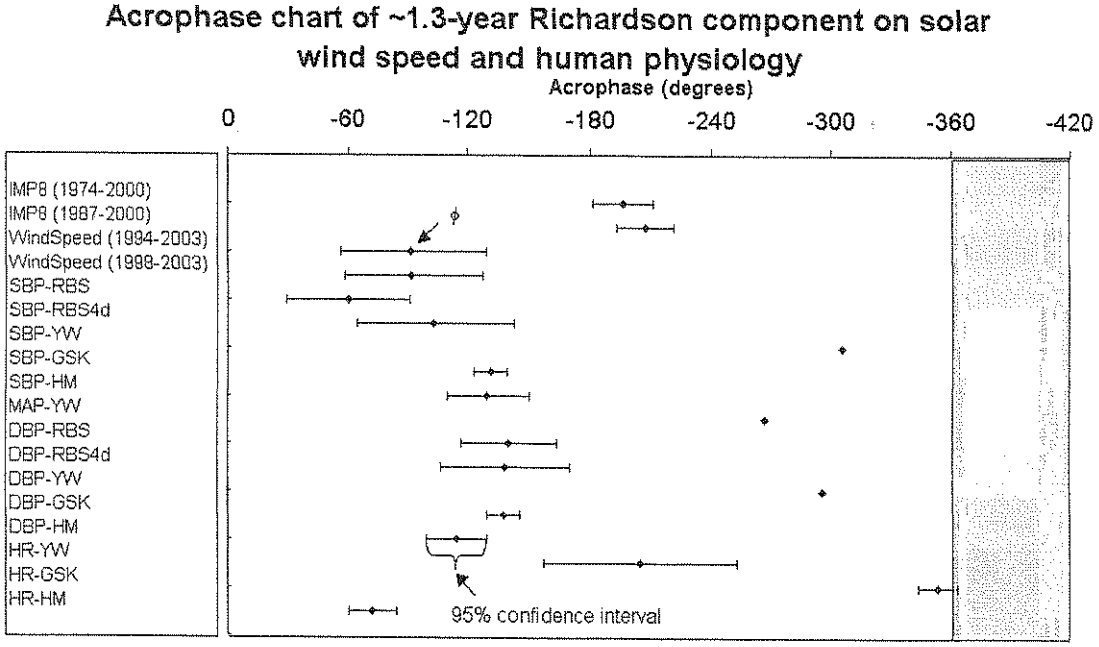
* Arrows point toward vertical bars (right) that are 95% confidence intervals (CI) for period length, fitted separately non-linearly; curves bracketing spectral estimates are tentative CI for amplitude (A); global spectral window suggests beating, validated by simulation of 2-component model with gliding spectral window (not shown) resembling result on actual data, left. **Linear in τ, interval = 2 y, increment = 10.5 d, trial τs from 2 y to 0.5 y, Δτ = 200 h. ***Of a treated MESOR-hypertensive man, 72-year old at the start of the 5-year record of mostly 0.5-hourly automatic "ambulatory" measurements.

Figure 14. **a.** Demonstration of a transyear component with a period of about 1.3 year, assessed nonlinearly with an estimate of 95% confidence interval, in all 12 longitudinal series of blood pressure and heart rate from 4 adults who monitored themselves for 5 to 36 years. Results are aligned with those of the velocity of the solar wind recorded by the IMP8 and Wind satellites. **b.** At the average period of 1.301 year, clustering of acrophases suggests possible synchronization of human blood pressure and heart rate with physical signal. **c.** The about 1.3-year component of blood pressure and heart rate of GSK (M, 72-77y) recorded during 1998-2003 has a period closer to the solar wind speed period characterizing the entire available record from the Wind satellite than that for the concurrent 5-year span. Physiological variables may resonate with non-photic environmental cycles that may have entered the genetic code during evolution (6, 23). © Halberg.

a)



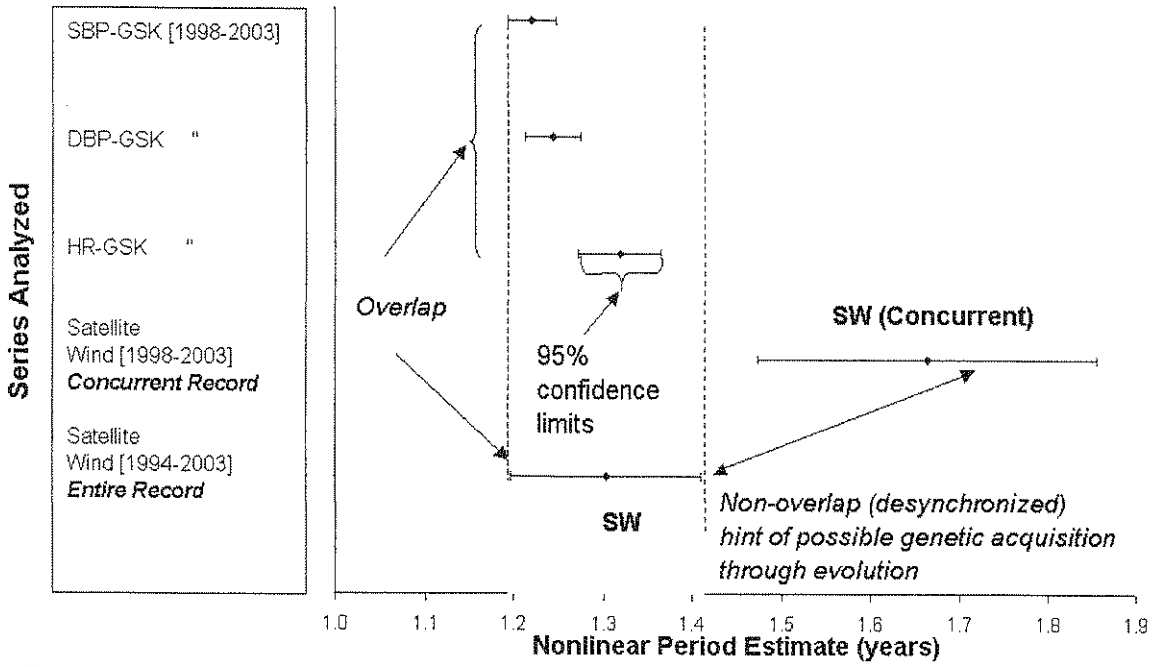
b)



Results at fixed average period of 1.301 year; acrophase reference: Jan 1, 1974.SBP and DBP are Systolic and Diastolic Blood Pressure; HR is Heart Rate.

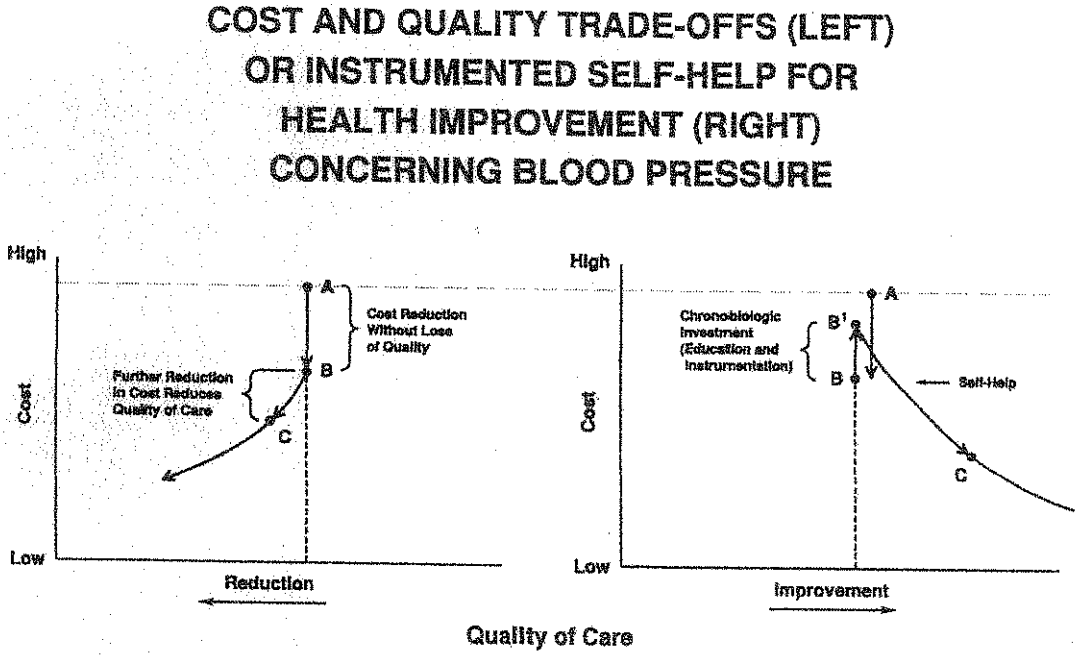
c)

Closer Agreement of About 1.3-Year Component in Human Blood Pressure (BP) and Heart Rate (HR) with Long-Term than with Concurrent Solar Wind (SW) Speed

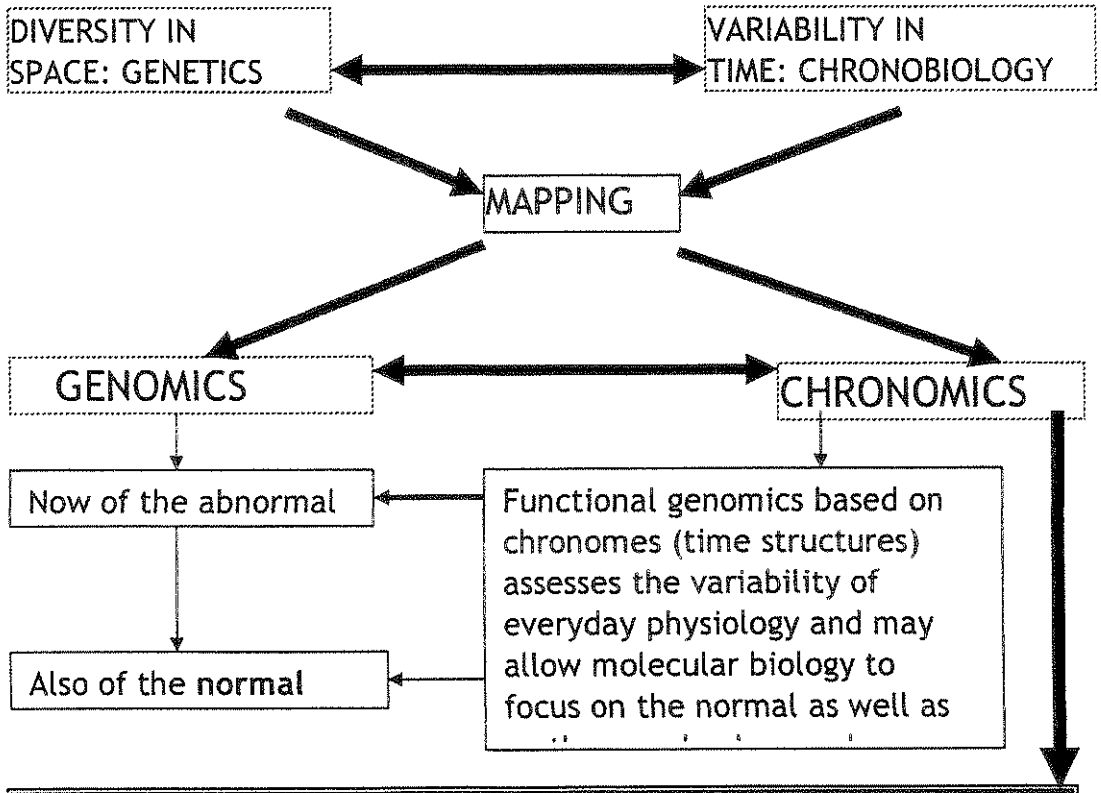


The non-overlapping 95% confidence intervals have to be qualified by the very wobbly nature of the Richardson rhythm in SW and physiology

Figure 15. Challenge to engineers, to civil servants dispensing government resources, and to each individual interested in self-help. Investment into physiological monitoring and education in chronobiology, to detect warning signs indicative of an elevated risk, rather than only of the fait accompli of disease, can prompt preventive intervention with the goal of avoiding the crippling of catastrophic diseases, also a major drain on financial resources. By placing added emphasis on prevention by general education in chronomic self-monitoring, health care costs could decrease while quality of care is individualized and improved (41, 45). © Halberg.



Insert 1
Complementarity in biological diversities in space-time*



The major merit of chronomics in its own right is the quantification of normalcy and the detection of earliest risk elevation, shadowing meritorious current, only circadian approaches to jet lag, shift-work and even the addition of a few years to a cancer patient's (partly high quality) life, achievements second only to prevention: PRE-habilitation rather than only REhabilitation

*An outcome of puzzles encountered in the 1950s

Table 1: Minnesotan work leading to 3-criteria-based chronomic vascular disease risk syndrome

Year[1]	Reference	Study	Comment (summary of results)
A. Survey of the circadian blood pressure (BP) amplitude (A) leading to transient, intermittent or sustained CHAT (Circadian Hyper-Amplitude-Tension)			
1980	Halberg J. et al. (1)	Spontaneously hype rtensive stroke-prone Okamoto rats (several groups of up to 40 rats with 4-hourly BP measurements for 24 hours repeated at different ages)	Before developing a high BP MESOR, the circadian BP amplitude may be transiently elevated under the loads of immobilization and heating during handling for manual measurements of tail BP **TRANSIENT CHAT**
1986	Scarpelli et al. (2; cf. 3-7 and 8, 9)	Several hundred schoolchildren in Florence, Italy, carried out self-measurements (SM) around the clock for one or several days : Results aligned with independent investigations across the USA, in Portugal and in China by SM, and in Baltimore, USA, with ambulatory BP monitoring (ABPM)	Feasibility studies on children showed that children with a *positive family history of high BP* and related cardiovascular diseases have a *larger circadian amplitude* of BP as compared to children with a negative family history
1986	Halberg et al. (10)	20 neonates monitored around the clock at 30-min intervals for 48 hours on 2 different days during the first week of life	The difference in circadian amplitude of BP as a function of the presence or absence of a family history of high BP is detectable during the first week of life
1990	Halberg et al. (11)	164 babies in Florence, Italy. Results replicate d in various geographic locations: Germany, Italy, Minnesota, Japan, Spain, USSR	Result on 20 neonates extended to a larger population of 164 babies, with qualification that difference in circadian amplitude of BP as a function of family history is detecte d only during years of minimal solar activity
1987	Scarpelli et al. (12)	10 patients with accelerated hypertension, taking SM ~5/day and followed up for up to 5 years	The 4 patients who died with malignant hypertension had a larger circadian BP amplitude than the 6 who were still alive
1990	Halberg et al. (11)	39 babies exposed *in utero* to betamimetic drugs vs. 113 control babies in Florence, Italy, each monitored around the clock at 30-min intervals for 48 hours during first week of life	Exposure *in utero* to betamimetic drugs is associated with an elevated circadian BP amplitude during the first week post-partum

[1]New findings opening a line of research with year flush with left margin in **bold**. Year for compleme ntary results indented. "Halberg" without initial refers to Franz Halberg.

Year[1]	Reference	Study	Comment (summary of results)
1995	Syutkina et al. (13)	18 children exposed *in utero* to betamimetic drugs vs. 25 control children in Moscow, Russia, studied by ABPM for 48 hours with measurements at 15-min intervals (results pooled with data from Spain)	The elevated circadian BP amplitude associated with exposure *in utero* to betamimetic drugs seen during the first week of life can still be observed during adolescence
1989	Cornélissen et al. (14)	221 pregnant women in Minnesota, clinically healthy at the outset, were studied by ABPM with hourly measurements around the clock for 2 days in each trimester of pregnancy, providing a total of 336 profiles	In addition to an 8 mm Hg difference in mean value between women who will or will not develop complications (gestational hypertension, preeclampsia) already observed during the first trimester of pregnancy, the occurrence of complications is also associated with BP profiles characterized by an elevated circadian BP amplitude. In particular, one case (JK) of CHAT where warning was not heeded, was followed 8 weeks later by severe pre-eclampsia, premature delivery and 26 months of hospitalization of offspring at a cost of about US $1 million
1991	Halberg et al. (15)		
1994	Cornélissen & Halberg (16)		
1997	Cornélissen et al. (17, 18)		
1992	Kumagai et al. (19)	30 men and 26 women 16-81 years of age, studied around the clock by ABPM in Tokyo, Japan. Characteristics of BP profile related to left ventricular mass index (LVMI).	MESOR-hypertension[2] may be preceded by a transient circadian amplitude elevation (an elevated circadian BP amplitude in the absence of an elevated mean value is observed at intermediate LVMI values, while an elevated MESOR is found only for the largest LVMI group)
1996	Otsuka et al. (20)	297 patients (121 normotensive + 176 treated hypertensive) studied around the clock by ABPM, with measurements at 15-min intervals for 48 hours, followed prospectively for 6 years. Characteristics of BP profile related to the actual incidence of adverse vascular events.	CHAT carries a relative risk of 8.2 (720% increase in risk) for ischemic cerebral events. This risk is larger than that associated with any other known risk factor, including MESOR-hypertension, and applies to normotensive as well as hypertensive patients. It is demonstrable in subpopulations of patients not presenting with any one of the other known risk factors.
1997	Otsuka et al. (21)		
1996	Watanabe et al. (22-24)	10 patients in Tokyo, Japan, monitored around the clock by ABPM with measurements at 15-min intervals for at least 1 week at monthly intervals while practicing autogenic training	Autogenic training decreases an excessive circadian BP amplitude

[2]MESOR = midline-estimating statistic of rhythm, a time structure (chronome)-adjusted average.

Year[1]	Reference	Study	Comment (summary of results)
1991 1995	Tamura et al. (25) see also Halberg & Cornélissen (26)	81 hypertensive patients studied by ABPM before and on treatment with one of 8 anti-hypertensive drugs.	Some but not all anti-hypertensive drugs lower an excessive circadian BP amplitude
1996	Cornélissen et al. (27)	Case report of fulminant CHAT in Minneapolis, MN, documented by ABPM.	CHAT had better predictive value than stress test and preceded by about 4 months the occurrence of a myocardial infarction in a 35-year-old man.
1998	Halberg et al. (28)		
1997	Watanabe et al. (29)	392 patients in Tokyo, Japan, studied around the clock by ABPM with measurements at 15-minute intervals for 1-7 days.	CHAT is more likely to occur in patients with borderline hypertension (transition between normotension and hypertension, in keeping with results by Kumagai et al. (19)
1998	Chen et al. (30)	424 patients studied by ABPM in Taiwan; characteristics of BP profile related to LVMI	CHAT is associated with an elevated LVMI
1999	Cornélissen et al. (31); see also Halberg et al. (28), Cornélissen et al. (32)	Meta-analysis of data from 297 patients of Otsuka et al. (19, 20) and Chen et al. (29)	Whereas vascular disease risk is linearly related with the BP MESOR (rhythm-adjusted mean value), the relation with the circadian BP amplitude is nonlinear
2001	Chen et al. (33); Cornélissen et al. (34)	2,039 patients (of whom 1,179 were untreated) studied by ABPM in Taiwan; characteristics of BP profile related to LVMI	Confirmation of an elevation in LVMI in association with CHAT, and of nonlinear relation of LVMI with circadian BP amplitude
2001	Schaffer et al. (35)	7-year follow-up (in terms of outcome) of 12 out of 24 dental patients who had been studied by ABPM with measurements at 15-minute intervals in 3 consecutive sessions of 4, 2 and 3 days for a total of 9 days bracketting 3 dental appointments	CHAT is associated with the occurrence of morbid events. (Only those who had abnormality in all 3 sessions had an adverse event.) Result is statistically significant, the small sample size notwithstanding, suggesting importance of risk related to CHAT
2001	Halberg et al. (36)	21 patients in Germany with staff measurements around the clock for 2 days followed up 28 years later	9 of 10 subjects without CHAT were alive whereas 7 of 11 subjects with CHAT had died. Difference is statistically significant, indicating that even manual measurements may serve for identifying abnormalities in BP variability

Year[1]	Reference	Study	Comment (summary of results)
2001	Shinagawa et al. (37)	18 patients monitored by ABPM at 30-min intervals for ≥24 h on 3 regimens (placebo, nifedipine and benidipine) using cross-over design	Treating CHAT may prevent adverse vascular events: as compared to placebo, nifedipine (10 mg b.i.d. at 08:00 and 20:00) increases and benidipine (4 mg/day at 08:00) decreases the circadian amplitude of BP. The resulting increase vs. decrease in the incidence of CHAT on nifedipine vs. benidipine may account for the corresponding difference between the number of stroke events of 7.6 and 3.5 and the total number of cardiovascular events of 20.4 and 8.8 per 1,000 person-years reported in large clinical trials in Asia
2001	Chen et al. (33) Cornélissen et al. (34)	Chrono-meta-analysis of 2,039 cases	Extension of results on 424 patients reported in 1998, with further demonstration that 1. risk associated with CHAT is larger than that of "non-dipping", which is found only for women and not for men, and 2. risk elevation is seen only for negative day-night ratios, indicating a need to assess the circadian amplitude and acrophase that can distinguish ecphasia from CHAT rather than day/night ratios that do not account for changes with gender and age of amplitude, acrophase and circadian waveform of BP.
2002	Halberg et al. (38)	Case report	Role of emotions on the circadian pattern of BP may bring about CHAT that can last for several days, supporting the recommendation to monitor for at least 7 days at the outset and preferably longitudinally for a lifetime.
2002	Cornélissen et al. (39)	Re-analysis of BP data from 297 patients in Tokyo, Japan	CHAT and a decreased heart rate variability are two separate risk factors accounting for the difference between <8% and 80% morbidity.
2002	Borer et al. (40)	Case report	CHAT present in African-American woman with documented high risk family history of cardiovascular disease
2002	Stinson et al. (41)	2 case reports comparing results from SM and ABPM	Approximation of ABPM results by SM is feasible only in some but not all subjects. A test is proposed to find out when CHAT can be identified by SM
B. DHRV (Decreased Heart Rate Variability)			

Year[1]	Reference	Study	Comment (summary of results)
1982	Orth-Gomér et al. (42)	50 patients with coronary artery disease (CAD) and 50 healthy controls undergoing 24-hour Holter monitoring in Stockholm, Sweden	The circadian pattern of premature ventricular contractions differs between the two groups
1990	Cornélissen et al. (43)	Meta-analysis of data from Huikuri et al. (44)	HRV is circadian periodic
1991 1994	Halberg et al. (45); Cornélissen et al. (46)	Daily incidence of myocardial infarctions (MI) in Moscow, Russia, between 1 Jan 1979 and 31 Dec 1981 (N=85,819)	There is a 7% increase in the daily incidence of MI after a magnetic storm
1997	Otsuka et al. (47); see also Otsuka et al. (21)	10 patients with CAD and 11 healthy men studied by 24-hour Holter monitoring in Tokyo, Japan	HRV, gauged by correlation dimension is reduced in patients with CAD; a decrease in HRV is demonstrable by night but not by day. In 297 patients studied by ABPM, a 550% increase in the risk of CAD was associated with a reduced HRV (24-h SD in lowest 7th percentile of distribution)
1997	Baevsky et al. (48)	Holter monitoring of 49 cosmonauts in space	HRV is reduced during a magnetic storm
1999	Cornélissen et al. (31)	129,205 deaths from MI in Minnesota from 1968 to 1996	Excess of 220 deaths from MI per year (5%) during solar maxima vs. solar minima
2000	Otsuka et al. (49) see also (31)	Longitudinal electrocardiographic (ECG) record for 7 days of clinically healthy man	HRV is reduced during a magnetic storm as compared to quiet conditions; HRV decrease observed in [0.003-0.04] Hz (~46.5 s) and [0.04-0.15] Hz (~10.5 s) but not in [0.15-0.40] Hz (~3.6 s) spectral region, suggesting involvement of the sympathetic rather than the parasympathetic nervous system
2000	Otsuka et al. (50-53)	Follow-up on students in Alta, Norway (70°N), each undergoing 7-day ECG monitoring bracketting magnetic storm	Confirmation on group of results observed in individual subject (49).
2000	Cornélissen et al. (54)	Alignment of long-term cycles in human pathology and physiology with solar activity cycle	There is a logical sequence of events suggesting non-photic influences from the sun
2000	Halberg et al. (55); see also Halberg et al. (56, 57)	Chronome maps of circadecadal and circadidecadal cycles, also extended to about 50-year cycles	Emergence of new field of **chronomics**
2002	Cornélissen et al. (58)	Review	Decreased HRV may be underlying mechanism for increased incidence of myocardial infarctions after magnetic storm

Year[1]	Reference	Study	Comment (summary of results)
2003	Halberg et al. (59), Cornélissen et al. (60) [Figure 6e]	Re-analysis of 297 patients in Tokyo	Decreased HRV notwithstanding, CHAT and an elevated pulse pressure are mostly separate risk factors

CONCLUSIONS:

1. The disease risk syndromes of circadian hyperamplitudetension (CHAT) and of decreased heart rate variability (DHRV) can be compared with the O-rings of the space shuttle Challenger as warnings before disaster [Figure 6d].
2. Timely detection and treatment of CHAT and/or of DHRV (and an excessive pulse pressure) may reduce health care costs [Figure 12].

1. Halberg J, Halberg E, Halberg F, Halberg Francine, Levine H, Haus E, Carandente F, Bartter FC, Delea C. Human and murine (SHRSP) blood pressure and telemetered core temperature variation and genetic disposition to mesor-hypertension. Chronobiologia 1978; 5: 342; Halberg J, Halberg E, Hayes DK, Smith RD, Halberg F, Delea CS, Danielson RS, Bartter FC. Schedule shifts, life quality and quantity modeled by murine blood pressure elevation and arthropod lifespan. Int J Chronobiol 1980; 7: 17-64.
2. Scarpelli PT, März W, Cornélissen G, Romano S, Livi R, Scarpelli L, Halberg E, Halberg F. Blood pressure self-measurement in schools for rhythmometric assessment of hyperbaric impact to gauge pressure "excess". In: Dal Palu C, Pessina AC (eds.), ISAM 1985, Proc. Int. Symp. Ambulatory Monitoring, Padua, March 29-30, 1985. Padua: CLEUP Editore, 1986: 229-237.
3. Halberg F, Ahlgren A, Haus E. Circadian systolic and diastolic hyperbaric indices of high school and college students. Chronobiologia 1984; 11: 299-309.
4. LaSalle D, Sothern RB, Halberg F. Sampling requirements for description of circadian blood pressure (BP) amplitude (A). Chronobiologia 1983; 10: 138.
5. Scheving LA, Scheving LE, Halberg F. Establishing reference standards by autorhythmometry in high school for subsequent evaluation of health status. In: Scheving LE, Halberg F, Pauly JE, editors. Chronobiology, Proc. Int. Soc. for the Study of Biological Rhythms, Little Rock, Ark. Stuttgart: Georg Thieme Publishers/Tokyo: Igaku Shoin Ltd., 1974: 386-393.
6. Johns KL, Halberg F, Cornélissen G, März W. Chronobiology at the American International School in Lisbon, Portugal. In: Halberg F, Reale L, Tarquini B, editors. Proc. 2nd Int. Conf. Medico-Social Aspects of Chronobiology, Florence, Oct. 2, 1984. Rome: Istituto Italiano di Medicina Sociale, 1986: 367-384.
7. Wan C, Wang Z, Cornélissen G, Halberg F. Age, gender and circadian or circasemidian blood pressure and heart rate variation of children. Chronobiologia 1992; 19: 121-129.
8. Cornélissen G, Wilson David, Halberg F, Ferencz C. Age and sex effects upon circadian characteristics in children of parents with and without a high blood pressure. Chronobiologia 1986; 13: 372-373.
9. Halberg F, Cornélissen G, Wilson David, Ferencz C. Circadian systolic amplitude separates boys of parents with or without a high blood pressure. Chronobiologia 1986; 13: 371-372.
10. Halberg F, Cornélissen G, Bingham C, Tarquini B, Mainardi G, Cagnoni M, Panero C, Scarpelli P, Romano S, März W, Hellbrügge T, Shinoda M, Kawabata Y. Neonatal monitoring to assess risk for hypertension. Postgrad Med 1986; 79: 44-46.
11. Halberg F, Cornélissen G, Bakken E. Caregiving merged with chronobiologic outcome assessment, research and education in health maintenance organizations (HMOs). Progress in Clinical and Biological Research 1990; 341B: 491-549.
12. Scarpelli PT, Romano S, Livi R, Scarpelli L, Cornélissen G, Cagnoni M, Halberg F. Instrumentation for human blood pressure rhythm assessment by self-measurement. In: Scheving LE, Halberg F, Ehret CF, editors. Chronobiotechnology and Chronobiological Engineering. Dordrecht, The Netherlands: Martinus Nijhoff; 1987. p. 304-309.
13. Syutkina EV, Cornélissen G, Halberg F, Grigoriev AE, Abramian AS, Yatsyk GV, Morozova NA, Ivanov AP, Shevchenko PV, Polyakov YA, Bunin AT, Sa?n SR, Maggioni C, Alvarez M, Fernandez O, Tarquini B, Mainardi G, Bingham C, Kopher R, Vernier R, Rigatuso J, Johnson D. Effects lasting into adolescence of exposure to betamimetics in utero. Clinical Drug Investigation 1995; 9: 354-362.

14. Cornélissen G, Kopher R, Brat P, Rigatuso J, Work B, Eggen D, Einzig S, Vernier R, Halberg F. Chronobiologic ambulatory cardiovascular monitoring during pregnancy in Group Health of Minnesota. Proc. 2nd Ann. IEEE Symp. on Computer-Based Medical Systems, Minneapolis, June 26-27, 1989. Washington DC: Computer Society Press, 1989: 226-237.
15. Halberg F, Cornélissen G, Carandente F. Chronobiology leads toward preventive health care for all: cost reduction with quality improvement. A challenge to education and technology via chronobiology. Chronobiologia 1991; 18: 187-193.
16. Cornélissen G, Halberg F. Introduction to Chronobiology. Medtronic Chronobiology Seminar #7, April 1994, 52 pp. (Library of Congress Catalog Card #94-060580; URL [accessed 1994] http://www.msi.umn.edu/~halberg/)
17. Cornélissen G, Halberg F, Bingham C, Kumagai Y. Toward engineering for blood pressure surveillance. Biomedical Instrumentation & Technology 1997; 31: 489-498.
18. Cornélissen G, Halberg F, Bingham C, Kumagai Y. More on software chronobioengineering for blood pressure surveillance. Biomedical Instrumentation & Technology 1997; 31: 511-513.
19. Kumagai Y, Shiga T, Sunaga K, Cornélissen G, Ebihara A, Halberg F. Usefulness of circadian amplitude of blood pressure in predicting hypertensive cardiac involvement. Chronobiologia 1992; 19: 43-58.
20. Otsuka K, Cornélissen G, Halberg F. Predictive value of blood pressure dipping and swinging with regard to vascular disease risk. Clinical Drug Investigation 1996; 11: 20-31.
21. Otsuka K, Cornélissen G, Halberg F, Oehlert G. Excessive circadian amplitude of blood pressure increases risk of ischemic stroke and nephropathy. J. Medical Engineering & Technology 1997; 21: 23-30.
22. Watanabe Y, Cornélissen G, Halberg F, Saito Yoshiaki, Fukuda K, Revilla M, Rodriguez C, Hawkins D, Otsuka K, Kikuchi T. Method and need for continued assessment of autogenic training effect upon blood pressure: case report. New Trends in Experimental and Clinical Psychiatry 1996; 12: 45-50.
23. Watanabe Y, Halberg F, Cornélissen G, Kikuchi T, Saito Y, Fukuda K, Revilla M Sr, Revilla M Jr, Rodriguez C, Wark DM, Otsuka K. Self-hypnosis lowers blood pressure swinging and overswinging in circadian hyperamplitudetension (CHAT). EuroRehab 1996; 2: 83-94.
24. Watanabe Y, Cornélissen G, Halberg F, Saito Yoshiaki, Fukuda K, Otsuka K, Kikuchi T. Chronobiometric assessment of autogenic training effects upon blood pressure and heart rate. Perceptual and Motor Skills 1996; 83: 1395-1410.
25. Tamura K, Kohno I, Saito Yuzo, Wakasugi K, Achiwa S, Imanishi Y, Cugini P, Halberg F. Antihypertensive individualized therapeutic strategy. Difesa Sociale 1991; 6: 109-124.
26. Halberg F, Cornélissen G, International Womb-to-Tomb Chronome Initiative Group: Resolution from a meeting of the International Society for Research on Civilization Diseases and the Environment (New SIRMCE Confederation), Brussels, Belgium, March 17-18, 1995: Fairy tale or reality ? Medtronic Chronobiology Seminar #8, April 1995, 12 pp. text, 18 figures. URL [accessed 1994] http://www.msi.umn.edu/~halberg/
27. Cornélissen G, Halberg F, Gubin D, Carandente F, Halberg J, Zaslavskaya R, Syutkin V, Kumagai Y, Watanabe Y, Otsuka K. Carpe diem mensuratem: Fulminant CHAT, its recognition a chronobiologic path to preventing a myocardial infarction? Abstract, 4° Convegno Nazionale, Societa Italiana di Cronobiologia, Gubbio (Perugia), Italy, June 1-2, 1996: 35-36.
28. Halberg F, Cornélissen G, Halberg J, Fink H, Chen C-H, Otsuka K, Watanabe Y, Kumagai Y, Syutkina EV, Kawasaki T, Uezono K, Zhao ZY, Schwartzkopff O. Circadian Hyper-Amplitude-Tension, CHAT: a disease risk syndrome of anti-aging medicine. J Anti-Aging Med 1998; 1: 239-259. (Editor's Note by Fossel M., p. 239.)
29. Watanabe Y, Cornélissen G, Halberg F, Bingham C, Siegelova J, Otsuka K, Kikuchi T. Incidence pattern and treatment of a clinical entity, overswinging or circadian hyperamplitudetension (CHAT). Scripta medica 1997; 70: 245-261.

30. Chen CH, Cornelissen G, Halberg F, Fiser B. Left ventricular mass index as "outcome" related to circadian blood pressure characteristics. Scripta medica 1998; 71: 183-189.
31. Cornélissen G, Halberg F, Schwartzkopff O, Delmore P, Katinas G, Hunter D, Tarquini B, Tarquini R, Perfetto F, Watanabe Y, Otsuka K. Chronomes, time structures, for chronobioengineering for "a full life". Biomedical Instrumentation & Technology 1999; 33: 152-187.
32. Cornélissen G, Otsuka K, Chen C-H, Kumagai Y, Watanabe Y, Halberg F, Siegelova J, Dusek J. Nonlinear relation of the circadian blood pressure amplitude to cardiovascular disease risk. Scripta medica (Brno) 2000; 73: 85-94.
33. Chen ChH, Cornélissen G, Siegelova J, Halberg F. Does overswinging provide an early warning of cardiovascular disease risk when non-dipping may fail ? A meta-analysis of 2039 cases. Scripta medica (Brno) 2001; 74: 75-80.
34. Cornélissen G, Chen ChH, Siegelova J, Halberg F. Vascular disease risk syndromes affecting both MESOR-normotensives and MESOR-hypertensives: a meta-analysis of 2039 cases. Scripta medica (Brno) 2001; 74: 81-86.
35. Schaffer E, Cornélissen G, Rhodus N, Halhuber M, Watanabe Y, Halberg F. Outcomes of chronobiologically normotensive dental patients: a 7-year follow-up. JADA 2001; 132: 891-899.
36. Halberg F, Cornélissen G, Katinas G, Hillman D, Schwartzkopff O. Season's Appreciations 2000: Chronomics complement, among many other fields, genomics and proteomics. Neuroendocrinol Lett 2001; 22: 53-73.
37. Shinagawa M, Kubo Y, Otsuka K, Ohkawa S, Cornélissen G, Halberg F. Impact of circadian amplitude and chronotherapy: relevance to prevention and treatment of stroke. Biomedicine and Pharmacotherapy 2001; 55 (Suppl 1): 125-132.
38. Halberg F, Cornélissen G, Otsuka K, Katinas GS, Schwartzkopff O, Halpin C, Mikulecky M, Revilla M, Siegelova J, Homolka P, Dusek J, Fiser B, Singh RB. Chronomics* (*the study of time structures, chronomes) detects altered vascular variabilities constituting risks greater than hypertension: with an illustrative case report. In: Mitro P, Pella D, Rybar R, Valocik G, editors. Proceedings, 2nd Congress on Cardiovascular Diseases, Kosice, Slovakia, 25-27 April 2002. Bologna: Monduzzi Editore; 2002. p. 223-258.
39. Cornélissen G, Otsuka K, Bakken EE, Halberg F, Siegelova J, Fiser B. CHAT (circadian hyper-amplitude-tension) and CSDD-HR (circadian standard deviation deficit of heart rate): separate, synergistic vascular disease risks ? Scripta medica (Brno) 2002; 75: 87-94.
40. Borer KT, Cornélissen G, Halberg F, Brook R, Rajagopalan S, Fay W. Circadian blood pressure overswinging in a physically fit, normotensive African American woman. American Journal of Hypertension 2002; 15: 827-830.
41. Stinson SM, Cornélissen G, Scarpelli PT, Halberg F. Self-measurement and ambulatory monitoring of blood pressure: a subject's chronobiological perspective. Biomed Pharmacother 2002; 56 (Suppl 2): 333s-338s.
42. Orth-Gomér K, Halberg F, Sothern R, Akerstedt T, Theorell T, Cornélissen G. The circadian rhythm of ventricular arrhythmias. In: Takahashi R, Halberg F, Walker C, editors. Toward Chronopharmacology, Proc. 8th IUPHAR Cong. and Sat. Symposia, Nagasaki, July 27-28, 1981. Oxford/New York: Pergamon Press, 1982: 191-202.
43. Cornélissen G, Bakken E, Delmore P, Orth-Gomér K, Akerstedt T, Carandente O, Carandente F, Halberg F. From various kinds of heart rate variability to chronocardiology. Am J Cardiol 1990; 66: 863-868.
44. Huikuri HV, Kessler KM, Terracall E, Castellanos A, Linnaluoto MK, Myerburg RJ. Reproducibility and circadian rhythm of heart rate variability in healthy subjects. Am J Cardiol 1990; 65: 391-393.
45. Halberg F, Breus TK, Cornélissen G, Bingham C, Hillman DC, Rigatuso J, Delmore P, Bakken E, International Womb-to-Tomb Chronome Initiative Group: Chronobiology in space. Keynote, 37th Ann. Mtg. Japan Soc. for Aerospace and Environmental Medicine, Nagoya, Japan, November 8-9, 1991. University of Minnesota/Medtronic Chronobiology Seminar Series, #1, December 1991, 21 pp. of text, 70 figures.

46. Cornélissen G, Wendt HW, Guillaume F, Bingham C, Halberg F, Breus TK, Rapoport S, Komarov F. Disturbances of the interplanetary magnetic field and human pathology. Chronobiologia 1994; 21: 151-154.
47. Otsuka K, Cornélissen G, Halberg F. Circadian rhythmic fractal scaling of heart rate variability in health and coronary artery disease. Clinical Cardiology 1997; 20: 631-638.
48. Baevsky RM, Petrov VM, Cornélissen G, Halberg F, Orth-Gomér K, Akerstedt T, Otsuka K, Breus T, Siegelova J, Dusek J, Fiser B. Meta-analyzed heart rate variability, exposure to geomagnetic storms, and the risk of ischemic heart disease. Scripta medica 1997; 70: 199-204.
49. Otsuka K, Yamanaka T, Cornélissen G, Breus T, Chibisov SM, Baevsky R, Halberg F, Siegelova J, Fiser B. Altered chronome of heart rate variability during span of high magnetic activity. Scripta medica (Brno) 2000; 73: 111-116.
50. Otsuka K, Ichimaru Y, Cornélissen G, Weydahl A, Holmeslet B, Schwartzkopff O, Halberg F, on behalf of the ICEHRV Working Group. Dynamic analysis of heart rate variability from 7-day Holter recordings associated with geomagnetic activity in subarctic area. Computers in Cardiology 2000; 27: 453-456.
51. Otsuka K, Cornélissen G, Weydahl A, Holmeslet B, Hansen TL, Shinagawa M, Kubo Y, Nishimura Y, Omori K, Yano S, Halberg F. Geomagnetic disturbance associated with decrease in heart rate variability in a subarctic area. Biomedicine and Pharmacotherapy 2001; 55 (Suppl 1): 51-56.
52. Otsuka K, Oinuma S, Cornélissen G, Weydahl A, Ichimaru Y, Kobayashi M, Yano S, Holmeslet B, Hansen TL, Mitsutake G, Engebretson MJ, Schwartzkopff O, Halberg F. Alternating-light-darkness-influenced human electrocardiographic magnetoreception in association with geomagnetic pulsations. Biomedicine and Pharmacotherapy 2001; 55 (Suppl 1): 63-75.
53. Otsuka K, Murakami S, Oinuma S, Kubo Y, Shinagawa M, Weydahl A, Cornélissen G, Halberg F. Altered heart rate variability on geomagnetically disturbed day and the following two days in subarctic. In: Mitro P, Pella D, Rybar R, Valocik G, editors. Proceedings, 2nd Congress on Cardiovascular Diseases, Kosice, Slovakia, 25-27 April 2002. Bologna: Monduzzi Editore; 2002. p. 271-283.
54. Cornélissen G, Halberg F, Gheonjian L, Paatashvili T, Faraone P, Watanabe Y, Otsuka K, Sothern RB, Breus T, Baevsky R, Engebretson M, Schröder W. Schwabe's ~10.5- and Hale's ~21-year cycles in human pathology and physiology. In: Schröder W, editor. Long- and Short-Term Variability in Sun's History and Global Change. Bremen: Science Edition, 2000. p. 79-88.
55. Halberg F, Cornélissen G, Otsuka K, Watanabe Y, Katinas GS, Burioka N, Delyukov A, Gorgo Y, Zhao ZY, Weydahl A, Sothern RB, Siegelova J, Fiser B, Dusek J, Syutkina EV, Perfetto F, Tarquini R, Singh RB, Rhees B, Lofstrom D, Lofstrom P, Johnson PWC, Schwartzkopff O, International BIOCOS Study Group. Cross-spectrally coherent ~10.5- and 21-year biological and physical cycles, magnetic storms and myocardial infarctions. Neuroendocrinol Lett 2000; 21: 233-258.
56. Halberg F, Cornélissen G, Otsuka K, Katinas G, Schwartzkopff O. Essays on chronomics spawned by transdisciplinary chronobiology: Witness in time: Earl Elmer Bakken. Neuroendocrinol Lett 2001; 22: 359-384.
57. Halberg F, Cornélissen G, Otsuka K, Katinas G, Delmore P, Schwartzkopff O. Earl Elmer Bakken: Pacing chronomics. Biomedicine and Pharmacotherapy 2001; 55 (Suppl 1): 19-20.
58. Cornélissen G, Halberg F, Breus T, Syutkina EV, Baevsky R, Weydahl A, Watanabe Y, Otsuka K, Siegelova J, Fiser B, Bakken EE. Non-photic solar associations of heart rate variability and myocardial infarction. J Atmos Solar-Terr Phys 2002; 64: 707-720.
59. Halberg F, Cornélissen G, Schack B. Self-experimentation on chronomes, time structures; chronomics for health surveillance and science: also transdisciplinary civic duty? Behavioral and Brain Sciences, http://www.bbsonline.org/Preprints/Roberts/Commentators/Halberg.html
60. Cornélissen G, Otsuka K, Halberg F. Circadian hyper-amplitude-tension (CHAT), a decreased (under-threshold) heart rate variability (DHRV) and an elevated pulse pressure (PP) are separate aspects of a chronomic cardiovascular disease risk syndrome. Abstract, 1st World Congress of Chronobiology, Sapporo, Japan, September 9-12, 2003, in press.

Table 2: Outcomes of chronobiological screens of blood pressure and heart rate*

N of patients (ref)	N at follow-up	Sampling	N measurements: Total (outcomes)	Finding
10 (1)	10 (up to 5 years)	5/day daily	Up to 9,125 (only partially analyzed)	Among P. Scarpelli's patients, the 4 who died with malignant hypertension had a larger c ircadian BP amplitude than the 6 who were still alive (SBP: t=1.84; P=0.103; DBP: t=2.99; P=0.017)
63 (2, 3)	21 after 28 years	~q4h for 2 days	756 (252)	9 of 10 subjects without CHAT are alive while 7 of 11 subjects with CHAT are dead 28 years later (chi -square=6.390; P<0.01)
56 (4)	56: Concomitant LVMI	q15 min for 24 h	5,376 (5,376)	Classification by Y. Kumagai of patients by LVMI (<100; 100 -130; >130 g/m^2) reveals elevation of circadian amplitude at LVMI in 100 -130 range whereas MESOR elevation occurs only at LVMI >130.
221 (5, 6)	221 (time of delivery)	q1 h/48 h in each trimester of pregnancy (336 profiles)	16,128 (16,128)	In addition to an 8 mm Hg difference in mean value between women who will or will not develop complications (gestational hypert ension, preeclampsia) already observed during the first trimester of pregnancy, the occurrence of complications is also associated with BP profiles characterized by an elevated circadian BP amplitude. In particular, one case (JK) of CHAT where warning was not heeded, was followed 8 weeks later by severe pre -eclampsia, premature delivery and 26 months of hospitalization of offspring at a cost of about $1 million
297 (7-12)	297 after 6 years	q15 min for 48 h	57,024 (57,024)	CHAT or a reduced circadian stan dard deviation of heart rate, or an excessive pulse pressure (>60 mm Hg) are large risk factors (larger than hypertension) for cerebral ischemic events, nephropathy and coronary artery disease, even when the blood pressure is within acceptable limits.
2039 (13-15)	2039 Concomitant LVMI	Hourly averages for 24 h	48,936 (48,936)	LVMI is increased in patients with CHAT, a reduced circadian standard deviation of heart rate, or an elevated pulse pressure. The relation between LVMI and the circadian endpoints i s nonlinear.
23 (16)	12 after 7 years	q15 min for 9 days	19,872 (10,368)	10 of 20 patients with no consistent BP abnormality are alive and well; 2 of 3 patients with consistent BP abnormality reported an adverse vascular event (P=0.015 by Fisher's Exact Test).
80 (17, 18)	80 Response to treatment administered 2 h before daily BP peak vs. control group treated 3 times a day	q4 h for 24 h before and on treatment	960 (960)	With smaller doses of medications, BP was lowered by R. Zaslavskaya to a larger ex tent and treatment was accompanied by fewer complications. Treatment: propranolol, clonidine, or alpha -methyldopa (P<0.05 for each effect)
18 (19)	18 (12 weeks)	q30 min (?24h) on 3 regimens	?2592 (?2592)	Treating CHAT may prevent adverse vascular events: As compared to placebo, nifedipine (1 mg b.i.d. at 08 & 20) increases and benidipine (4 mg/day at 08) decreases the circadian amplitude of blood pressure. The resulting increase vs. decrease in the incidence of CHAT on nifedipine vs. benidipine may account for the corresponding difference between the number of stroke events of 7.6 vs. 3.5 and the total number of cardiovascular events of 20.4 vs. 8.8 per 1,000 person -years.
Totals: 2,807	2,754		160,769 (>141,636)	

*SBP and DBP: Systolic and Diastolic blood pressure; HR: heart rate; CHAT: Circadian Hyper -Amplitude-Tension, a condition defined by a circadian a exceeding the upper 95% prediction limit of acceptability (in healthy peers matched by gender and age); LVMI: left ventricular mass index. By comparison wit classical studies, the number of measurements in chronobiological work completed thus far is likely to be larger, and confounding by inter -subject variabili (20).

1. Scarpelli PT, Romano S, Livi R, Scarpelli L, Cornélissen G, Cagnoni M, Halberg F. Instrumentation for human blood pressure rhythm assessment. In: Scheving LE, Halberg F, Ehret CF (eds) Chronobiotechnology and Chronobiological Engineering. Dordrecht, The Netherlands: Martinus Nijhoff, 1987: 304-309.
2. Halhuber MJ, Cornélissen G, Bartter FC, Delea CS, Kreze A, Mikulecky M, Müller-Bohn T, Siegelova J, Dusek J, Schwartzkopff O, Halberg F. Circadian urinary glucocorticoid and rhythmic blood pressure coordination. Scripta medica 2002; 75: 139-144.
3. Halberg F, Cornélissen G, Katinas G, Hillman D, Schwartzkopff O. Season's Appreciations 2000: Chronomics complement, among many other fields, genomics and proteomics. Neuroendocrinology Letters 2001; 22: 53-73.
4. Kumagai Y, Shiga T, Sunaga K, Cornélissen G, Ebihara A, Halberg F. Usefulness of circadian amplitude of blood pressure in predicting hypertensive cardiac involvement. Chronobiologia 1992; 19: 43-58.
5. Cornélissen G, Halberg F, Bingham C, Kumagai Y. Toward engineering for blood pressure surveillance. Biomedical Instrumentation & Technology 1997; 31: 489-498.
6. Cornélissen G, Rigatuso J, Syutkina EV, Schwartzkopff O, Johnson DE, Halberg F, International Womb-to-Tom Chronome Group. Case report of an acceptable average but overswinging blood pressure in Circadian Hyper-Amplitude-Tension, CHAT. Neuroendocrinology Letters, in press.
7. Otsuka K, Cornélissen G, Halberg F. Predictive value of blood pressure dipping and swinging with regard to vascular disease risk. Clin Drug Invest 1996; 11: 20-31.
8. Otsuka K, Cornélissen G, Halberg F, Oehlert G. Excessive circadian amplitude of blood pressure increases risk of ischemic stroke and nephropathy. J. Medical Engineering & Technology 1997; 21: 23- 30.
9. Otsuka K, Cornélissen G, Halberg F. Circadian rhythmic fractal scaling of heart rate variability in health and coronary artery disease. Clinical Cardiology 1997; 20: 631-638.
10. Halberg F, Cornélissen G, Halberg J, Fink H Chen C-H., Otsuka K, Watanabe Y, Kumagai Y, Syutkina EV, Kawasaki T, Uezono K, Zhao ZY, Schwartzkopff O. Circadian Hyper-Amplitude-Tension, CHAT: a disease risk syndrome of anti-aging medicine. J. Anti-Aging Med. 1998; 1: 239-259.
11. Cornélissen G, Otsuka K, Chen C-H, Kumagai Y, Watanabe Y, Halberg F, Siegelova J, Dusek J. Nonlinear relation of the circadian blood pressure amplitude to cardiovascular disease risk. Scripta medica 2000; 73: 85-94.
12. Cornélissen G, Otsuka K, Bakken EE, Halberg F, Siegelova J, Fiser B. CHAT (circadian hyper-amplitude-tension) and CSDD-HR (circadian standard deviation deficit of heart rate): separate, synergistic vascular disease risks? Scripta medica 2002; 75: 87-94.
13. Chen CH, Cornélissen G, Halberg F, Fiser B. Left ventricular mass index as 'outcome' related to circadian blood pressure characteristics. Scripta medica 1998; 71: 183-189.
14. Chen C-H, Cornélissen G, Siegelova J, Halberg F. Does overswinging provide an early warning of cardiovascular disease risk when non-dipping may fail? A meta-analysis of 2039 cases. Scripta medica 2001; 74: 75-80.
15. Cornélissen G, Chen C-H, Siegelova J, Halberg F. Vascular disease risk syndromes affecting both MESOR-normotensives and MESOR-hypertensives: a meta-analysis of 2039 cases. Scripta medica 2001; 74: 81-86
16. Schaffer E, Cornélissen G, Rhodus N, Halhuber M, Watanabe Y, Halberg F. Outcomes of chronobiologically normotensive dental patients: a 7-year follow-up. JADA 2001; 132: 891-899.
17. Zaslavskaya RM. Chronodiagnosis and chronotherapy of cardiovascular diseases. 2nd ed. Translation into English from Russian. Moscow: Medicina, 1993, 397 pp.
18. Cornélissen G, Zaslavskaya RM, Kumagai Y, Romanov Y, Halberg F. Chronopharmacologic issues in space. J Clin Pharmacol 1994; 34: 543-551.
19. Shinagawa M, Kubo Y, Otsuka K, Ohkawa S, Cornélissen G, Halberg F. Impact of circadian amplitude and chronotherapy: relevance to prevention and treatment of stroke. Biomedicine and Pharmacotherapy 2001; 55 (Suppl 1): 125-132.

20. Halberg F, Cornélissen G, Halberg E, Halberg J, Delmore P, Shinoda M, Bakken E. Chronobiology of human blood pressure. Medtronic Continuing Medical Education Seminars, 1988, 4th ed., 242 pp.

DEVELOPMENT OF CIRCADIAN RHYTHMICITY IN THE RAT SUPRACHIASMATIC NUCLEUS

Helena Illnerová, Zdenka Bendová, Martin Sládek, Martin Jáč and Alena Sumová,

Institute of Physiology, Academy of Sciences of the Czech Republic, Prague

Address correspondence to:

Doc. RNDr. Helena Illnerová, DrSc
President of The Academy of Sciences of the Czech Republic
Národní třída 3
117 20 Praha

Mammalian circadian rhythms are controlled by a pacemaker located in the suprachiasmatic nucleus (SCN) of the hypothalamus. Morphologically and functionally the SCN consists at least of two parts, the dorsomedial (dm) and the ventrolateral (vl) SCN. The waveform of rhythms in both parts is affected by the photoperiod. A molecular clockwork consisting of clock genes and their negative and positive transcriptional - translational feedback loops is responsible for the SCN rhythmicity. In the rat SCN, circadian rhythms in the metabolic and electrical activity appear already prenatally. The aim of the present study was to find out whether rhythmicity of the rat dm - and vl-SCN develops in parallel, when the photoperiod starts to affect the SCN rhythmicity and what is ontogenesis of clock genes expression.

A typical rhythm of the dm-SCN, i.e., the endogenous rhythm in c-Fos production, was present already in 3-day old rats. However, even at 10 days of age, it was not yet affected significantly by the photoperiod. A typical rhythm of the vl-SCN, i.e., the rhythm in c-Fos photoinduction, was not yet expressed in the SCN of 3-day old rats. At 10 days of age, the rhythm was already significant and started to be affected by the photoperiod. The molecular clockwork developed gradually from the fetal to the postnatal stage. In 3- and 10- day old rats, there were already significant rhythms in clock gene mRNAs, namely, in Per1, Per2 , BMal1 and Cry1 mRNAs, but not in the Clock mRNA. The amplitude of the rhythms increased gradually with age. The study indicates that the dm-SCN rhythmicity develops earlier than the vl-one, however a response to the photoperiod may develop sooner in the vl-SCN than in the dm - one. The molecular clockwork develops only gradually through increasing the amplitude of rhythms in clock genes expression.

CAN SOCIETY AFFORD NOT TO FOLLOW A CHRONOBIOLOGICAL APPROACH TO BLOOD PRESSURE SCREENING, DIAGNOSIS AND TREATMENT?

Germaine Cornélissen[1], Franz Halberg[1], Earl E Bakken[2], Zhengrong Wang[3], Roberto Tarquini[4], Federico Perfetto[4], Giacomo Laffi[4], Cristina Maggioni[5], Yuji Kumagai[6], Pavel Prikryl[7], Pavel Homolka[7], Jiri Dusek[7], Jarmila Siegelova[7], Bohumil Fiser[7]

[1]Chronobiology Center, University of Minnesota, Minneapolis, Minnesota, USA
[2]North Hawaii Community Hospital Inc., Kamuela, HI, USA
[3]West China Medical Center, Sichuan University, Chengdu, China
[4]University of Florence, Florence, Italy
[5]University of Milan, Milan, Italy
[6]Kitasato University East Hospital, Sagamihara, Japan
[7]Masaryk University, Brno, Czech Republic

Address for correspondence:

Germaine Cornélissen
Halberg Chronobiology Center
Mayo Mail Code 8609
University of Minnesota
420 Delaware Street SE
Minneapolis, MN 55455, USA
Tel: 612-624-6976
Fax: 612-624-9989
E-mail: corne001@umn.edu

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INTRODUCTION

There is a growing body of evidence suggesting that time structures in us and around us are intrically interwoven. Most if not all components of variation found in biota are also found in the environment, and vice versa [1]. For instance, about daily changes are seen in almost every biological variable under 24-hour synchronized conditions. It has also long been known that the phase of circadian rhythms can be manipulated by changing the phase of the environmental cycles [2]. At least for the case of circadian rhythms, their genetic inheritance has been demonstrated on a molecular basis [3, 4], suggesting that the influence from the environment has been acquired genetically during the course of evolution.

The mapping of chronomes should benefit our understanding of human health and disease in several ways. The study of human chronomes can serve the derivation of refined reference values to better define health and to identify pre-disease, so that prophylactic interventions can be instituted as early as possible, preferably before disease sets in [5-7]. The focus is thus put on pre-habilitation, in the hope that the need for re-habilitation will thereby be reduced [8-10]. The study of chronomes of other organisms such as bacteria [11-13] is also meritorious so that actions can be taken to protect humans and other animals from possible infections, and to apply any eradicating methods at the most opportune time so as to achieve highest efficacy with least side effects. Finally, the study of time structures in the environment may help safeguard the integrity of the environment while also gaining a better understanding of the relations between biota and their environment [14, 15].

BIOCOS, the project aimed at studying **BIO**logical systems in their **COS**mos has obtained a great deal of expertise in the fields of blood pressure (BP) and heart rate (HR) monitoring and of marker rhythmometry for the purposes of screening, diagnosis, treatment and prognosis. Information gained from this work suggests a close link between mental health and cardiovascular health [16]. Too often the argument is presented that a chronobiological approach relying on longitudinal monitoring of vital signs and on a computer-assisted analysis for an inferential statistically guided interpretation of the results is too complex and too expensive and hence cannot be generally introduced to developing countries where a cost-effective health care system is needed the most. In the following, these misconceptions are dispelled and the case is made for cardiovascular health that chronobiology offers the most promising approach to improving the quality of care at a reduced cost.

COST BENEFIT OF LONGITUDINAL MONITORING VERSUS SINGLE SAMPLES.

Several studies [17, 18] comparing the classification of patients based on single office measurements with that based on ambulatory monitoring for one to seven days suggest that the incidence of misdiagnosis is around 40%, in keeping with the 48% response to placebo in the Australian Therapeutic Trial [19, 20]. Comparison of circadian characteristics from day to day in records spanning at least two days further indicates the shortcomings of monitoring limited to a single 24-hour span [21-23]. Prolonging the monitoring from one to two days reduces the uncertainty in the estimation of circadian parameters by about 35% [24], whereas further information on the biological week [25-28] requires monitoring for at least 7 days, the current recommendation of BIOCOS for everybody at the outset [29]. It is now widely accepted that prognosis of target organ damage is by far superior when it is based on around the clock monitoring than on single

office measurements [30-32].

There can also be large day-to-day changes in the circadian characteristics of blood pressure and heart rate in some people, as illustrated in Figure 1. The mistaken impression that the circadian variation in blood pressure and heart rate is sufficiently stable to be approximated by a single 24-hour profile stems in large part from the use of statistical methods on groups of subjects rather than focusing on the individual patient. Correlation analyses applied to large groups of subjects with a wide range of average values emphasize similarity. Statistical analyses focusing on individual differences observed from one profile to another, however, yield information more likely to help the patient in need of treatment [21]. Several case reports document this point [7, 33-36]. Continued monitoring is the most logical solution. Feasible today by telemetry for the lifetime of laboratory animals, it still awaits industrial developments for application in human beings.

Longitudinal monitoring does not need to be costly. The high cost of ambulatory blood pressure monitoring prevailing today stems in large part from the practice to limit the procedure to special cases. Should the recommendation to screen every citizen be embraced, the cost of monitors would drop drastically as was the case for many commodities (such as the ball pen, the wrist watch, and the pocket calculator) when they became widely accessible. Serial blood pressure and heart rate data can also be obtained affordably by self-measurements, as already advocated by Janeway in 1904 [37]. Self-measurements taken 5 to 8 times a day during waking, preferably with at least occasional nightly readings (which could be taken by a family member so as not to interrupt sleep), have been successful to help the treatment of patients with malignant hypertension [38]. Manual measurements have also been successful to separate children with or without familial antecedents of high blood pressure and/or related cardiovascular disease and to predict outcomes [38, 39] by the assessment of the circadian amplitude of blood pressure interpreted in the light of reference values derived specifically for self-measurements [24].

MERIT OF A CHRONOBIOLOGICAL ASSESSMENT AND INTERPRETATION OF THE DATA.

Taking serial measurements a few times each day is important to greatly reduce the error associated with single measurements. The assessment by cosinor [2, 40] of the circadian amplitude and acrophase in addition to the MESOR further reduces the error term since blood pressure and heart rate are usually characterized by a circadian variation of large extent. Taking only one or two measurements a day, always at awakening and/or at bedtime may fail to reveal abnormalities seen only at other times of day, or abnormalities that apply only to the variability in blood pressure or heart rate.

By enlarging the monitoring to the population at large, clinically healthy individuals can provide the reference values needed to identify any abnormality that may occur within the physiological range. In view of gender differences and of changes in circadian characteristics as a function of age, illustrated in Figures 2 and 3, reference values are best specified by gender and age, and, whenever possible, also by ethnicity. Outcome studies could further refine the reference standards by relying primarily on the data provided by low-risk subjects. Reference values are needed not only for the MESOR and for the amplitude and acrophase of the 24-hour component and all pertinent harmonic terms, but also for the interpretation of time-specified single values [7]. Whereas the presence of a prominent circadian rhythm in blood pressure is no longer contested, this

knowledge has not been applied to time-specify reference values, so that the diagnosis does not depend on the clock hour of the clinical examination, as was demonstrated theoretically, Figure 4 [34] and clinically, Figure 5 [41].

A double-barrelled approach has been developed for the interpretation of blood pressure and heart rate records. It consists of a parametric and a non-parametric analysis of the data, in which the results from the individual subject are being compared with gender- and age-specified reference values in health [7]. Reference values for parameters have led to the identification of new disease risk syndromes, such as CHAT (circadian hyper-amplitude-tension, a condition characterized by a circadian amplitude exceeding the upper 95% prediction limit), BP-ecphasia (a condition characterized by a deviant circadian acrophase of blood pressure), and DHRV (decreased heart rate variability, defined as a below-threshold standard deviation of heart rate measurements collected around the clock). Together with an excessive pulse pressure (above 60 mmHg), CHAT and DHRV can make the difference between <4% and 100% morbidity in a 6-year prospective study, Figure 6 [42].

NEED FOR INTERNATIONAL RELATIONAL DATABASES LINKING PATTERNS AND TREATMENT MODALITIES TO OUTCOMES.

A systematic organization into databases of all records, preferably with regular follow-ups of the subjects who provided the records, would be invaluable to build evidence-based archives [43]. Information could thus be gathered to determine systematically and rigorously optimal chronotherapeutic regimens and to identify pre-disease conditions in a timely fashion, so that prophylactic interventions may be instituted. In addition to the data, outcomes and pertinent clinical data could be added to the existing databases. Information from both prospective trials and from retrospective analyses could add to the current knowledge. An example is provided in Figure 7, where a treatment regimen found to be superior in large clinical trials in reducing the incidence of strokes and other cardiovascular events is shown in a cross-over chronobiologic pilot study to decrease the incidence of CHAT. Because different treatment regimens can affect the circadian blood pressure amplitude differently [44, 45], some schedule may induce iatrogenic CHAT, which in turn may increase the risk of stroke, whereas another schedule may have the opposite effect [46]. A change in morbidity/mortality could thus be associated with changes in blood pressure and/or heart rate characteristics occurring naturally or intentionally induced by a given intervention.

An important distinction needs to be made between lessons learned from large clinical trials and their application for the individual patient. Differences and trends uncovered in studies on groups, even when each subject provides only one or a few measurements, cannot be similarly assessed in medical practice when a decision needs to be made for treating the individual patient. In order to be able to reach an informed decision for the given patient, serial rather than single data should be collected. When time series are available, it becomes possible to assess risk elevation or the response to treatment for that particular patient. Available procedures include parameter tests [47] and cumulative control charts [33, 35]. Treatment kind, dosing and timing then all become amenable to optimization for the given patient. The point also needs to be made that timing should routinely be introduced as a major factor in clinical trials. Without any added cost, or rather with considerable saving, chronobiological designs can offer important new information regarding the optimization of treatment by timing its administration as a

function of circadian and other rhythms. 'Larger' is not necessarily better when timing is ignored, as documented both theoretically [48] and clinically [49, 50].

SPIN-OFFS FROM ORGANIZED ARCHIVES OF LONG-TERM RECORDS.

Once databases are organized to include archives of longitudinal records from test pilots, special analyses may also serve basic science. For instance, these analyses may further the understanding of the influence of environmental factors near and far on human physiology and pathology [1]. Cases in point include the biological week [51, 52], the half-year [53], the trans-year [54, 55], and circadecadal and circamultidecadal cycles [1, 56], notably an about 10.5-year cycle in mortality from myocardial infarctions in Minnesota [57]. In particular, the recent detection of an about 1.3-year change in blood pressure and heart rate resembling the about 1.3-year variation in the velocity of the solar wind illustrates the fact that organisms are influenced by non-photic as well as photic effects from the sun [54, 55]. Problems related to the recent increase in mortality from stroke [1, 57-60] could also be more readily investigated, also accounting for a putative about 50-year cycle.

NEW TECHNOLOGICAL DEVELOPMENTS.

Not so many years ago, patients with diabetes used single daily injections of slow-acting insulin preparations aimed at covering 24 hours. Any adjustment in the daily dose relied on glucose tests in urine. Today, programmable insulin pumps use fast-acting insulin and patients are encouraged to test their glucose concentrations in blood samples several times a day to achieve tighter control and thereby reduce their risk of complications. In a first attempt to closing the loop, the new Paradigm 512 insulin pump from Medtronic/MiniMed has recently been combined with the Paradigm Link blood glucose meter, the two devices communicating with each other by means of radio waves to automatically adjust the dosage of insulin delivery [61]. In the case of blood pressure, 24-hour formulations of anti-hypertensive agents are still prevalent today, with the tacit implication that the need for medication remains constant from moment to moment and from one day to another. The technology needed to close the loop between the monitoring of blood pressure and the administration of anti-hypertensive drugs, however, should no differ too much from the technology already in use for the treatment of diabetes.

For a wider acceptance of the monitoring of blood pressure, new approaches may have to be considered. One possibility is the use of minimally invasive devices relying on an implanted sensor to measure blood pressure beat to beat. Another area of rapid development relates to the use of electronic textiles [62]. Shirts are being designed to monitor a patient's vital signs and alert a doctor by means of a wireless signal at the first sign of trouble. Medical textiles incorporate current-carrying fibers into fabrics to power electronic sensors that can monitor the wearer's breathing, temperature and heart rate, and may soon be capable of monitoring also oxygen and blood glucose concentrations. The clothing thus becomes the monitoring device, whereas software controls the communications inside the on-fabric network and can send radio signals using Bluetooth or any other IEEE 802.11 wireless standard to personal computers and over the Internet [63]. This technology offers diverse applications among which the prevention of sudden infant death syndrome (SIDS) is already within reach.

CONCLUDING REMARKS.

Optimization in manufacturing and marketing relies on monitoring, analysis and outcome studies. Similar systems are in place for tracking infections, as we recently witnessed in the case of SARS [64]. But this is not yet the case for personal health, the most precious commodity of all. It may be high time to remedy the situation, notably since all ingredients for doing so are available, namely the monitoring devices, the analytical procedures, treatment modalities and ways to optimizing their scheduling of administration, and also drug delivery devices [65]. Closing the loop has started for diseases such as diabetes (insulin pump linked to glucose sensor) [61], for certain cardiovascular conditions (pacemaker-cardioverter-defibrillators), but not yet for blood pressure disorders that place a patient at a higher risk of cardiovascular disease before there is overt disease.

Blood pressure can be as variable as blood sugar. Rather than focusing on 24-hour formulations (one size fits all), tighter control may perhaps be achieved using first a radically different strategy relying on faster acting anti-hypertensives that can be titrated as needed not only in response to blood pressure measurements recorded automatically and continuously but also in anticipation of the usual daily, weekly and even longer (e.g., circannual) patterns known to characterize blood pressure, including an increase prior to awakening, in preparation the next day's activities [66]. Whereas this may not happen overnight, we should not wait until tomorrow to use the technology that is available today. Education in chronobiology literacy at all ages can go a long way at an affordable cost, a small investment with potential great reward if by pre-habilitation the very high cost of after-the-fact care can be drastically curtailed, an approach equally applicable in developed, developing, and, where it is particularly needed, in under-developed countries.

REFERENCES.

1. Halberg F, Cornélissen G, Otsuka K, Watanabe Y, Delyukov A, Gorgo Y, Zhao ZY, Weydahl A, Sothern RB, Siegelova J, Fiser B, Dusek J, Syutkina EV, Perfetto F, Tarquini R, Singh RB, Rhees B, Lofstrom D., Lofstrom P, Sonkowsky R, Johnson PWC, Schwartzkopff O, International BIOCOS Study Group. Cross-spectrally coherent ~10.5- and 21-year biological and physical cycles, magnetic storms and myocardial infarctions. Neuroendocrinoogy Letters 2000; 21: 233-258.
2. Halberg F. Chronobiology. Annual Reviews of Physiology 1969; 31: 675-725.
3. Pennisi E. Multiple clocks keep time in fruit fly tissues. Science 1997; 278: 1560-1561.
4. Plautz JD, Kaneko M, Hall JC, Kay SA. Independent photoreceptive circadian clocks throughout Drosophila. Science 1997; 278: 1632-1635.
5. Halberg F, Cornélissen G, Carandente A, Bakken E, Young E. Chronobiologic perspectives of international health care reform for the future of children. Chronobiologia 1993; 20: 269-275.
6. Cornélissen G, Delmore P, Bingham C, Rutledge G, Kumagai Y, Kuwajima I, Suzuki Y, Kuramoto K, Otsuka K, Scarpelli PT, Tarquini B, Cagnoni M, Garcia L, Zaslavskaya RM, Syutkina E, Carandente F, Rapoport SI, Romanov YA, Tamura K, Bakken E, Halberg F. A response to the health care crisis: a 'health start' from 'womb to tomb'. Chronobiologia 1993; 20: 277-291.
7. Cornélissen G, Otsuka K, Halberg F. Blood pressure and heart rate chronome mapping: a complement to the human genome initiative. In: Chronocardiology and Chronomedicine: Humans in Time and Cosmos, Otsuka K, Cornélissen G, Halberg F (eds.), Life Sciences Publishing, Tokyo, 1993, pp. 16-48.

8. Cornélissen G, Halberg F, Schwartzkopff O, Delmore P, Katinas G, Hunter D, Tarquini B, Tarquini R, Perfetto F, Watanabe Y, Otsuka K. Chronomes, time structures, for chronobioengineering for "a full life". Biomedical Instrumentation & Technology 1999; 33: 152-187.
9. Halberg F, Cornélissen G, Wall D, Otsuka K, Halberg J, Katinas G, Watanabe Y, Halhuber M, Müller-Bohn T, Delmore P, Siegelova J, Homolka P, Fiser B, Dusek J, Sanchez de la Pena S, Maggioni C, Delyukov A, Gorgo Y, Gubin D, Carandente F, Schaffer E, Rhodus N, Borer K, Sonkowsky RP, Schwartzkopff O. Engineering and governmental challenge: 7-day/24-hour chronobiologic blood pressure and heart rate screening: Part I. Biomedical Instrumentation & Technology 2002; 36: 89-122.
10. Halberg F, Cornélissen G, Wall D, Otsuka K, Halberg J, Katinas G, Watanabe Y, Halhuber M, Müller-Bohn T, Delmore P, Siegelova J, Homolka P, Fiser B, Dusek J, Sanchez de la Pena S, Maggioni C, Delyukov A, Gorgo Y, Gubin D, Carandente F, Schaffer E, Rhodus N, Borer K, Sonkowsky RP, Schwartzkopff O. Engineering and governmental challenge: 7-day/24-hour chronobiologic blood pressure and heart rate screening: Part II. Biomedical Instrumentation & Technology 2002; 36: 183-197.
11. Halberg F, Cornélissen G, Katinas G, Hillman D, Schwartzkopff O. Season's Appreciations 2000: Chronomics complement, among many other fields, genomics and proteomics. Neuroendocrinology Letters 2001; 22: 53-73.
12. Faraone P, Cornélissen G, Katinas GS, Halberg F, Siegelova J. Astrophysical influences on sectoring in colonies of microorganisms. Scripta medica 2001; 74: 107-114.
13. Cornélissen G, Halberg F, Gheonjian L, Paatashvili T, Faraone P, Watanabe Y, Otsuka K, Sothern RB, Breus T, Baevsky R, Engebretson M, Schröder W. Schwabe's ~10.5- and Hale's ~21-year cycles in human pathology and physiology. In: Schröder W (ed.): Long- and Short-Term Variability in Sun's History and Global Change. Bremen: Science Edition, 2000, pp. 79-88.
14. Halberg F, Wendt H, Cornélissen G, Hawkins D, Sothern RB, Haus E, Garcia Alonso L, Portela A, Syutkina EV, Breus TK, Vernova YS, Kleitman E, Kleitman N, Stebbings JH, Johnson D. Chronobiologic monitoring of health and environmental integrity. Human Physiology 1998; 24: 728-733.
15. Halberg F, Syutkina EV, Cornélissen G. Chronomes render predictable the otherwise-neglected human "physiological range": position paper of BIOCOS project. Human Physiology 1998; 24: 14-21.
16. Otsuka K, Murakami S, Kubo Y, Yamanaka T, Mitsutake G, Ohkawa S, Matsubayashi K, Yano S, Cornélissen G, Halberg F. Preface. Chronomics for chronoastrobiology with immediate spin-offs for life quality and longevity. Biomedicine and Pharmacotherapy, in press.
17. Kumagai Y, Kuwajima I, Suzuki Y, Kuramoto K, Otsuka K, Cornélissen G, Halberg F. Untenable acceptance of casual systolic/diastolic blood pressure readings below 140/90 mm Hg. Chronobiologia 1993; 20: 255-260.
18. Schaffer E, Cornélissen G, Rhodus N, Halhuber M, Watanabe Y, Halberg F. Outcomes of chronobiologically normotensive dental patients: a 7-year follow-up. J American Dental Association 2001; 132: 891-899.
19. Management Committee. Australian National Blood Pressure Study: The Australian Therapeutic Trial in Mild Hypertension. The lancet (June 14) 1980: 1261-1267.
20. Halberg F, Cornelissen G, Halberg E, Halberg J, Delmore P, Shinoda M, Bakken E. Chronobiology of human blood pressure - Medtronic Continuing Medical Education Seminars, 1988, 4th ed., 242 pp.
21. Cornélissen G. Instrumentation and data analysis methods needed for blood pressure monitoring in chronobiology. In: Scheving LE, Halberg F, Ehret CF (eds). Chronobiotechnology and Chronobiological Engineering. Dordrecht, The Netherlands: Martinus Nijhoff, 1987, pp. 241-261.
22. Tamura K, Wu J, Cornélissen G, Halberg F. Agreement between consecutive ambulatory 24-hour blood pressure and heart rate pro?les in Japanese hospital staff. Progress in Clinical and Biological Research 1990; 341A: 263-272.
23. Watanabe Y, Cornélissen G, Halberg F, Bingham C, Siegelova J, Otsuka K, Kikuchi T. Incidence pattern and treatment of a clinical entity, overswinging or circadian hyperamplitudetension (CHAT). Scripta medica 1997; 70: 245-261.

24. Halberg F, Scheving LE, Lucas E, Cornélissen G, Sothern RB, Halberg E, Halberg J, Halberg Francine, Carte J Jr, Straub KD, Redmond DP. Chronobiology of human blood pressure in the light of static (room-restricted) automatic monitoring. Chronobiologia 1984; 11: 217-247.
25. Carandente F, Cornélissen G, Halberg F Further data and analyses. Chronobiologia 1994; 21: 311-314.
26. Halberg F, Cornelissen G, Raab F, Schaffer E, Siegelova J, Fiser B, Dusek J, Prikryl P, Otsuka K. Automatic physiologic 7-day monitoring and chronobiology. Japanese J of Electrocardiology 1995; 15 (Suppl. 1): S-1-5 - S-1-11.
27. Siegelova J, Homolka P, Dusek J, Fiser B, Cornélissen G, Halberg F. Extracircadian-to-circadian variance transpositions early and vice versa late in life in the human circulation. Proceedings, 1st International Symposium, Workshop on Chronoastrobiology & Chronotherapy (Satellite Symposium, 7th Annual Meeting, Japanese Society for Chronobiology), Kudan, Chiyodaku, Tokyo, 11 Nov 2000, pp. 58-60.
28. Singh RB, Cornélissen G, Siegelova J, Homolka P, Halberg F. About half-weekly (circasemiseptan) pattern of blood pressure and heart rate in men and women of India. Scripta medica 2002; 75: 125-128.
29. Halberg F, Smith HN, Cornélissen G, Delmore P, Schwartzkopff O, International BIOCOS Group. Hurdles to asepsis, universal literacy, and chronobiology-all to be overcome. Neuroendocrinology Letters 2000; 21: 145-160.
30. Mancia G, Gamba PL, Omboni S, Paleari F, Parati G, Sega R, Zanchetti A. Ambulatory blood pressure monitoring. J Hypertension (Suppl) 1996; 14: S61-S66.
31. Mallion JM, Baguet JP, Siche JP, Tremel F, De Gaudemaris R. Clinical value of ambulatory blood pressure monitoring. J Hypertension 1999; 17: 585-595.
32. Mancia G, Parati G. Ambulatory blood pressure monitoring and organ damage. Hypertension 2000; 36: 894-900.
33. Halberg F, Cornélissen G, International Womb-to-Tomb Chronome Initiative Group. Resolution from a meeting of the International Society for Research on Civilization Diseases and the Environment (New SIRMCE Confederation), Brussels, Belgium, March 17-18, 1995: Fairy tale or reality? Medtronic Chronobiology Seminar #8, April 1995, 12 pp. text, 18 ?gures.
34. Cornélissen G, Halberg F. Impeachment of casual blood pressure measurements and the ?xed limits for their interpretation and chronobiologic recommendations. Annals. NewYork Academy of Sciences 1996; 783: 24-46.
35. Cornélissen G, Halberg F, Hawkins D, Otsuka K, Henke W. Individual assessment of antihypertensive response by self-starting cumulative sums. J Medical Engineering & Technology 1997; 21: 111-120.
36. Halberg F, Cornélissen G, Otsuka K, Katinas GS, Schwartzkopff O, Halpin C, Mikulecky M, Revilla M, Siegelova J, Homolka P, Dusek J, Fiser B, Singh RB. Chronomics detects altered vascular variabilities constituting risks greater than hypertension: with an illustrative case report. In: Mitro P, Pella D, Rybar R, Valocik G (eds). Proceedings, 2nd Congress on Cardiovascular Diseases, Kosice, Slovakia, 25-27 April 2002. Bologna: Monduzzi Editore, 2002, pp. 223-258.
37. Janeway TC. The clinical study of blood pressure. New York: D. Appleton & Co., 1904, 300 pp.
38. Scarpelli PT, Romano S, Livi R, Scarpelli L, Cornélissen G, Cagnoni M, Halberg F. Instrumentation for human blood pressure rhythm assessment by self-measurement. In: Chronobiotechnology and Chronobiological Engineering, Scheving LE, Halberg F, Ehret C. (eds), Martinus Nijhoff, Dordrecht, The Netherlands, 1987, pp. 304-309.
39. Halhuber MJ, Cornélissen G, Bartter FC, Delea CS, Kreze A, Mikulecky M, Müller-Bohn T, Siegelova J, Dusek J, Schwartzkopff O, Halberg F. Circadian urinary glucocorticoid and rhythmic blood pressure coordination. Scripta medica 2002; 75: 139-144.
40. Cornélissen G, Halberg F. Chronomedicine. In: Encyclopedia of Biostatistics, Armitage P, Colton T (eds), v. 1, John Wiley & Sons Ltd, Chichester, UK, 1998, pp. 642-649.
41. Bartter FC. Periodicity and medicine. In: Scheving LE, Halberg F, Pauly JE (eds). Chronobiology. Tokyo: Igaku Shoin Ltd., 1974, pp. 6-13.

42. Cornélissen G, Otsuka K, Chen CH-H, Halberg F. Circadian Hyper-Amplitude-Tension (CHAT) and an elevated pulse pressure are separate cardiovascular disease risk factors. Clin Exp Hypertens, in press.
43. Halberg F, Cornélissen G, Bakken E. Caregiving merged with chronobiologic outcome assessment, research and education in health maintenance organizations (HMOs). Progress in Clinical and Biological Research 1990; 341B: 491-549.
44. Tamura K, Kohno I, Saito Y, Wakasugi K, Achiwa S, Imanishi Y, Cugini P, Halberg F. Antihypertensive individualized therapeutic strategy. Difesa Sociale 1991; 6: 109-124.
45. Watanabe Y, Cornélissen G, Watanabe M, Watanabe F, Otsuka K, Ohkawa S-i, Kikuchi T, Halberg F. Effects of autogenic training and antihypertensive agents on circadian and circaseptan variation of blood pressure. Clin Exp Hypertens 2003; 25: 405-412.
46. Shinagawa M, Kubo Y, Otsuka K, Ohkawa S, Cornélissen G, Halberg F. Impact of circadian amplitude and chronotherapy: relevance to prevention and treatment of stroke. Biomedicine and Pharmacotherapy 2001; 55: 125-132.
47. Bingham C, Arbogast B, Cornélissen Guillaume G, Lee JK, Halberg F. Inferential statistical methods for estimating and comparing cosinor parameters. Chronobiologia 1982; 9: 397-439.
48. Bingham C, Cornélissen G, Halberg F. Power of 'Phase 0' chronobiologic trials at different signal-to-noise ratios and sample sizes. Chronobiologia 1993; 20: 179-190.
49. Halberg F, Bingham C, Cornélissen G. Clinical trials: the larger the better? Chronobiologia 1993; 20: 193-212.
50. Cornélissen G, Halberg F, Prikryl P, Dankova E, Siegelova J, Dusek J, International Womb-to-Tomb Chronome Study Group. Prophylactic aspirin treatment: the merits of timing. JAMA 1991; 266: 3128-3129.
51. Halberg F. The week in phylogeny and ontogeny: opportunities for oncology. In vivo 1995; 9: 269-278.
52. Cornélissen G, Engebretson M, Johnson D, Otsuka K, Burioka N, Posch J, Halberg F. The week, inherited in neonatal human twins, found also in geomagnetic pulsations in isolated Antarctica. Biomedicine and Pharmacotherapy 2001; 55 (Suppl 1): 32-50.
53. Cornélissen G, Halberg F, Pöllmann L, Pöllmann B, Katinas GS, Minne H, Breus T, Sothern RB, Watanabe Y, Tarquini R, Perfetto F, Maggioni C, Wilson D, Gubin D, Otsuka K, Bakken EE. Circasemiannual chronomics: half-yearly biospheric changes in their own right and as a circannual waveform. Biomedicine and Pharmacotherapy, in press.
54. Cornélissen G, Masalov A, Halberg F, Richardson JD, Katinas GS, Sothern RB, Watanabe Y, Syutkina EV, Wendt HW, Bakken EE, Romanov Y. Multiple resonances among time structures, chronomes, around and in us. Is an about 1.3-year periodicity in solar wind built into the human cardiovascular chronome? Human Physiology, in press.
55. Halberg F, Cornélissen G, Schack B. Self-experimentation on chronomes, time structures; chronomics for health surveillance and science: also transdisciplinary civic duty? Behavioral and Brain Sciences. http://www.bbsonline.org/Preprints/Roberts/Commentators?Halberg.html
56. Halberg F, Cornélissen G, Katinas G, Appenzeller O, Otsuka K, Sothern RB, Tarquini R, Perfetto F, Maggioni C, Wilson D, Schröder W, Schwartzkopff O, Kessler T, Wang Z, Burioka N, Watanabe Y, Bakken E. System times and time horizons for biospheric near-matches of primarily non-photic environmental cycles. Biomedicine and Pharmacotherapy 2002; 56 (Suppl 2): 266s-272s.
57. Cornélissen G, Halberg F, Breus T, Syutkina EV, Baevsky R, Weydahl A, Watanabe Y, Otsuka K, Siegelova J, Fiser B, Bakken EE. Non-photic solar associations of heart rate variability and myocardial infarction. J Atmospheric Solar-Terrestrial Physics 2002; 64: 707-720.
58. Fiser B, Cornélissen G, Siegelova J, Dusek J, Homolka P, Mazankova V, Halberg F. Increase in stroke deaths after 1997 in the Czech Republic. Scripta medica 2002; 75: 95-100.
59. Johansson B, Norrving B, Lindgren A. Increased stroke incidence in Lund-Orup, Sweden, between 1983 to 1985 and 1993 to 1995. Stroke 2000; 31: 481-486.

60. Otsuka K (ed). Proceedings, First International Symposium, Workshop on Chronoastrobiology and Chronotherapy (Satellite Symposium, Seventh Annual Meeting, Japanese Society for Chronobiology), Kudan, Chiyodaku, Tokyo, 11 Nov 2000.
61. Kordella T. A pump/meter duo. Pump and meter talk via radio waves. Diabetes Forecast. Nov 2003, p 45.
62. Service RF. Electronic textiles charge ahead. Science 2003; 301: 909-911.
63. Marculescu D, Marculescu R, Park S, Jayaraman S. Ready to ware. IEEE Spectrum. Oct 2003, pp. 28-32.
64. Yang W. Severe acute respiratory syndrome (SARS): infection control. Lancet 2003; 361: 1386-1387.
65. Hrushesky JM, Langer R, Theeuwes F (eds). Temporal control of drug delivery. Annals of the New York Academy of Sciences 618, 1991, 641 pp.
66. Halberg F. Some physiological and clinical aspects of 24-hour periodicity. J Lancet 1953; 73: 20-32.

Figure 1. Seven-day profile of systolic blood pressure of an untreated 27-year old asymptomatic woman illustrates how variable blood pressure can be from one day to another. The data have been analyzed for each day separately by the least squares fit of a model consisting of cosine curves with periods of 24 and 12 hours. The daily MESORs (middle horizontal line segments) are bracketed by the 90% prediction limits for the 24-hour amplitude determined for each day separately (upper and lower horizontal line segments). Whereas all parameters can be within acceptable limits on some days, large deviations are seen to occur on other days, when CHAT (circadian hyper-amplitude-tension) is diagnosed in the absence or in the presence of MESOR-hypertension. Evidence from longitudinal records suggests that circadian characteristics can vary greatly from one day to another in some patients. These data cast doubt on the wisdom of using 24-hour profiles for diagnosis and fixed-dose 24-hour formulations of anti-hypertensive drugs for treatment.

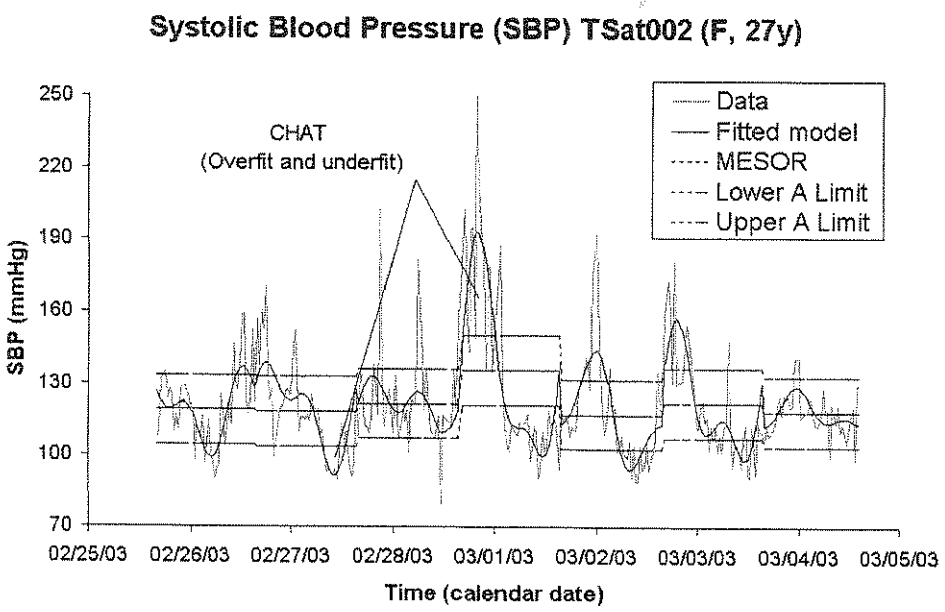


Figure 2. Changes with age in the MESOR of systolic (left) and diastolic (right) blood pressure of clinically healthy men. Note that the MESOR of SBP increases up to about 80 years of age, after which it starts decreasing slightly. By contrast, the MESOR of DBP reaches a maximum around 50 years of age. Consequently, pulse pressure tends to increase after 50 years of age. Specification as to gender, age and ethnicity can be complemented in this case by geography (USA) but not yet by social class.

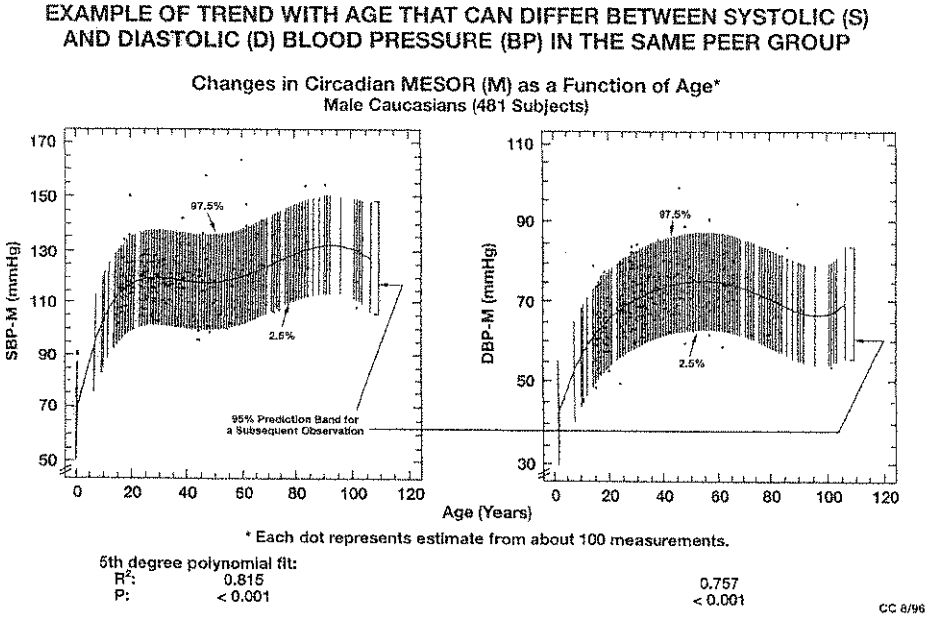


Figure 3. Changes as a function of age in the circadian waveform of systolic blood pressure of clinically healthy women. With increasing age, the post-prandial dip in SBP becomes more pronounced, resulting in an increase in the relative prominence of the 12-hour versus the 24-hour amplitude. After 80 years of age, the circadian amplitude decreases and the circadian acrophase becomes less stable. These changes account for the lack of a clear circadian rhythm in the group of centenarians (bottom right), although individually, the circadian rhythm in blood pressure was shown to remain demonstrable.

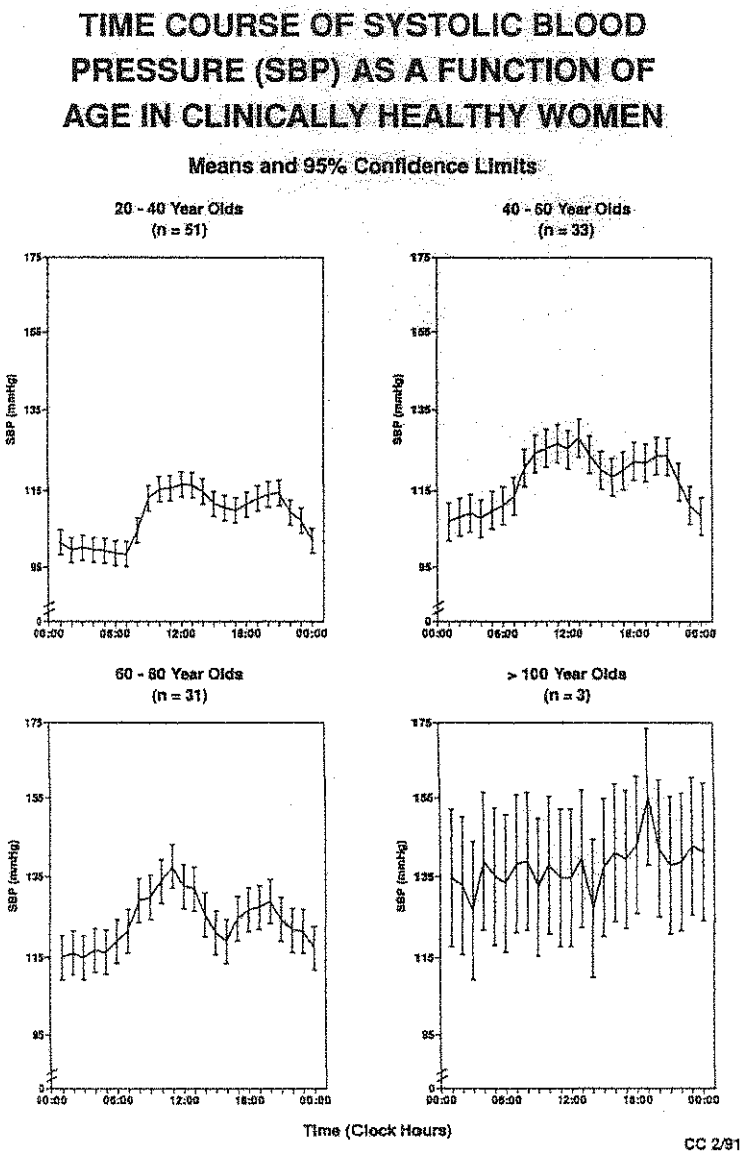


Figure 4. Limitations in dealing with blood pressure interpreted by fixed limits (and casual measurements or automatic office hour profiles). Theoretical evidence points to the need to replace fixed thresholds by time-varying reference standards (chronodesms) and to rely on more than one or a few casual blood pressure measurements. In the absence of measurement error, the circadian rhythm in blood pressure results in contradictory diagnoses. For a wide range of MESORs, subjects with a large but acceptable circadian amplitude will be diagnosed as MESOR-normotensive in the morning but as MESOR-hypertensive in the afternoon. The diagnosis and the decision to treat or not to treat should not depend on the clock hour of measurement!

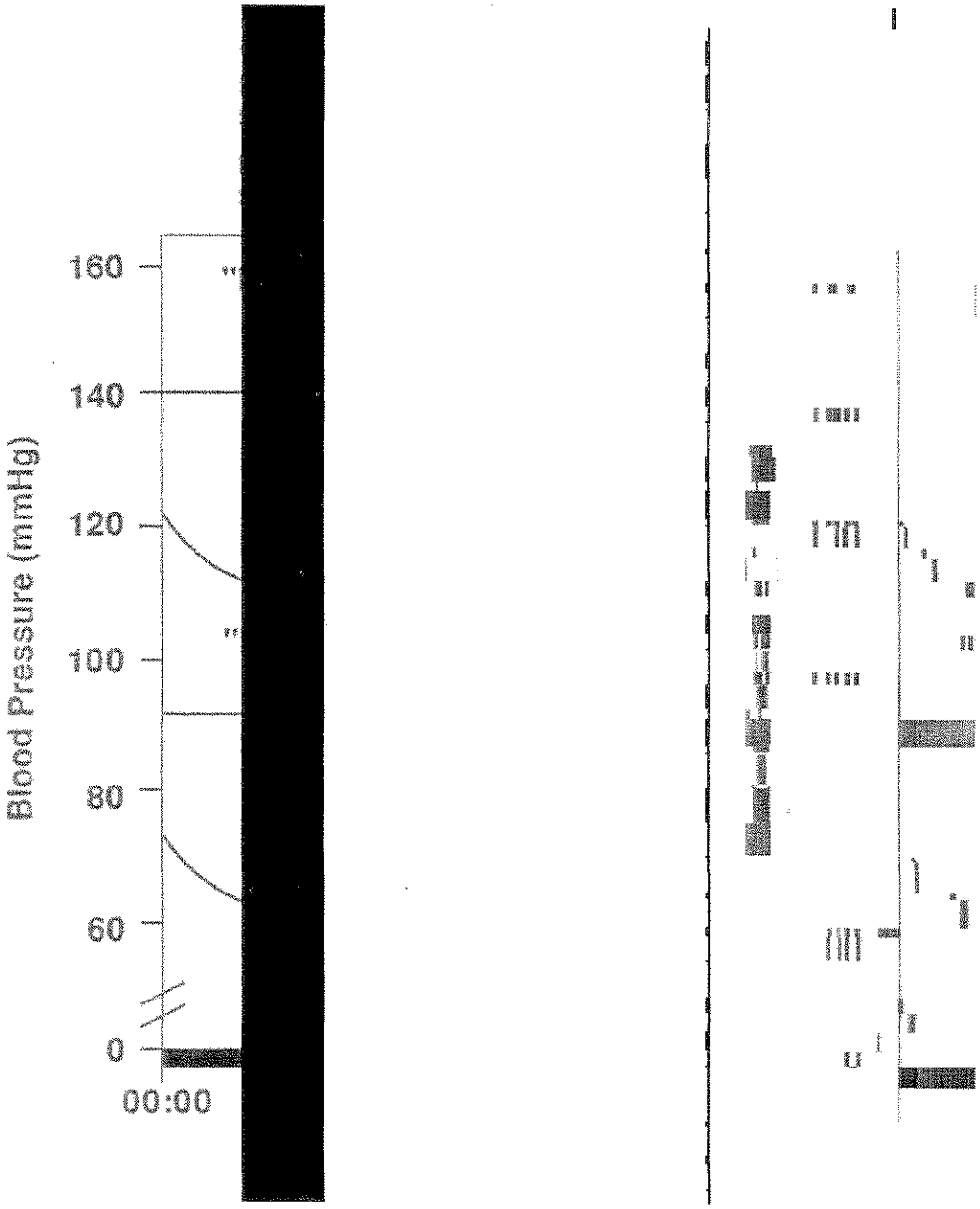


Figure 5. JW, a 61-year old man, was diagnosed as 'normotensive' by a physician he saw only in the morning, but as 'hypertensive' by another physician he saw only in the late afternoon. To clear the matter, he was monitored at NIH around the clock for over two weeks. The daily alternation between hypertension and normotension is clearly apparent from around the clock staff measurements at NIH. The danger of having a conventional diagnosis dependent upon the appointment's time, predicted theoretically in Figure 4, was thus validated clinically in this patient, whose staff measurements can hardly be questioned, their variability notwithstanding.

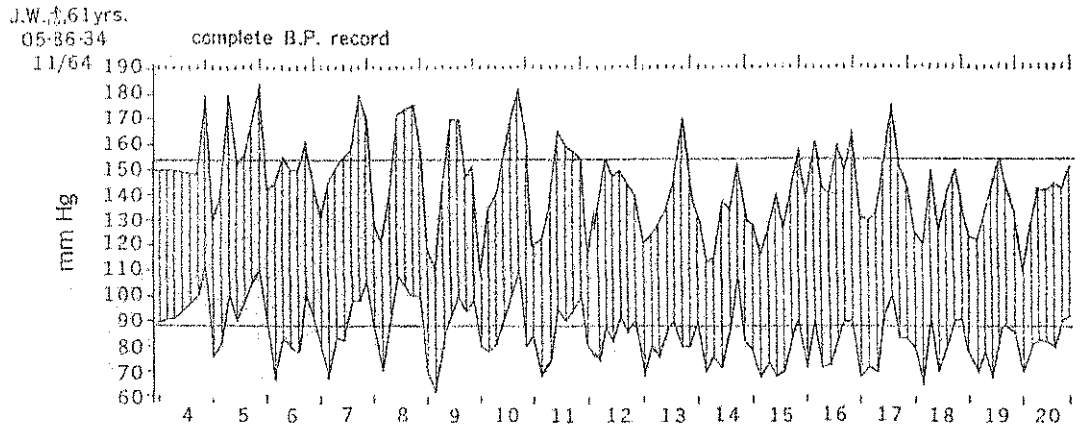


Figure 6. The incidence of morbidity among 121 normotensive and 176 treated hypertensive patients (so diagnosed by their time structure or chronome-adjusted mean, MESOR) with no cardiovascular disease at the outset is compared in a 6-year prospective study among patients presenting without or with 1, 2 or all 3 of 3 risks factors. The risk factors considered are:

1. CHAT (brief for circadian hyper-amplitude-tension) is a condition characterized by an excessive circadian amplitude of (diastolic) blood pressure (above the upper 95% prediction limit of clinically healthy peers of the same gender and a similar age);
2. An elevated pulse pressure (EPP) is defined as a difference between the MESORs of systolic and diastolic blood pressure above 60 mmHg; and
3. A decreased heart rate variability (DHRV) is defined as a standard deviation of heart rate measurements at 15-min intervals for 48 hours in the lowest 7% percentile of the patient population.

Risk was determined at the start of study, based on a 48-hour profile of automatic measurements of blood pressure and heart rate at 15-min intervals with an ambulatory monitor. Morbidity was checked about 6-monthly thereafter. Diagnoses considered were: coronary artery disease, cerebral ischemic events, nephropathy and retinopathy (related to blood pressure disorder). After 6 years, morbidity was diagnosed in 39 of the 297 patients.

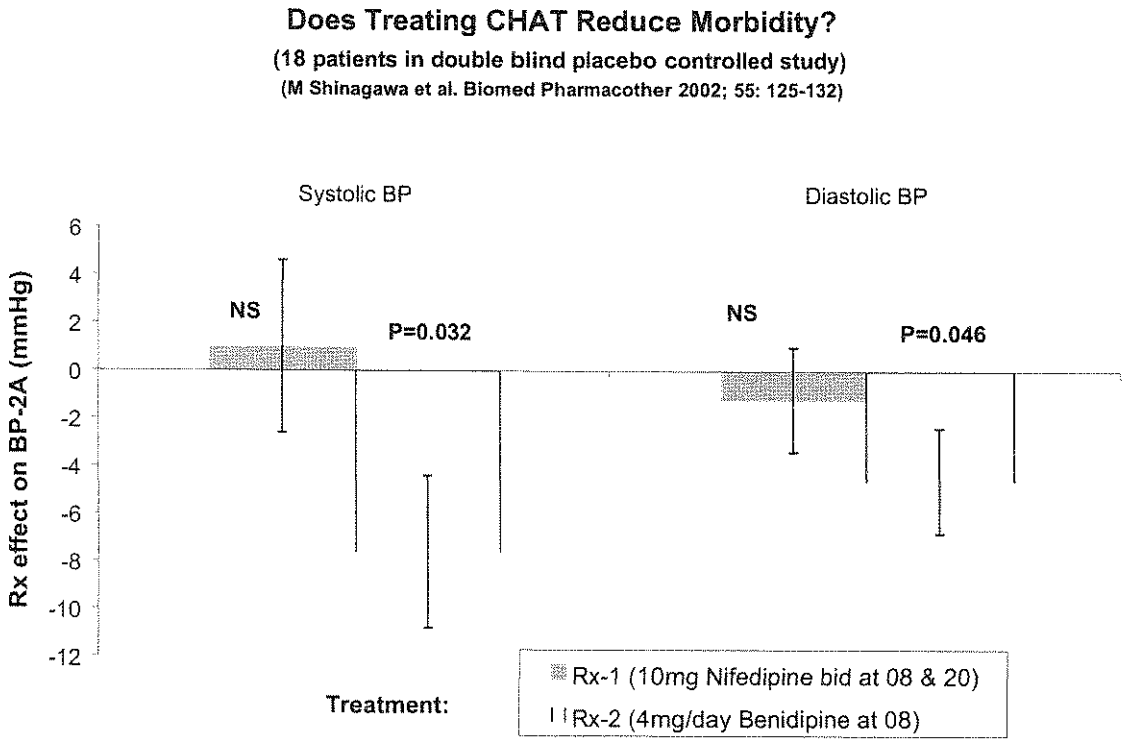
In the reference population of 214 patients presenting none of the 3 risk factors, morbidity was found in 8 cases (3.7%) (top left). The presence of DRHV or EPP alone raises the incidence of morbidity to 30.8% (top middle). When these two risks are both present, morbidity is doubled (66.7%) (top right). The presence of CHAT (bottom) invariably increases the incidence of morbidity, from 3.7% to 23.5% in the absence of the other two risk factors (bottom left), from 30.8% to 50% or 100% when either DHRV or EPP is also present (bottom middle), or from 66.7% to 100% when all 3 risk factors are present (bottom right).

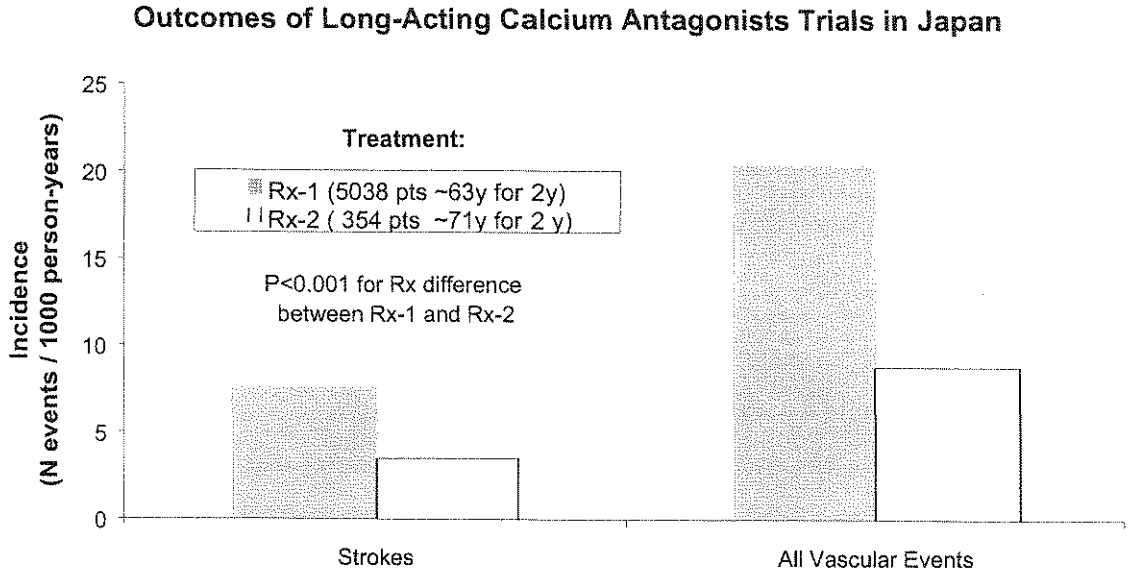
Except for a weak relation between pulse pressure and the standard deviation of heart rate, the 3 risk factors are mostly separate and additive. The results suggest the desirability to routinely assess blood pressure

variability in addition to heart rate variability since even in MESOR-normotension, CHAT is associated with a statistically significant increase in cardiovascular disease risk (not shown), and it can be successfully treated.

Whereas the number of morbid events and the number of patients in this study are small, the results are supported by several other propective and retrospective chronobiological investigations.

Figure 7. Treating CHAT (circadian hyper-amplitude-tension) is likely to reduce morbid events: outcomes of two calcium antagonists are better for drug administered according to a schedule associated with a reduction in the circadian amplitude of blood pressure than for a drug schedule associated with an increase in the circadian blood pressure amplitude.





SELF-EXPERIMENTATION -- DANGEROUS WHEN FIRST DONE BY A FEW, NOW SAFE FOR EVERYBODY'S HEALTH ?

Othild Schwartzkopff*, Franz Halberg*, Germaine Cornélissen*, George Katinas*, Zhengrong Wang@, Matthias Kröz•, Karl Hecht§. Roberto Tarquini¶, Federico Perfetto¶, Cristina MaggioniΔ, Jarmila Siegelova◊, Jiri Dusek◊, Bohumil Fiser◊°, R.B. Singh♦, Earl Bakken#

*University of Minnesota, Minneapolis, MN, USA; @West China University of Medical Sciences, Chengdu, P.R. China; •Forschungsinstitut Havelhöhe, Berlin, Germany; §Institut für Psychosoziale Gesundheit GbRmbH, Berlin, Germany; ¶University of Florence, Italy; ΔUniversity of Milan, Italy; ◊Masaryk University, Brno, Czech Republic; °World Health Organization, Geneva, Switzerland; ?Medical Hospital and Research Centre, Centre for Nutrition and Heart Research, Moradabad, U.P., India; #North Hawaii Community Hospital Inc., Kamuela, HI, USA; ‡Tokyo Women's Medical University, Daini Hospital, Tokyo, Japan

Address correspondence to:

Othild Schwarzkopff, M.D.
Assistant professor of pediatrics emerita
Halberg Chronobiology Center,
University of Minnesota
715 Mayo Building
Mayo Mail Code 8609
420 Delaware St. S.E.
Minneapolis, MN 55455, USA

Support: MSM 141 100004 (CZ)

As a pediatrician by profession, the senior author tackles the task of pleading for education in chronobiology and chronomics in a historical and philosophical view, without graphs, glancing back into the history of self-experimentation mostly over the last few hundred years. We consider the aspects of self experimentation, based on self-surveillance, that may have been part of general medicine as it branched out into multiple specialties and subspecialties. This is the background in particular to the more recent development over the last 50 years of the wide field of the no longer new science of chronobiology and the somewhat newer science of chronomics. Originating and developing during the last half century of research to an important extent in the chronobiology laboratories in Minneapolis, Minnesota, chronobiology and chronomics rest on many earlier fruitful examples of self-experimentation and self-surveillance, that are the topic of this essay.

SETTING THE STAGE

A scholarly book by Lawrence K. Altman (1) was the major source for this presentation and should be consulted when no specific references are given. This book provides an in-depth discussion of the motivation of many experimenters who wished to examine, rather than to prove or disprove, an up-to-then widely held assumption of the cause of a certain disease or condition. For instance, the possibility that certain diseases are contagious was an opinion widely held for pellagra and beriberi, before they were found to be caused by a certain deficiency in the diet. By contrast, during centuries, epidemics, such as the plague, cholera or typhus, were not always perceived as contagious. It took a long time to identify bacteria and viruses, flukes, spirochetes and the ways of their transmission. Of course, many, if not most, diseases are multifactorial. Some diseases, such as yellow fever, played a role in determining the course of history. The construction of the Panama Canal was delayed by an outbreak of yellow fever among the construction workers. It is said that Napoleon's decision to sell the Louisiana Territory to America so easily and cheaply in 1803 was prompted by great losses his army suffered due to yellow fever.

During the Spanish-American war in 1898 yellow fever ravaged the soldiers and the population in Cuba and the states of the west coast of America. In Washington a commission was formed to investigate the cause of yellow fever, with Walter Reed at the helm with his physician coworkers Aristide Agramonte, James Carroll and the bacteriologist Jesse Lazear. The belief that the disease was spread through the 'black vomit' was widely held, though some people also considered the transmission of the yellow fever through mosquito bites. The members of the commission pledged to let themselves be bitten by mosquitoes which had fed on a patient with active yellow fever. James Carroll was the first to let himself be bitten. He got sick but eventually recovered, not without a likely damage to his heart. Lazear had less luck. Five days after being bitten he became severely ill and died 12 days later. Ultimately, Carroll later identified an ultramicroscopic organism as the virus causing the yellow fever.

There is a long tradition in the art of self-experimentation among physicians and other scientists. Many investigators experimented during the last part of the 19th century with substances such as opium (morphine) and cocaine, curare, nitrous oxide, ether, and chloroform in search for a painkiller for surgical or dental procedures. At those times self-experimentation was not subjected to any controls by governmental or institutional review boards (33). Researchers in the field of infectious or dietary diseases were numerous and were unhampered by restrictions to unneeded and unwanted interventions. Many were free to put themselves in harm's way. It became clear to what extent physicians and scientists were willing to go to try to clarify a long held belief about the nature and mode of transmission of certain diseases.

Though motivations might have been of different kinds, without the dedication and enthusiasm of researchers and without their willingness to take personal risks, our knowledge in medicine could not have advanced to its present state. We now face new kinds of decisions around new kinds of problems at new crossroads when, e.g., the cloning of humans is being considered. But this is beyond what we wish to consider herein.

Our grandfathers in medicine -- Hippocrates (c460-c377 BC), Galen (125-199), Avicenna (980-1037) and in Renaissance times Valerius Cordus (1515-1544) -- were limited in their healing practices, relying mainly on herbal medicines, but surely they must have tasted their herbal concoctions. Isaac Newton (1643-1727), though not a physician, is assumed to have experimented on himself, as his hair contained an unusual amount of mercury, among other substances. Beginning in the 18th century and continuing further in the 19th

and 20th centuries, research in many fields such as chemistry, biology, physiology, bacteriology, pharmacology and physics in the end helped the practicing physician at the bedside or at the office, to make a correct diagnosis and to guide the treatment for the individual patient. Thus, some infectious diseases were mostly conquered, some of them before causative agents, such as bacteria and viruses, were identified. Now we have antibiotics, sulfonamides and vaccines available, while surgery has been made comfortable with advances in the field of anesthesia. We wish to provide examples from the developments in the fields of anesthesia and vaccines to note that self-experimentation and self-surveillance played a major role in what we are able to do today.

Aims. We sketch historical developments leading to anesthesia and follow up on these (today useful) feats, e.g., for surgical cardiac rehabilitation. We conclude, however, that self-surveillance, Table 1, that contributed to the development of Minnesota chronobiology, the study of mechanisms underlying chronomes (time structures), and to chronomics, the mapping of these chronomes may deserve at least equal resources, primarily in developing areas.

The term *anesthesia* was coined by Oliver Wendell Holmes (1809-1894) in an 1846 letter to Dr. William Morton, a pioneer in the use of ether for surgical pain relief. By a history of self-experimentation, we wish to pay respect to the sometimes-sacrificial devotion of a series of leaders in medicine and surgery; they set an example for broader self-surveillance and, if need be, self-experimentation with no pain but perhaps even greater returns.

Here we list the accomplishments of pioneers in anesthesia for those who worry that carrying a blood pressure (BP) and heart rate (HR) monitor may not be fully socially acceptable. They may reconsider contributing immediately to the prevention of their own potential stroke and/or other severe disease and/or into the prevention of such conditions in others, an important achievement in its own right (2).

MILESTONES ON THE PAINFUL WAY TO PAINLESS INTERVENTIONS (E.G., SURGERY)

In 1803, Friedrich Wilhelm Sertürner, an apprentice in a pharmacy in Germany, in looking for different components in opium, isolated the specific pain relieving substance and named it morphine. He was one of the first investigators to apply chemical analysis in pharmacology. (While experimenting he swallowed ten times the amount of morphine now used and had numerous brushes with death.) (3-5)

In 1821, Enoch Hale, chemist and physician at Massachusetts General Hospital in Boston, introduced the intravenous application of drugs, now a standard way to administer medicines, by trying it first with castor oil on himself.

("Having been persuaded, from my observations, and those of others, that some of the milder medicines may be injected into the vein with safety, I resolved to make the experiment upon myself. Accordingly I filled a half ounce measure with cold-pressed castor oil, ... drew into a syringe ... sat down and counted my pulse, and found it to beat 80 times a minute ... opened the median vein by a pretty large orifice; ... He (the assistant) then attempted to introduce the silver tube ... but being a little agitated, he was not able to get the tube into the orifice in the vein ... I took the tube myself, and after several ineffectual trials, which gave considerable pain, I succeeded in introducing it") (6, 7)

In 1844-1845, Horace Wells, dentist, introduced laughing gas - nitrous oxide - into dental practice for its analgesic properties. His official presentation of the use of nitrous oxide during a tooth extraction was unsuccessful as the patient cried out in pain before the

end of the procedure, presumably due to the too early withdrawal of the mask.

Suicidal death of Dr. Wells: In his letters written by himself immediately before his death, he says: "I had, during the week, been in constant practice of inhaling chloroform for the exhilarating effects produced by it; and on Friday evening last I lost all consciousness before I removed the inhaler from my mouth ...". (8). While under the influence of chloroform he cut his left inguinal vein and bled to death.

In 1846, William Morton, dentist, discoverer of surgical anesthesia, used ether in extensive studies on himself and on dental patients, also demonstrating its use on a surgical patient at the Massachusetts General Hospital (9; cf. 10).

(Morton's claim to be the first to apply ether at a surgical operation was heavily contested by Horace Wells and Charles Jackson. It remained a lifelong struggle for him and he never got the award money from the government, which later after the Civil War was withdrawn.) (10)

In 1847, Sir James Young Simpson, obstetrician in Edinburgh (11) (who could claim the chronobiologist-implementer of the chronobra, Hugh Simpson, as his progeny [12]), in his practice preferred chloroform (12; see also 13), discovered 1831 by Eugene Soubeiran in France. Chloroform became popular after it was used in the delivery of the two youngest children of Queen Victoria.

"Latterly, in order to avoid, if possible, some of the inconveniences and objections pertaining to sulphuric ether (particular its disagreeable and very persistent smell, its occasional tendency to irritation of the bronchi during its first inspiration, and the large quantity of it occasionally required to be used, more especially in protracted cases of labor), I have tried upon myself and others the inhalation of different other volatile fluids, with the hope that some one of them might be found to possess the advantages of ether, without its disadvantages. For this purpose, I selected for experiment, and have inhaled several chemical liquids of more fragrant or agreeable odor, such as the chloride of hydrocarbon (or Dutch liquid)... I have found, however, one infinitely more efficacious than any other, viz., Chloroform, or the Perchloride of Formyle, and I am enabled to speak most confidently of its superior anesthetic properties, having now tried it upon upwards of thirty individuals." (11)

In 1883, Sigmund Freud, psychoanalyst, was also recognized as a pioneer of psychopharmacology in Vienna, because of his experiments with cocaine, suggesting its possible medical use as a local anesthetic (14, 15).

In 1884, Richard Hall and William S. Halsted, physicians at Roosevelt Hospital in New York, used cocaine as a local anesthetic by injection through the skin for many operations. By trying to set appropriate doses for the desired effect they self-experimented and both became addicted (16).

In 1886, August Bier, surgeon in Kiel, Germany, with the help of his assistant August Hildebrandt self-experimented, introducing spinal anesthesia, each by injecting cocaine into the other's lumbar spine. (Bier describes extensively the mutual self-experiments with his assistant Dr. Hildebrandt.) (17)

In 1944, Frederick Prescott, physician and chemist, director of the British drug company Burroughs Wellcome, which had isolated curare in pure form (Tubarin), experimented with incremental doses to correlate them with the degree of paralysis. Curare was injected into his arm until a completely paralyzed state was reached without loss of consciousness or sensibility. A second experiment proved the feasibility to combine the i.v. and i.m. modes of application (18).

In 1946, Scott M. Smith, anesthesiologist at the University of Utah Medical School, not

aware of Prescott's experiments, self-experimented in a similar way and combined initial i.v. application of the drug with i.m. injections, demonstrating that curare could be given also in this manner. Neostigmine was available to be used as the antidote (19).

THE FIRST VACCINES: SMALLPOX AND RABIES

In the development of vaccines their safety was confirmed by the self-experimentation of the researchers. A first self-experimenter, however, is no more than an example of how far, in the pursuit of science, more than 2 centuries ago, inquisitive minds went, to seek an answer to their question at a time when no Institutional Review Board (IRB) had to be asked for permission (33).

In 1767, John Hunter (1728-1793), a teacher and friend of Edward Jenner and a well respected and esteemed physician and surgeon to King George III, inoculated himself with pus from a patient with gonorrhea, to find an answer to the question of the mode of transmission of gonorrhea. He not only got the disease but at the same time also infected himself with syphilis since the patient had both infections. John Hunter presumably died with syphilis at the age of 65.

In 1796 Edward Jenner (1749-1823), though he did not test his smallpox vaccine on himself (it is said that he had suffered from a case of smallpox in his youth) inoculated an 8-year old youngster with cowpox. The youngster came down with cowpox and recovered. Jenner then inoculated him with smallpox and repeated it twenty times: the boy stayed healthy.

It was nearly 150 years later, only after the availability of the electron microscope, that the actual causative micro-organism could be identified -- at a time when, through the use of the vaccinations, the disease had almost but disappeared.

In 1880 Louis Pasteur (1822-1895), chemist and physicist, at the age of 58, began his work on a vaccine against rabies. Pasteur never tried the vaccine on himself. On several occasions, when he was ready to self-test the vaccine, he was kept from doing so by his collaborators. His first successful human trial in 1885 of his vaccine was on a 9-year old boy who was bitten by a rabid dog. The boy received 17 injections of increasing strength and did not get sick.

The Pasteurian Club consisted of a loose group of researchers, contemporaries of Pasteur, in search of vaccines for different communicable diseases, who were also committed to try the vaccine first on themselves, mainly to examine its tolerance:

– In 1903 Romuald Nitsch in Poland injected himself with his own version of the rabies vaccine into the skin of his abdomen with no ill effects.

– In 1945 Harold N. Johnson of the Rockefeller Foundation passed the Flury strain (which he recovered from the spinal cord of a girl named Flury, who died from rabies) 136 times through chicks, assuming that there was a lesser likelihood of allergic reactions, rather than with passes through mammalian tissue.

– Also in 1945, Hilary Koprowski, a virologist at Lederle Laboratories, started trying to find a vaccine for rabies which had to be given only as a single injection. Using the Flury strain he got from Johnson and after 180 passes through chick eggs, the strain had lost its ability to infect, yet produced antibodies in large amounts.

– While in South America, Koprowski also worked on a vaccine for Colorado Tick Fever (Malta fever) which he tried on himself and his colleagues and proved the vaccine to be effective against the disease, he and 5 of his collaborators had afterwards exposed themselves to the bite of the mosquito.

– In 1957 Koprowski, then director of the Wistar Institute, developed the Wistar strain WI-38 of killed rabies virus grown on human fetal cells (HDCV-human diploid cell vaccine). About 1960, after the development of the electron-microscope, the rabies virus finally could be identified, 80 years after Pasteur started to seek a vaccine.
– In 1971 Koprowski himself and his co-workers test-tried the new single dose vaccine against rabies, almost a century after Pasteur started his work on the rabies vaccine. The first test of the effectiveness of the vaccine was caried out in Germany on 42 people, bitten by rabid dogs; all survived.

CHOLERA, PLAGUE AND TYPHUS VACCINE

In 1884 James Ferran y Clua developed a cholera vaccine, tested it on himself and on a co-worker but it failed in an epidemic due to its relative crudeness.

In 1892 Waldemar Mordecai Haffkine (1860-1930) was injected with cholera vaccine to test himself first, had only minor side effects. In 1897 he developed a vaccine against plague. He got sick with fever after trying it on himself. The wider use of the vaccine in India was quite successful.

In 1893 Almroth E. Wright (1861-1947) of St. Mary's Hospital in London developed a typhoid vaccine, from killed bacteria, using it first on himself.

In 1897. Almroth E. Wright developed a killed vaccine against Brucellosis (Malta fever) and first tried it on himself; he then exposed himself to live Brucella bacteria and got sick. The vaccine was not strong enough to protect him. (Wright was a well-known teacher and scientist "who was later nicknamed 'Almost' Wright when his theories led to far more significant discoveries made by his assistant Alexander Fleming" [20]. He was a friend of Bernard Shaw who writes about his conversations with him: "we move from one intellectual category to another, and obviously keener one").

SHIFT IN FOCUS

As epidemics, such as cholera, typhoid, typhus and others, seem to be largely under control, new infections do appear and other afflictions come to the forefront. Currently one of the most urgent tasks to focus upon is heart disease, which, with obesity, appears to be the new epidemic, where self-experimenting and self-surveillance come into play and are the most urgent requirements from the individual. Over the last half-century numerous research projects have been developed and executed and still continue today in this field.

Werner Forssmann (1904-1979) was an early pioneer in modern cardiac research. As a young graduate in medicine from the University of Berlin during his internship in Eberswalde, he had the idea that one could try to enter the heart by pushing a catheter into a vein, as he had seen done to a horse. At the time it was believed that touching the heart would mean immediate death. Forssmann did not get the ahead from his supervisor but then tried successfully to interest a nurse to help him with his project. He inserted a catheter, generally used for catheterizations of the bladder, into his cubital vein and pushed it further as far as he could into the right atrium. To document this he went to take an x-ray picture, which showed the position of the tip of the catheter. In order the see himself on the screen he had a mirror held in front of him. When he found resistance from a colleague, who thought he was out of his mind and tried to stop him, Forssmann kicked him in the leg to get him out of the way.

When all was done, the chief surgeon, Dr. Schneider, recognized the importance of what Forssmann had done and was cooperative further on. Forssmann tried also the injection of radiopaque material (he used sodium iodide) into the catheter to be able to outline the different chambers of the heart, but was not too successful. Nevertheless, he was the first investigator to try angiography on humans. His paper, published in 1929, found the interest of a famous surgeon in Berlin, Sauerbruch, who offered him a place in Berlin, but he did not like it there, was fired and went back to Eberswalde. He did the heart catheterization on himself 17 times in all, using his leg veins when his elbow veins were scarred. He received the Nobel Prize for Physiology or Medicine in 1956 sharing it with 2 Americans, André Cournand and Richard Dickinson, who had read Forssmann's paper and started experimenting on dogs and chimpanzees in 1936. In 1940 they did their first experiment on a human, a patient dying of cancer in New York at Columbia-Presbyterian Medical Center. Neither of the two physicians did self-experimentation. It haunted Cournand for the rest of his life.

Not all of the self-experimenters lived a full life. A few succumbed to their self-inflicted disease by trying to find its secret, whether infections (as in the case of Jesse Lazear, already discussed in connection with yellow fever) or nutritional deficiency. The pursuit of finding the cause of scurvy dates back 250 years. A British ship's physician, James Lind (1716-1794) published "A treatise on Scurvy" in 1753, proclaiming that it could be prevented and/or cured with citrus fruits and vegetables but still included meteorological reasons as being necessary to acquire scurvy. Some years later a young physician in London, William Stark, experimented with different diets. He inflicted himself with scurvy by taking only bread and water. At another time he ate only flour, oils, bred and honey for lengthy spans of time, developing gradually the symptoms of scurvy. Obviously unaware of Lind's book, he continued with his diet and continued with his diet. He died from scurvy less than 9 months after he started his self-experiment.

More examples of daring and of enterprising spirit for the betterment of the world:
– John Paul Stapp (1910-1999), an Air Force physician, tested human tolerance limits to crest forces on fast track and rocket sled rides, reaching a peak velocity of 639 miles/hour in 5 seconds then slammed to a stop in 1.5 sec, withstanding pressure almost 40 times his own weight. He made his first rocket- sled run in 1947. He continued with ever greater g-forces and sudden stops to see, how sudden a deceleration a human can withstand and doing this hundreds of times over 10 years.
– During wartime in 1943, William Lovelace II, a surgeon and Army Lieutenant Colonel, tried out high altitude escape. He jumped from a bomber at an altitude of 40,200 feet, trying out his emergency oxygen container. He was knocked unconscious by the opening of the parachute in 40 degree below zero, lost his glove, his hand being instantly frostbitten. He regained consciousness somewhere during his descent and landed in a wheat field 23 minutes later with a wrenched back. Following his experiment a delayed automatic opening device for parachutes was developed.

Experimentation on humans was grossly abused during World War II in the Nazi concentration camps. At the Nuremberg Trial there were no guidelines as yet of how to judge and punish. Out of this situation developed the Nuremberg code by a then newly created international military tribunal. Ever since human experimentation has been welcome, grant money is given out for it yet, academic advancement often depends upon it and so does many scientists' livelihood, with strict supervision and rules of conduct to a degree sometimes more restrictive than useful and necessary.

PURKINJE

Against this background, we turn to THE local hero, one of Bohemia's most famous sons, Jan Evangelista Purkinje. Purkinje's scientific bent showed early while working at the Golden Crown pharmacy in Prague. His trials with ipecacuanha and emetine were well known and widely discussed in his time. He swallowed camphor and turpentine and reported on his reaction in great detail. Well known today are the morphological structures carrying his name, and for as long as histology matters, they are a document of his broad contributions that reach beyond his self-experimentations: cells in the cerebellar cortex and also certain conducting fibres in the heart are known by his name.

Purkinje's most discussed self-experimentation is with digitalis. He describes the effect of an overdose of digitalis which he concocted with leaves of digitalis purpurea (2 drams to half a quart, boiled for half an hour) and further the toxic effects of nausea and vomiting, slow heart rate with many dropped beats, cardiac distress and feeling of oppression with each beat. The following day he noticed flickering in his eyes, which lasted up to the fifteenth day. He denied any affection of the brain, as he had stated to have during his experiments with opium and camphor. Experiments like that were certainly not discouraged. It was an environment when no Institutional Review Board had to be asked to give its opinion and its approval or disapproval (33). Thus we know a lot on account of Purkinje's inquisitive nature. His self-experimenting stopped, however, after his wife died (1835) and he had to look after his two sons.

Johann Wolfgang von Goethe (1749-1832) gave his young friend Purkinje a high approval rate: "It was remarkable to me how he rose from the abyss of clericalism relying on his own spiritual strength, a self-educated man, concentrating himself on his inner being, and undergoing voluntary martyrdom to acquire knowledge by studying his own self, in details and generally, just to get to understand himself".

Another role model is provided by the story of Santorio Santorio (1561-1636) as an example of his sense of purpose, dedication and perseverance. For 30 years Santorio calculated the difference between the amount of food he had consumed and the subsequent excretions while sitting on a homemade scale and so established the concept of insensible loss through the skin. He set an example of how long a dedicated and motivated individual is willing to continue to learn about his physiology.

Minnesotan chronobiology and chronomics, for ourselves first and foremost

For his kind of self-experimentation Purkinje was right to stop after his wife died. Not so for self-surveillance and self-experimentation in one's own health care. The older one gets, the more self-surveillance and self-experimentation are recommended. Santorio's endeavor was unsurpassed until Robert Sothern in Minnesota, who is now in his 37th year of self-testing eleven of his psychophysiological variables, including his BP and HR ~5 times/day, a new record, surpassing many others listed in Table 1. With our increasing knowledge in the field of chronobiology we have come to realize that, as all physiological processes are rhythmic, the timing of an intervention has to be considered also, as many of Minnesotan researchers have documented, those in the footnote to table 1 in particular.

By comparison with the history of anesthesia, self-surveillance for prehabilitation is neither life-threatening nor painful (2). Avoiding a massive, incapacitating stroke is just as useful an aim today as the avoidance of pain was in a figurative yesterday. As shown in Table 1, chronomics seeks, with basic knowledge, also harbingers and preventive countermeasures. Thereby, it tries to avoid the need for rehabilitation and extensive surgical intervention, too frequent today, albeit fortunately performed with anesthesia. In

the interim, extensive research on experimental animals has also documented that the same dose of an anesthetic can be survived or is lethal only as a function of timing (21-25). These findings as yet have no clinical application, but may be considered, e.g., when the dose of an anesthetic may have to be reduced.

With increasing knowledge in the field of chronobiology, however, we have to come to realize that most physiological processes examined have a time structure, irrespective of any merit from the timing of an intervention, such as exposure to an anesthetic. The task on hand is the penetration of the normal range by appropriate surveillance so that problems are recognized and countermeasures are instituted, the topics presented elsewhere (26-28).

In summary, against the backgrounds of the development of anesthesia, including a suicidal example, or of vaccines, it is much easier to practice self-surveillance in the service of

1. oneself, for one's health care, notably prehabilitation instead of rehabilitation (2); also in the service of
2. general health care by providing one's accumulating physiological or pathological data that can be mined and can be aligned with outcomes from others also contributing data, while the individual is alive and may benefit from the epidemiological results; and, thus, we are led to
3. the establishment of new kinds of archives for transdisciplinary science as well as health, all in one, proposed to the World Health Organization and national governments, since already some of the individual's time series are (if not dense enough) long enough to be aligned with dense and long physical environmental data that are monitored for centuries on earth and now also for decades via satellites (29, 30). The corresponding human monitoring becomes possible with automatic recorders and leads to new discoveries, not only in human (chrono) epidemiology (31) but in broader physical and other transdisciplinary science (2). This last kind of service resulted in the recent discovery of the biological transyear, namely of an ~1.3 year in human blood pressure and heart rate, that is statistically significantly longer than one calendar year and shorter than 2 calendar years (32). Chronobiology, of course, also relies heavily on laboratory animals that often "go first", e.g., in relation to temporal aspects of anesthesia (21-25) and of immunology more broadly. We conclude by concurring with Alexander Pope (1688-1744) that "the proper study of Mankind is Man", and that the only critical measures of an individual are those of that given individual, not spotchecks but time series, of necessity since variability is the essence of life, to be resolved in scientific health care cost-effectively by self-surveillance and self-experimentation.

REFERENCES

1. Altman LK. Who Goes First? The Story of Self-Experimentation in Medicine. Berkeley: University of California Press; 1987 (new preface 1998). 430 + xxvi pp.
2. Halberg F, Cornélissen G, Schack B. Self-experimentation on chronomes, time structures; chronomics for health surveillance and science: also transdisciplinary civic duty? Behavioral and Brain Sciences, http://www.bbsonline.org/Preprints/Roberts/Commentators/Halberg.html
3. Hanzlik PJ. 125th anniversary of the discovery of morphine by Sertürner. J Am Pharm Assn 1929; 18: 375-384.
4. Macht DI. The history of opium and some of its preparations and alkaloids. J Am Med Assoc 1915; 64: 477-481.
5. Sertürner FWA. Darstellung der reinen Mohnsäure (Opiumsäure), nebst einer chemischen Untersuchung des Opiums. J Pharmacol 1806; 14: 47-93.
6. Hale E. Boylston medical prize dissertations for the years 1819 and 1821: Experiments and observations on the communication between the stomach and the urinary organs, and On the propriety of administering medicine by injection into the veins / By E. Hale, jun. Boston: Oliver Everett and Joseph W. Ingraham; 1821. p. 79.
7. Hale E. New England J of Medicine and Surgery 1822; 11: 163-175.
8. Death of Dr. Horace Wells - Reprint of newspaper comments. Boston Med Surg J 1848; 38: 24-25.
9. Morton WTG. Remarks on the proper mode of administering sulphuric ether by inhalation. Boston. Dutton and Wentworth, 1847. 44 pp.
10. Woll D. The discovery and controversy over the first use of surgical anesthesia. http://www.gotessays.com/print/458/index.php
11. Clendening L. Source Book of Medical History. New York: Hoeber; 1942. p. 373-377.
12. Simpson HW. Sir James Young Simpson Memorial Lecture 1995. Breast cancer prevention: a pathologist's approach. J R Coll Surg Edinb 1996; 41: 359-370.
13. Simpson EB. Sir James Simpson's introduction of chloroform: By his daughter. The Century (New York) 1894; 47: 412-421. Cornell University Library, Making of America: http://cdl.library.cornell.edu/cgi-bin/moa/moa-cgi?notisid=ABP2287-0047-109
14. Freud EL (ed.). Letters of Sigmund Freud. New York: Basic Books; 1960. Letter 43, April 21, 1884.
15. Freud S. Über Coca. St. Louis Med Surg J 1884; 47: 502-505.
16. Penfield W. Halsted of Johns Hopkins. J Am Med Assn 1969; 210: 2214-2218.
17. Bier A. Versuche über Cocainisierung des Rückenmarkes. Dtsch Zschr Chirurgie 1899; 51: 361-369.
18. Prescott F, Organe G, Rowbotham S. Tubocurarine chloride as an adjunct to an anesthesia. Lancet 1946; ii: 80-84.
19. Smith SM et al. The lack of cerebral effects of d-tubarine. Anesthesiology 1947; 8: 1-14.
20. Andrews JK. The playwright's prescription. The Shakespeare Theatre, ©1999-2003. http://www.shakespearedc.org/pastprod/docessay.html
21. Marte E, Halberg F. Circadian susceptibility rhythm of mice to librium. Fed Proc 1961; 20, 305.
22. Matthews JH, Marte E, Halberg F. A circadian susceptibility-resistance cycle to fluothane in male B1 mice. Canad anesthesiol Soc J 1964; 11: 280-290.
23. Matthews JH, Marte E, Halberg F. Fluothane toxicity in mice studied by indirect periodicity analysis. In: Fink BR, ed. Toxicity of Anesthetics. Baltimore: Williams and Wilkins; 1968. p. 197-208.
24. Marte E, Nelson DO, Matthews JH, Halberg F. Circadian rhythm in murine susceptibility to anesthetics halothane and methohexital. Proc Int Symp Clin Chronopharmacol, Chronotherapeutics and Chronopharmacy, Tallahassee, Florida, February 9-12, 1978. p. 25-26 (abstract).
25. Marte E, Nelson DO, Halberg F, Matthews JH. Circadian rhythms in murine susceptibility to the anesthetics halothane and methohexital. In: Walker CA, Winget CM, Soliman KFA, editors.

Chronopharmacology and Chronotherapeutics. Tallahassee, Florida: Florida A & M University Foundation; 1981. p. 89-94.

26. Halberg F, Cornélissen G, Carandente F. Chronobiology leads toward preventive health care for all: cost reduction with quality improvement. A challenge to education and technology via chronobiology. Chronobiologia 1991; 18: 187-193.
27. Halberg F, Schwartzkopff O, Cornélissen G, Katinas G, Wang ZR, Bakken EE. Greetings on Bohumil Fiser's 60th birthday, to be followed by many added timely, healthy, and hence chronomic, reciprocal years and trans-years, resolved as simply as possible, but not simpler: Salutationes ad sexagesimum diem natalem in multos annos et trans annos adjunctos tempestive sanos -- et ergo chronomicos, quam simplicissime sed non simplicius. This volume.
28. Cornélissen G, Halberg F, Bakken EE, Wang ZR, Tarquini R, Perfetto F, Laffi G, Maggioni C, Kumagai Y, Prikryl P, Homolka P, Dusek J, Siegelova J, Fiser B. Can society afford not to follow a chronobiological approach to blood pressure screening, diagnosis and treatment? This volume.
29. Cornélissen G, Halberg F, Breus T, Syutkina EV, Baevsky R, Weydahl A, Watanabe Y, Otsuka K, Siegelova J, Fiser B, Bakken EE. Non-photic solar associations of heart rate variability and myocardial infarction. J Atmos Solar-Terr Phys 2002; 64: 707-720.
30. Halberg F, Cornélissen G, Otsuka K, Watanabe Y, Katinas GS, Burioka N, Delyukov A, Gorgo Y, Zhao ZY, Weydahl A, Sothern RB, Siegelova J, Fiser B, Dusek J, Syutkina EV, Perfetto F, Tarquini R, Singh RB, Rhees B, Lofstrom D, Lofstrom P, Johnson PWC, Schwartzkopff O, International BIOCOS Study Group. Cross-spectrally coherent ~10.5- and 21-year biological and physical cycles, magnetic storms and myocardial infarctions. Neuroendocrinol Lett 2000; 21: 233-258.
31. Halberg F, Cornélissen G, Sothern RB, Wallach LA, Halberg E, Ahlgren A, Kuzel M, Radke A, Barbosa J, Goetz F, Buckley J, Mandel J, Schuman L, Haus E, Lakatua D, Sackett L, Berg H, Wendt HW, Kawasaki T, Ueno M, Uezono K, Matsuoka M, Omae T, Tarquini B, Cagnoni M, Garcia Sainz M, Perez Vega E, Wilson D, Griffiths K, Donati L, Tatti P, Vasta M, Locatelli I, Camagna A, Lauro R, Tritsch G, Wetterberg L. International geographic studies of oncological interest on chronobiological variables. In: Kaiser H, editor. Neoplasms-Comparative Pathology of Growth in Animals, Plants and Man. Baltimore: Williams and Wilkins; 1981. p. 553-596.
32. Cornélissen G, Masalov A, Halberg F, Richardson JD, Katinas GS, Sothern RB, Watanabe Y, Syutkina EV, Wendt HW, Bakken EE, Romanov Y. Multiple resonances among time structures, chronomes, around and in us. Is an about 1.3-year periodicity in solar wind built into the human cardiovascular chronome? Human Physiology, in press.
33. Hendricks M. Do unto yourself. Johns Hopkins Magazine , 1996 (April issue).
http://www.jhu.edu/-jhumag/496web/yourself.html

Table 1: Illustrative (very incomplete; see footnote) self-surveillance with groups, family or singly as a basis for experimentation, as need be for health care and for science*

Table 1: Illustrative (very incomplete; see footnote) self-surveillance with groups, family or singly as a basis for experimentation, as need be for health care and for science*

Investigator	Year (reference)	Comments
Franz Halberg (FH) with 12 medical and 4 graduate students**	1951 [a]	Blood drawn every 90 minutes around the clock on 2 days for blood cell counts, validated as an indirect circadian rhythmic gauge of the adrenal cycle (by added studies on patients after gland removal and substitution treatment
Erna † (EH), Francine, Julia Halberg & FH	Since 1950s [b-m]	Self- or automatic measurements of core temperature (CT), blood pressure (BP) and heart rate (HR), CT via rectal probes worn for years, mapping circadian and circaseptan spectra, leading to rules of phase-shifting rhythms of several frequencies; transmeridian dyschronism [b]; changes in relation to menarche and other age effects [c, d]
EH †		Harbinger: change in circadian CT acrophase, hours before the occurrence of fever in a nosocomial infection [e]
EH †		Timing cancer chemotherapy by marker rhythms prolonged life by several years [f], according to the founder of the subspecialty of oncology in the USA [g]
EH †		HR-MESOR elevation harbinger years before the diagnosis of an ovarian cancer [h]
FH		Changing R_x to Lisinopril yields cusum-validated BP lowering [i, j]
		Changing timing of Diltiazem 240 mg from 08:30 to 04:30 lowered blood pressure MESOR ($P<0.001$) from individualized parameter test and CUSUM [i]
FH	1995 [k, l]	CHAT (circadian hyper-amplitude-tension) was eliminated (as checked for >1 month) by reducing sotalol dose (from 160 to 80 mg) and timing 80 mg in the morning [k, l]
FH	2003 [m]	During a worrisome span of his wife's hospitalization (heart surgery), CHAT was eliminated for >1 month by switching Flomax from the evening to the morning [m]. Months later, during a relatively unworrisome span, as earlier, Flomax in the evening was not connected with CHAT
Robert B. Sothern with parents	1967-present [n]	Starting at 21 years of age, mapped systolic, mean arterial and diastolic BP, HR, R rate, PEF, CT, 1-minute estimation, eye-hand coordination, mood and vigor about 5 times a day, continuing in 37th year. CT measured for 19 years by parents
Günther & Knapp with FH	1969 [o]	10 clinically healthy students mapped circadians of CT, HR, respiration (R), HR/R, BP, peak expiratory flow (PEF), dynamometry (right/left), body weight, urinary variables, bead stringing (right/left), and 2-minute estimation
Howard Levine †	1969-1981 [p]	Self-measured blood pressure several times/day beginning at age 53 and continuing until the day of his death from amyotrophic lateral sclerosis
Frederic C. Bartter †	1973-1983 [q]	Self-measured blood pressure beginning at age 59 and continuing until his fatal stroke
Germaine Cornélissen and parents	1975-present [r-v]	Multiple studies involving self-monitoring of BP, HR, ECG, temperature, other vital signs, hormones, cancer markers and other variables, in blood, urine and saliva
Yoshihiko Watanabe and family	1987-present [w]	BP and HR mapping at 0.5-h on himself and for shorter spans on family
George Katinas	1998-present [x]	Half-hourly BP and HR mapping in MESOR-hypertensive circadian hyper-amplitude-tension at 0.5-h intervals, continuing for >5 years
Gen Mitsutake	2001 [y]	Self-monitoring of BP, HR, ECG and mood
Anatoly Delyukov	2001 [z]	ECG monitoring for 50 days & then for 70 days

*Separate studies on themselves and/or others were set up independently or with us, among many others, by Christian Hamburger †, Erhard Haus, Marianne Haus †, Nathaniel Kleitman † and his family, Yuji Kumagai and his family, Nelson and Mirian Marques and their family, Gordon Northrup, Kuniaki Otsuka, Ana Portela Alvarez, Alain Reinberg, Miguel Revilla and his family, Hugh Simpson, Michael Smolensky, Keiko Uezono, and especially Eugene A. Kanabrocki (with Lawrence E. Scheving † who also studied mitoses on the skin of his own back), and mapped, from 1969 to the present, nearly 200 different variables, many now routines in the clinical laboratory, many variables on repeated occasions as a function of age. Interested readers are directed to these senior authors. Further self-surveillance/self-experimentation is found among the titles in the bibliography of FH on his website (http://www.msi.umn.edu/~halberg/)

**K. Berge †, R. Borreson, B. Clark, J. Huff, V. Kuhlman, G. Merkel, A. Nisswandt, J. Norquist, M. Robbins, G. Schabacker, J. Trautmann, L. Wood

† deceased

a. Halberg F, Visscher MB, Flink EB, Berge K, Bock F. Diurnal rhythmic changes in blood eosinophil levels in health and in certain diseases. Journal-Lancet (Mpls) 1951; 71: 312-319.

b. Halberg F. Chronobiology. Annu Rev Physiol 1969; 31: 675-725.

c. Halberg E, Halberg Francine, Halberg J, Halberg F. Forging chronobiology and pediatrics as well as geriatrics: a birthday greeting for Theodor Hellbrügge. Int J Chronobiol 1979; 6: 135-143.

d. Halberg E, Halberg J, Halberg Francine, Sothern RB, Levine H, Halberg F. Familial and individualized longitudinal autorhythmometry for 5 to 12 years and human age effects. J Gerontol 1981; 36: 31-33.

e. Halberg E, Fanning R, Halberg F, Cornélissen G, Wilson D, Griffiths K, Simpson H. Toward a chronopsy: Part III. Automatic monitoring of rectal, axillary and breast surface temperature and of wrist activity; effects of age and of ambulatory surgery followed by nosocomial infection. Chronobiologia 1981; 8: 253-271.

f. Halberg F, Cornélissen G, Bingham C, Fujii S, Halberg E. From experimental units to unique experiments: chronobiologic pilots complement large trials. in vivo 1992; 6: 403-428.

g. Kennedy BJ. A lady and chronobiology. Chronobiologia 1993; 20: 139-144.

h. Halberg E, Cornélissen G, Halberg Francine, Halberg J, Halberg Franz: Heart rate monitoring as a gauge of cardiotoxicity guides individualized chronochemotherapy. In: Proc. XX Int. Conf. Chronobiol., Tel Aviv, Israel, June 21-25, 1991, p. 15.23.

i. Halberg F, Cornélissen G, International Womb-to-Tomb Chronome Initiative Group: Resolution from a meeting of the International Society for Research on Civilization Diseases and the Environment (New SIRMCE Confederation), Brussels, Belgium, March 17-18, 1995: Fairy tale or reality ? Medtronic Chronobiology Seminar #8, April 1995, 12 pp. text, 18 figures. URL [accessed 1994] http://www.msi.umn.edu/~halberg/

j. Cornélissen G, Halberg F, Hawkins D, Otsuka K, Henke W. Individual assessment of antihypertensive response by self-starting cumulative sums. J Medical Engineering & Technology 1997; 21: 111-120.

k. Halberg F, Cornélissen G, Otsuka K, Katinas G, Schwartzkopff O. Essays on chronomics spawned by transdisciplinary chronobiology: Witness in time: Earl Elmer Bakken. Neuroendocrinol Lett 2001; 22: 359-384.

l. Cornélissen G, Halberg F, Otsuka K, Shinagawa M, Kubo Y, Ohkawa S, Siegelova J. Iatrogenic CHAT (excessive blood pressure variability) within the acceptable range of BP values: implications for chronotherapy? Abstract 9, MEFA 9th International Fair of Medical Technology and Pharmacy, Brno, November 6-11, 2001.

m. Katinas G, Halberg F, Cornélissen G, Otsuka K, Tarquini R, Perfetto F, Maggioni C, Schwartzkopff O, Bakken E. Transient circadian hyper-amplitude-tension (CHAT) may be intermittent: case reports illustrating gliding spectral windows. Biomed Pharmacol, in press.

n. Sothern RB, Halberg F. Longitudinal human multifrequency structure of blood pressure self-measured for over 2 decades. Proc. 2nd Ann. IEEE Symp. on Computer-Based Medical Systems, Minneapolis, June

26-27, 1989. Washington DC: Computer Society Press; 1989. p. 288-294.

o. Günther R, Halberg F, Knapp E. Circadiane Rhythmometrie mittels elektronischer Rechner zur Beurteilung von Kurwirkungen, Kurverlauf und Kurerfolgsbeurteilung. In: Teichmann W, editor. Symposium II. Bad Wörishofen: Sanitas-Verlag; 1968. p. 106-111.

p. Levine H, Halberg F. Circadian rhythms of the circulatory system. Literature review. Computerized case study of transmeridian flight and medication effects on a mildly hypertensive subject. U.S. Air Force Report SAM-TR-72-3. Brooks AFB, Texas: USAF School of Aerospace Medicine, Aerospace Medical Division (AFSC); April 1972. 64 pp.

q. Bartter FC, Delea CS, Baker W, Halberg F, Lee JK. Chronobiology in the diagnosis and treatment of mesor-hypertension. Chronobiologia 1976; 3: 199-213.

r. Cornélissen Guillaume G, Halberg F, Fanning R, Kanabrocki EL, Scheving LE, Pauly JE, Redmond DP, Carandente F. Analysis of circadian rhythms in human rectal temperature and motor activity in dense and short series with correlated residuals. In: Gautherie M, Albert E, editors. Biomedical Thermology, Strasbourg, June 30-July 4, 1981. New York: Alan R. Liss; 1982. p. 67-184.

s. Cornélissen G, Carandente F, Halberg F. Circadian and circaseptan periodicity in differential skin surface temperature automatically monitored by polychronor. In: Halberg F, Reale L, Tarquini B, editors. Proc. 2nd Int. Conf. Medico-Social Aspects of Chronobiology, Florence, Oct. 2, 1984. Rome: Istituto Italiano di Medicina Sociale; 1986. p. 271-287.

t. Cornélissen G, Carandente F, Halberg F, Norgren P, Phansey S, Tagatz G. Circatrigintan hormonal and skin surface temperature rhythms following hysterectomy. Progress in Clinical and Biological Research 1987; 227A: 493-505.

u. Halberg F, Cornélissen G, Halberg E, Halberg J, Delmore P, Shinoda M, Bakken E. Chronobiology of human blood pressure. Medtronic Continuing Medical Education Seminars, 4th ed. Minneapolis: Medtronic Inc.; 1988. 242 pp.

v. Cornélissen G, Masalov A, Halberg F, Richardson JD, Katinas GS, Sothern RB, Watanabe Y, Syutkina EV, Wendt HW, Bakken EE, Romanov Y. Multiple resonances among time structures, chronomes, around and in us. Is an about 1.3-year periodicity in solar wind built into the human cardiovascular chronome? Human Physiology, in press.

w. Watanabe Y, Cornélissen G, Halberg F, Otsuka K, Ohkawa S-I. Association by signatures and coherences between the human circulation and helio- and geomagnetic activity. Biomedicine and Pharmacotherapy 2001; 55 (Suppl 1): 76s-83s.

x. Katinas G, Cornélissen G, Halberg F. Chronomicro- macroscopy of circadian blood pressure waveforms in CHAT by spectra with plexograms fitted by moving polynomials. This volume.

y. Mitsutake G, Otsuka K, Cornélissen G, Herold M, Günther R, Dawes C, Burch JB, Watson D, Halberg F. Circadian and infradian rhythms in mood. Biomedicine and Pharmacotherapy 2001; 55 (Suppl 1): 94-100.

z. Delyukov A, Gorgo Y, Cornélissen G, Otsuka K, Halberg F. Infradian, notably circaseptan testable feedsidewards among chronomes of the ECG and air temperature and pressure. Biomedicine and Pharmacotherapy 2001; 55 (Suppl 1): 84-92.

SYNCHRONIZATION AND SYMMORPHOSIS

Thomas Kenner

Department of Physiology, Medical University in Graz

Address correspondence to:
Prof. Dr. Thomas Kenner
Physiologiches Institut der Karl-Franzens-Universität Graz
Harrachgasse 21
A-8010 Graz

SUMMARY

Nearly all biological variables have the trend to oscillate. This phenomenon appears to be essential for the optimal adjustment and therefore, for the effectivity of control. There seems to be an optimal magnitude of the oscillation amplitude. Missing oscillations or low amplitudes are signs of disease as well as increased amplitudes. Biological oscillators tend to synchronize. There are indications that in biological systems parameters as well as variables are adjusted according to rules, which include adaptation as well as optimization. In an outlook the behavior of large complex biological systems like universities is considered.

REPORT AND ANALYSIS

It is well known that the term synchronization is composed of the Greek words syn (together) and chronos (time). In contrast to the later mentioned Greek word symmorphos (similar in shape), a combined word synchronos was not used in old Greek language.

The observation of time and the attempt to interpret the meaning of time goes certainly back to early human thought. The names of Augustinus, Newton, Kant, Mach and Einstein have to be mentioned. In the attempt to measure time, the control of ship-navigation played an important role. Here the term synchronicity plays a role in the sense to synchronize a clock on a ship with the clock at home on land. This is necessary to determine location and course of a ship. One special attempt to synchronize clocks is attributed to the Spanish Habsburg emperor Karl 5 th (1500 - 1558). In his last 2 years of retirement in San Yuste he attempted in vain to synchronize the hands and the ringing of several clocks. The first experiment on the synchronization of the pendulum swing of two clocks which were hanging on a wooden stick, has been performed by Christiaan Huygens (1629 - 1695). The question of measuring time, synchronization of clocks and the role of the speed of information transfer was the basic question in the development of the theory of relativity (1).

In physics and engineering, nonlinear systems which are loosely coupled have a trend

to synchronize (2). Since biological systems use to be nonlinear it is not surprising that many synchronization phenomena can be observed in living organisms and also between living systems and external oscillations.

An interesting additional aspect of the Greek term synchronous, which should be mentioned here, is the phenomenon of simultaneously related phenomena in psychology. Carl Gustav Jung put forward a new concept which he called synchronicity. This term means a "meaningful coincidence" of outer and inner events, which are themselves causally connected. The emphasis lies on the word "meaningful" (3).

It is evident that synchronous variation of two variables does not necessarily imply synchronization. Furthermore it is important to note that there is no synchronization without oscillations in autonomic systems (2). This means, that each of two synchronized systems has to be autonomous and to generate self sustained oscillatons.

The following question is of interest: Is in biology the phenomenon of synchronization an accidental effect like the synchronization of Huygens' two pendulum clocks? Or has the phenomenon of synchronization a biological function?

Erich von Holst has studied the synchronization of the movement of fins in fishes and of extremities in mammals (4). He called the observation of synchronized movement "coordination". The obvious goal of coordination is the propagation of the animal. Since each fin or each extremity can work as independent oscillator it seems justified to speak of synchronization in the sense of the description by Pikovsky and Rosenblum (2). In contrast, pathologically generated oscillations like tremor in Parkinsons' disease may lead to synchronized movements of both legs (4) without any biological advantage. There are other examples of pathological movements like "cramped synchronized" movements in babies with cerebral lesions, described by H. Prechtl (5), which also have no apparent biological meaning except that they are signs of neural dysfunction.

The heart beat is easy measurable by feeling the arterial pulse or by recording the EKG. The periodic contraction of the heart is basically generated by a so called "integrate-and-fire-oscillator" (2) and is influenced by the interaction with a surprising number of internal, and a variety of external rhythms. The same is true for the respiration, which is controlled by an autonomous neural oscillator. The heart rate under normal conditions varies depending on the respiration rate. Therefore, an interrelation between the two rhythms is existing.

We have studied in 1976 the synchronization between heart beat and respiration in anesthetized rabbits (6). By using a description which is now called synchrogram we could nicely demonstrate time sequences of synchronization, desynchronization and entrainment. Entrainment is the process of striving of one oscillator for synchronization. Interestingly, from time to time the animal took a deep sigh followed by a change of synchronization. Later we recorded similar phenomena in babies and in adults under different conditions.

In summary there are three important phenomena which are of particular interest. 1) Nearly all biological variables have the trend to oscillate. This phenomenon appears to be essential for the optimal adjustment and therefore, for the effectivity of control. There seems to be an optimal magnitude of the oscillation amplitude. Missing oscillations or low amplitudes are signs of disease as well as increased amplitudes. 2) Biological oscillators tend to synchronize. 3) There are indications that in biological systems parameters as well as variables are adjusted according to rules, which include adaptation as well as optimization. The magnitude of biological variables in animals of different size (weight), can be described statistically by so-called "allometric" functions of the body mass. These

functions describe a phenomenon which in physiology is called "biological similarity". In particular, concerning time, it appears that each organism has its own system time, again depending on body mass. Time periods of heart beat and the breath to breath period are longer in larger animals, whereas pressure values are nearly the same in animals of different size.

We can see that oscillations and synchronization are characteristic features of all biological variables. It is proposed that such oscillations may be important for an optimization according to a search mechanism as it was first discussed by I. Priban (1965). Synchronization in biological systems may be found in any region of frequencies. High frequency synchronization is found in neural cells. Low frequency synchronization is well known in connection with circadian and longer oscillation periods. Synchronization appears to be important for rehabilitation and relaxation - including sleep.

The allometric relations describe the relation between function and global mass of an organism. An additional problem is the coordination of structure and function. Ewald Weibel has summarized the adjustment of variables, parameters and the adequate development of structures in relation to their function by the Greek term symmorphosis - which means "similarity according to shape". The title of Weibels's book is "Symmorphosis" (2000). From the physiological viewpont symmorphosis and synchronization - "similarity with respect to time" are closely related.

Weibel describes the definition of symmorphosis as follows: "state of structural design commensurate to functional needs resulting from regulated morphogenesis whereby the formation of structural elements is regulated to satisfy but not exceed the requirements of the functional system. It is obvious that the principles of adaptation, integration and economy are satisfied if structural design is commensurate to functional needs throughout the organism."

One example for illustration is the average length of the lung capillaries. They have to provide optimal gas exchange under condition of rest as well as under condition of extreme exercise. The structure of the pulmonary vascular tree has to be adjusted to variation of volume flow, flow velocity and of ventilation. The obvious question if synchronization plays a role in improving this adjustment is not clarified.

Outlook

Entities like cells or organs or an organism may be called biological system if their basic functions concerning metabolism, energy, growth, movement etc. are considered. A human individual or groups of individuals, societies are also biological systems. However in these latter examples, psyche and social aspects have to be considered. Recent discussions in our country direct the attention towards consideration of cost and effectiveness, e.g. of hospitals or of universities. Although simple allometric relations can not directly be applied concepts like synchronization and symmorphosis may be applied. The additional condition is, that a set of behavioral, psychological and ideological factors gain a major influence: emotions, development of a pecking order, strain for power and prestige, politics etc. play a role.

From the viewpoint of a physician the question is of interest if there is some similarity in the generation of a general failure of an organism by development of a disease or in a large system, including many individuals, by mismanagement. In fact, it seems that as a most simple approach a "triple hypothesis" is applicable in both cases. In a disease the three items are: critical person, critical time and critical stress (9). In a large complex

system the three items are the following: critical system, critical time and critical situation. In the example of our university the critical system is the university in the state of increasing ratio of demand versus support. The critical time can be defined by the condition of unfinished implementation of a new organization law. The critical situation is the enforcement of again another newer law, which in contradiction to political prognoses turns out to be very expensive and troublesome. This example proofs the simple approach of triple hypothesis even in very complex biological systems. One most critical effect ofthis new law is the actual "amputation" of the medical faculty from the body of the Alma Mater in order to generate a new and separate Medical University. This separation is much more expensive than expected by the government. The prediction of the minister had been that the procedure would save money.

LITERATURE

1) Gallison G: Einsteins Uhren, Poincares Karten.
S. Fischer, Frankfurt (2003)

2) Pikovsky A, Rosenblum M, Kurths J: Synchronization, a universal concept in nonlinear science, Cambridge University Press (2001)

3) Jung CG: Man and his symbols (page 211)
Doubleday & Co, N.Y. (1964)

4) Holst E von: Die relative Koordination als Phänomen und als Methode zentralnervöser Funktionsanalyse.
Erg. Physiologie 42: 228 - 306 (1939)

5) Prechtl HFR:
General movement assessment as a method of developmental
Neurology: new paradigms and their consequences.
Developmental Medicine & Child Neurology 43: 836 - 842 (2001)

6) T. Kenner, H. Pessenhofer, G. Schwaberger: Method for the analysis of the
entrainment between heart rate and ventilation rate.
Pflügers Arch. 363: 263-265 (1976)

7) Weibel ER: Symmorphosis, on form and function in shaping life.
Harvard University Press, Cambridge, Mass. (2000)

8) Priban IP, Fincham: Self-adaptive control and the respiratory system.
Nature 208: 339 - 343 (1965)

9) Kurz R, Kenner T, Poets C (Hrsg.): Der Plötzliche Säuglingstod.
Springer-Verlag, Wien, New York, (2000)

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BLOOD FLOW CONTROL OF EXTRA AND INTRACRANIAL ARTERIAL VESSELS DURING MODERATE HYPOTENSION

Jean-Paul Martineaud [1], Philipe Bonnin[1], Etienne Savin [1], Pavel Vank[2], Jiří Jančík[2], Jiří Dušek[2], Bohumil Fišer[2], Jarmila Siegelová[2]

[1] Faculte de Medicine Lariboisiere, Université Paris 7, France, [2] Masaryk University, Brno, CZ

Prof. Dr. Jean-Paul Martineaud
Service d´ Explorations Fonctionnells Multidisciplinaires
Faculte de Medicine Lariboisiere, Université Paris, France
2, rue Ambroise-Paré
F-75010 Paris

Support: MSM 141 100004 (CZ)

ABSTRACT

The study was carried out in ten healthy subjects to precise characteristics of autoregulation in the cephalic circulation. It consisted of measurements of blood velocities in middle cerebral artery (MCA), intracranial branch of internal carotid artery, and in temporal superficial artery (TSA), extracranial branch of external carotid, during a sudden and moderate fall (-9 %) in arterial systemic pressure. In this situation, flow velocities in MCA and TSA decreased abruptly, -16 % and -30 % respectively, during the first cardiac cyclel. The recovery was different in the branches of the common carotid artery: it was fast in MCA, beginning with the second cardiac cycle and completed in five seconds; while it was longer in TSA, remaining low till approximately the thirtieth second. It is suggested that myogenic autoregulation can be present in extracranial as well as in intracranial arterial vessels and would be efficient in one minute roughly. In cerebral arteries, however, other mechanisms developed and a faster recovery is probably explained by intervention of the autonomic nervous system, perhaps via the baroreflex. Then autoregulation protects effectively the cerebral blood flow in the case of a sudden hypotension.

INTRODUCTION

Cerebral circulation depends on multitude of control mechanisms allowing rather a constant flow, better than in any vascular territory, in spite of the important haemodynamic modification (Lassen, 1959), consequently, a steady cellular environment can be maintained , which is necessary for an optimal nervous function. The first known factor to explain a constant cerebral blood flow was the myogenic autoregulation, Baylis reflex (Bayliss, 1902). More recently there was discovered the proeminent intervention of

partial pressure of carbon dioxide in arterial blood (Pco2) for constancy or modifications of the cerebrovascular bed (Mamo, 1967). In the first case, the decrease in arterial blood pressure induces vasodilatation of cerebral arteries and inversely the increase brings vasoconstriction; in the second situation, the decrease of local pH determined by an increasing Pco2 induces a marked vasodilatation and inversely the decrease of Pco2. Hence in both situations, the cellular environment is preserved.

But numerous and recent studies showed that many other factors, nervous, humoral and metabolic, act in the control of cerebrovascular resistances, so that it is very dificult to establish a complete and coherent diagram of the regulation of cerebral circulation (Aaslid 1989, Stragaard 1995). Consequently, this study was undertaken in order to get more information about the time course of vasoreactivity in intra and extracranial beds, after abrupt changes in haemodynamic situation, using non-invasive methods (Jorgensen 1992, Larsen 1994) and physiological stimuli.

MATERIAL AND METHODS

Ten healthy men (mean age: 38.9 yr, height: 180.3 cm, body weight: 73.2 kg) participated in this experiment. They were studied in supine position, at neutral ambient temperature (21 +/- 1 °C).

Two inflatable cuffs were positionned on both thighs of each subject. After a 20 min rest the cuff pressure was rapidly increased to 180 mmHg and kept constant during 5 min, bringing about a complete circulatory arrest in both lower extremities (Savin et al.,1997). After this 5 min occlusion the cuff pressure was lowered abruptly to 60 mmHg, so that an abnormal venous return from lower limbs was avoided.

Blood pressure (BP), blood velocities in mean cerebral artery (MCA) and temporal superficial artery (TSA) were measured simultaneously, before and during occlusion, during and after the cuffs release (1.5 and 10 s, 1 min). Heart rate was evaluated from pressure recordings, beat to beat.

Blood velocities were determined using ultrasonic velocimeters: a transcranial Doppler velocimeter (AngioDop DMS, 2 MHz) for the right middle cerebral artery and a usual velocimeter (Gamma Dop Technimed, 8 MHz) for the left temporal superficial artery at the temple. BP was recorded by means of the Penaz´s photoplethysmograph (Finapress Ohmeda) giving systolic, diastolic and mean systemic pressure and heart rate.

The results presented in this paper are expressed as mean values of ten subjects with standard deviations. Statistical significances of observed differencies were calculated the Wilcoxon test for paired data: only p values less than 0.05 were considered as significant.

RESULTS

Maen values for heart rate, systemic blood pressure, blood velocities in MCA and TSA before, during and after occlusion of lower limbs are presented with statistical differences in the Table I.

Blood pressure

Immediately after the inflation of occluding cuffs systolic and diastolic BP increased: mean BP increased by 15mmHg, but decreased rapidly and returned to the original level within two minutes. After the cuff release, all pressures decreased and mean BP was reduced by 8.9 % during the first systole and 12.2 % at the 5th s and returned to the preocclusion level only after half a minute.

Heart rate

Immediately after the cuff release HR increased to initial value: The difference from the value observed during occlusion was 7.3 % at the first second and after 5 s this difference was 12.2 %, which was significant. After 10 s the preocclusion level was practically restored.

Blood velocities

The values at rest and during the third minute of occlusion were not different. They decreased significantly with deflation of cuffs.

In MCA, the velocity after release decreased by 16.7 % at the first second and then increased very rapidly. The original level was reached at the fifth second and even exceeded at the tenth second, despite the fact that hypotension was sustained.

In TSA, the blood velocity following the cuff release decreased by 30.4 % immediately and for a much longer time than in MCA; indeed it was only between the thirtieth second and one minute that the rest value was reestablished.

The differences between the relative values of velocities in MCA and TSA at the first, fifth and tenth second were significant: in these three cases the values of the decrease were higher for TSA (p 0.05).

DISCUSSION

In non-invasive studies of vascular reactivity the Doppler velocity measurements are of importance, especially for evaluating instantaneous changes in the brain circulation by means of transcranial method (Aaslid 1982, Madsen 1993). If it is assumed that MCA and TSA diameters were not modified during the experiments, the Doppler method allows us to record the flow modifications and to evaluate the resistance changes (Jorgensen 1995). In these circumstances the changes in flows are exactly proportional to the changes in velocities (Larsen 1994), even if we cannot quantify these changes. Furthermore TSA and MCA are generally considered as representative of cephalic cutaneovisceral vascular territories and cerebral vessels respectively. Then we can consider this study as valuable for conclusions about autoregulation.

The inflation of pressure cuffs around thighs creates an acute ischemia in a large peripheral bed with anaerobic metabolism. Then general haemodynamic disturbances can be observed, especially an increase in systemic pressure (Jorgensen 1992), but in our study it was only for a few minutes. The decrease in heart rate induced by this initial hypertension was still present at the fifth minute. During the ischemic period vasodilatation developed in both lower limbs and explains the fall in arterial pressure after the cuff release. This fall elicits the baroreflex and control reactions appear rapidly. In this stimulation, hypotension is the most important circulatory signal because venous back pressure simultaneously established (60 mmHg) prevents an abnormal venous return flow and stimulation of volumoreceptors in pulmonary circulation.

In every experiment of this study the development of moderate but acute hypotension induced an immediate and significant decrease (-15 to -30 %) in blood flows in territories of common carotid artery. This purely passive effect could be enhanced by circulating vasodilator agents, such as CO2, formed in legs during ischemia: however the apparition time seems too short for metabolic effects out of legs. The secondary vasomotor responses to hypotension are different in the branches of common carotid.

The blood flow in one extracranial branch of the external carotid artery, TSA, remained low for ten seconds and then increased slowly, taking one minute approximately for

reaching initial level. The flow recovery was quite parallel with that of blood pressure: this suggests that superficial arterial vessels are essentially non reactive or passive vascular beds as defined by Green (1964). Nevertheless the fall in TSA blood velocity was proportionately greater than in systemic pressure and this could indicate a concomittant local vasoconstriction, in order to attenuate a general haemodynamic stress. Intervention of an opposite myogenic reaction cannot be excluded in the recovery with delay, even if it was not prominent in this situation. In contrast with this response, after the initial decrease the blood velocity in MCA, intracranial branch of internal carotid artery, increased much more rapidly than in TSA as well as blood pressure due to different mechanisms. In any physiological situation, compensatory vasodilatation (or vasoconstriction) which is the classical mechanism for constant cerebral circulation is present and needs only some seconds to be completely activated and efficient (Pinard 1985). Certainly this autoregulatory reaction has been known for many years, in humans as well as in laboratory animals (Meyer 1965, Yoshida 1966, Florence 1992). What is new in this study is the extreme quickness and efficiency of the control mechanisms becasuse velocimetry allows instantaneous determinations of blood velocity in physiological conditions. With a delay of one cardiac cycle only, the reincrease was beginning and was achieved during the fifth second. It is suggested at first that control by the autonomic adrenergic system can play an important role, probably via the baroreflex which influences autoregulation of the cerebral blood flow at least during hypertension (Talman 1994). In our experiment the increase in heart rate, which is a specific indicator of baroreceptor stimulation, occurs immediately after the fall of blood pressure (Siegelová et al, 1994,1997,2002). It has been established that the secondary increase in cerebral blood flow is mediated partially by beta-adrenergic receptors during the weightlessness stress (Bryan 1990). Myogenic autoregulation which is a classical control mechanism of cerebral blood flow needs some seconds to be activated and efficient as well as biochemical and humoral factors locally released (Pinard 1985, Meadow 1994). Inversely during an acute hypertension, the same mechanisms will be used in the opposite direction.

REFERENCES

1. Aaslid R.T., Markwalder M., Normes H.: Noninvasive transcranial Doppler ultrasound recording of flow velocity in basal cerebral arteries. J. Neurosurg. 57, 1982, 769-774.
2. Aaslid R.T., Lindegaard K.F., Sorteberg W., Normes H.: Cerebral autoregulation dynamics in humans. Stroke 20, 1989, 45-52.
3. Bayliss W.M.: On the local reactions of the arterial wall to chasnges of internal pressure. J. Physiol. London 28, 1902, 220-231.
4. Bryan R.M.: Cerebral blood flow and energy metabolism during stress. Am. J. Physiol. 259, 1990, H269-H280.
5. Florence G., Seylaz J.: Rapid autoregulation of cerebral blood flow: a laser-Doppler flowmetry study. J. Cereb. Blood flow Metab. 12, 1992, 674-680.
6. Green H.D., Rapela C.E.: Blood flow in passive vascular beds. Circ. Res. 15, suppl I, 1964, 1-11.
7. Jorgensen L.G.: Transcranial Doppler ultrasound for cerebral perfusion. Acta Physiol. Sc. 625, Suppl.,1995, 1-44.

8. Jorgensen L.G., Perko M., Hanel B., Schroeder T.V., Secher N.H.: Middle cerebral artery flow velocity and blood flow during exercise and muscle ischemia. J. Appl. Physiol. 72, 1992, 1123-1132.
9. Larsen F.S., Olsen K.S., Hansen B.A., Paulson O.B., Knufdsen G.M.: Transcranial Doppler is valid for determination of the lower limit of cerebral blood flow autoregulation. Stroke 25, 1994, 1985-1988.
10. Lassen N.A.: Cerebral blood flow and oxygen consumtiopn in man. Physiol. Rev. 39, 1959, 183-238.
11. Madsen P.L., Sperling B.K., Warming t., Schmidt J.F., Secher N.H., Wildschiodtz G., Holm S., Lassen N.A.: Middle cerebral artery blood velocity and cerebral blood flow and O2 uptake during dynamic exercixe. J. Appl. Physiol. 74, 1993, 245-250.
12. Mamo H., Seylaz J.: La circulation cérébrale. Données méthodologiques et physiologiques. Rev. Electroenc. Neurophysiol. Clin. 4, 1967, 194-209.
13. Meadow W., Rudinsky B., Bell A., Lonzon M., Randle C., Hipps R.: The role of prostaglandins and endothelium-derived factor in the regulation of cerebral blood flow and cerebral oxygen utilisation in the piglet. Pediatric Research 35, 1994, 649-656.
14. Meyer J.S., Handa J., Huber P., Yoshida K.: Effect of hypotension on internal and external carotid flow. Demonstration of a homeostatic mechanism peculiar to cerebral vessels and its importance in cerebrovascular occlusion. J. Neurosurg. 23, 1965, 191-196.
15. Pinard E., Lacombe P., Reynier-Rebuffel A.M., Seylaz J.: Intrinsic and extrinsic mechanisms involved in the cerebro vacular reaction elicited by immobilization stress in rabbits. Brain Res. 340, 1985, 305-314.
16. Savin E, Siegelova J, Fiser B, Bonnin P : Intra and extracranial artery blood velocity during a sudden blood pressure decrease in humans. Eur.J.Physiol 1997; 76 : 289-296
17. Siegelová J, Fišer B, Dušek J, AL-Kubati M: Baroreflex-Senstivitaetsmessung bei Patienten mit essentieler Hypertonie: Einfluss von Enalapril. Nieren und Hochdruckkrankheiten 1995;24:20-22.
18. Siegelová J, Fišer B, Dušek J. Baroreflex heart rate sensitivity in essential hypertension: the effect of Isoptin SR 240 therapy. In: Varró V, de Chantel R, eds. 22nd Congress of International Society of Internal. Medicine, Bologna: International Proceeding Div. Monduzzi Editore, 1994:99-102.
19. Siegelová J, Hofírek I, Fišer B, Dušek J, Placheta Z, Jančík J, Dobšák P, Svačinová H, Vank P, Toman J. Relationship between pulse pressure and baroreflex sensitivity in patients with essential hypertension. Scripta medica 2002:75(2):101-104
20. Siegelova J, Fiser B, Savin E, Dusek J : Baroreflex mediated vasomotor response in hypertensives. J.Hypertension 1997 ; 15 : 194
21. Strandgaard S., Paulson O.B.: Cerebral blood flow in untreated and treated hypertension. Neth. J. Med. 47, 1995, 180-184.
22. Talman W.T., Dragon D.N., Ohta H.: Baroreflexes influence autoregulation of cerebral blood flow during hypertension. Am. J. Physiol. 267, 1994, H1183-H1189.
23. Yoshida K., Meyer J.S., Sakamoto K., Honda J.: Autoregulation of cerebral blood flow. Electromagnetic flow measurement during acute hypertension in the monkey. Circ. Res. 19, 1966, 726-738.

Tab. 1: Middle cerebral artery (MCA), superficial artery (TSA) velocities (cm.s-1) and common carotid artery blood flow (CCA) ml.min-1 and blood pressure (mmHg) at rest, during occlusure and after cuff release (1,5 and 10 s) for 10 subjects (values are means SD, * = $p < 0.05$; comparidon with rest value).

	Rest	Occluding	Cuff release		
			1s	5s	10s
MCA $cm.s^{-1}$	50.3 ±11.6	50.1 ±10.2	* 41.8 ±11.6	49.3 ±10.9	51.6 ±10.5
TSA $cm.s^{-1}$	10.9 ±2.5	10.6 ±2.6	* 7.4 ±2.5	* 8.2 ±2.2	* 8.2 ±2.4
CCA $ml.min^{-1}$	* 305.1 ±23.4	299.1 ±23.7	232.7 ±33.2	281.5 ±43.3	292.5 ±29.6
Blood pressure mm.Hg	101.4 ±9.9	98.4 ±9.9	* 89.8 ±10.3	* 86.4 ±10.0	* 90.5 ±10.0
Heart rate $Beats.min^{-1}$	87.0 ±17.0	82.0 ±14.0	88.0 ±14.0	* 92.0 ±12.0	86.0 ±15.0

SHORT TERM BLOOD PRESSURE REGULATION

Skrabal F., Mayer H., Lindenmann J., Hitachi O., Fortin J., Gratze G., Beitzke M.
Dept. of Physiology, Karl-Franz-University, Graz,Austria

Prof. Dr. Falko Skrabal
Karl-Franzens-Universität Graz
Department of Physiology
Falko.Skrabal@kfunigraz.ac.at

Support: MSM 141 100004 (CZ)

Short term haemodynamic and blood pressure (BP) regulation is achieved mainly by Frank Starling`s law and by the reflex arcs of the baroreceptor-vasoconstriction system. The latter includes the afferent nerves from the high pressure baroreceptors in the carotis, the NTS in the medulla oblongata and vagal and sympathetic efferents to the heart, kidney and resistance vessels. Since interruptions of blood flow to the brain of only 10 seconds duration will lead to cerebral hypoperfusion with all its consequences, diagnostic instruments should be able to detect haemodynamic disturbances of that order of duration. Neither oscillometric nor auscultatory blood pressure measurements will detect blood pressure falls of about 10 seconds duration and short lasting falls of cardiac output cannot be detected by conventional non-invasive or invasive methods including thermodilution. We present the clinical experience of several years with a non-invasive haemodynamic monitor, which measures stroke volume, BP, total peripheral resistance, central sympathetic and parasympathetic drive and baroreceptor reflex sensitivity online on a beat to beat basis

(Task Force™ Monitor,by www.cnsystems.at).

The standard protocol to evaluate possible disturbances of short term haemodynamics include head up tilting on the tilt table and carotid massage. We have investigated more than 1000 patients with suspected syncope by the above protocol and present our experiences and many clinical examples. Autonomic and baroreceptor failure is very common in patients with syncope especially in elderly patients. The degree of autonomic failure varies enormously and it is commonly incomplete so that it may not be detected by the conventional recording of an ECG and of oscillometric BP. Incomplete cases show only marked impairment of peripheral vasoconstriction which is (partly) compensated by a rise in stroke volume and cardiac output. Atherosclerotic encephalopathy, diabetes mellitus , vitamin B12 deficiency and malignancy are common causes of autonomic failure. Vasovagal Syncope may also be diagnosed with great sensitivity if complete beat to beat haemodynamic and autonomic assessment is obtained, since the condition may be diagnosed even if the syncopal event does not occure during the tilt test. Tilting commonly also induces arrhythmias in susceptible patients, so that their haemodynamic consequences can be evaluated. Ventricular arrhythmias may or may not result in reduced cardiac output dependent on their origine.

An accurate diagnosis enables a more focused therapy. A wide range of differential therapeutic options is available, which will be discussed in detail.

Complete haemodynamic monitoring including beat to beat stroke volume and BP for the evaluation of syncope should and will become mandatory in the future.

SEASONAL VARIATIONS OF PAIN THRESHOLD ARE MODULATED BY SEASON OF BIRTH: COMPARISON OF HEALTHY WOMEN WITH EATING DISORDERS PATIENTS

Yamamotová A.[1], Papežová H.[2], Vurmová I.[2], Rubešová I.[1], Kočová M.[3]

Charles University, 3rd Faculty of Medicine, [1]Department of Physiology, 1st Faculty of Medicine, [2]Department of Psychiatry, [3]Faculty of Natural Sciences, Prague, Czech Republic

OBJECTIVES

Physiological and behavioral data are influenced by two types of seasonality: seasonality of external environmental factors (temperature, photoperiod, nutrition) which modulate neurohormonal regulations, and season of birth that can affect perinatal neurodevelopment and contribute to predisposition to several diseases. Psychiatric disorders are associated with the season of birth including eating disorders with higher incidence of patients born in summer months (Eagles et al. 2001). Until now, both types of seasonality have been analyzed separately.

AIM OF STUDY

In our study we compared interactions of these two types of seasonality on the pain threshold in healthy women and patients with eating disorders, who have decreased nociceptive sensitivity.

MATERIAL AND METHODS

Thermal pain threshold was measured in 124 women (73 anorexia and bulimia nervosa and 51 healthy controls) on the dorsal surface of three fingers. Experiments were performed from September to July between 10 AM and 2 PM.

RESULTS

The month of birth did not influence pain threshold either in controls or in patients ($p = 0.401$, $p = 0.094$, respectively). ANOVA revealed a tendency to higher pain threshold in winter experiments (January-February, $F(11,112) = 1.89$; $p = 0.0478$), which became more significant when interaction with season of birth was included into analysis ($F(1,115 = 3.58$; $p = 0.0087$). In both groups, the greatest seasonal variations in nociception were observed in persons born during March-June with the lowest values in spring and the highest in winter. On the contrary, persons born during November-February show no seasonal changes in pain sensitivity. In the whole sample, irrespectively to diagnoses, the

lowest seasonal values were associated with the season of birth (cosinor analysis: acrophase = -198.2°, p = 0.0076, acrophase relative to time zero = month of birth).

CONCLUSIONS

These preliminary results show that the month of birth should determine reactivity of organism to various stressors including pain. We can hypothesize that the highest pain sensitivity observed during the season of birth might reflect increased arousal and the lowest pain threshold observed in March-April should be linked to the lowest serotonin turnover observed in persons born in these months (Chotai, Adolfsson 2002).

REFERENCES

Chotai J, Adolfsson R. Converging evidence suggests that monoamine neurotransmitter turnover in human adults is associated with their season of birth. Eur Arch Psychiatry Clin Neurosci 2002; 252:130-134.

Eagles JM, Andrew JE, Johnston MI, Easton EA, Millar HR. Season of birth in females with anorexia nervosa in Northeast Scotland. Int J Eat Disord 2001; 30:167-175.
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CHRONOTHERAPY: RATE-PRESSURE PRODUCT IN ESSENTIAL HYPERTENSION

Siegelová Jarmila[1], Fišer Bohumil[2], Dušek Jiří[1], Placheta Zdeněk[1], Vlček Jiří [1], Svoboda Ladislav [1], Pazdírek Jiří [1], Cornelissen Germaine[3], Halberg Franz[3]

[1]Department of Functional Diagnostics and Rehabilitation, [2] Department of Physiology, Faculty of Medicine, Masaryk University, Brno
[3]Halberg Chronobiology Center, University of Minnesota, USA

Address for correspondance:

Prof. MUDr. Jarmila Siegelová, DrSc.
Dept. Functional Diagnostics and Rehabilitation
Faculty of Medicine, Masaryk University
St. Anna Teaching Hospital
Pekařská 53
656 91 Brno, Czech Republic

INTRODUCTION

In essential hypertension, an increased sympathetic activity is present, which further increases a risk of cardiaovascular morbidity and mortality. Nowadays, the effect of antihypertensive therapy is generally evaluated by means of ambulatory 24-hour blood pressure monitoring and the value of the decrease in blood pressure is taken as an ultimate goal of treatment. This is true for the majority of patients because of a correlation between blood pressure values and the development of organ pathological changes, e.g., hypertrophy of left ventricular mass and arterial smooth muscles. In patients with angina pectoris, heart rate and systolic blood pressure values have been related to the onset of pain in angina pectoris (1). The rate-pressure product (RPP), also called the Robinson index (expressed as systolic blood pressure x heart rate/100), is a major determinant of cardiac oxygen consumption (2). Therefore, in cardiac patients, it is necessary to lower blood pressure and also achieve a decrease in heart rate and in oxygen consumption of the heart. The aim of this study was to analyze the circadian RPP in patients with essential hypertension who received enalapril therapy.

MATERIALS AND METHODS

Four groups of subjects were examined: 11 normotensive men (C), 10 patients with moderate essential hypertension without therapy (EH), 10 patients with essential hypertension (EH E) who had been treated with a single daily dose of 12.5 mg enalapril

in the morning for one year and 10 patients with nephrogenous hypertension (N) who had been on a therapy of 25 mg enalapril every morning for one year.

The diagnosis of essential hypertension was determined according to the WHO criteria. The patients had been followed up in our clinic for at least 3 years before the beginning of the study; the secondary hypertension was excluded and essential hypertension was established. The patients with nephrogenous hypertension had previously been followed up in a nephrological department at least 3 years and their diagnosis was verified clinically and by examination of histological specimens.

The study was approved by the Ethical Committee of Masaryk University and all patients gave their informed consent.

An accutracker II was used for 24-hour ambulatory blood pressure (BP) and heart rate (HR) monitoring. The rate-pressure product (systolic blood pressure x heart rate/100, RPP) was calculated for a 24-hour period. The statistical evaluation was carried out by means of the Wilcoxon test.

RESULTS

The characteristics of the normotensives (C), untreated (EH) and treated hypertensives (EH E) and patients with nephrogenous hypertension (N) are shown in Table 1.

Tab.1: Characteristics of the subjects and patients

Group	No.	age (years)	height (cm)	body weight (kg)
C	11	48 ± 6	180 ± 6	82 ± 5
EH	10	49 ± 5	179 ± 8	83 ± 6
EH E	10	49 ± 5	178 ± 5	86 ± 5
N	10	49 ± 9	176 ± 5	85 ± 3

The subjects differed neither in age nor in basic anthropologic data. The values of systolic BP, diastolic BP and RPP during 24 hours are given in Tables 2 and 3.

Tab. 2: The 24-hour mean (SD) values of systolic blood pressure (SBP), diastolic blood pressure (DBP) and rate - pressure product (RPP)

Group	SBP(mmHg)	DBP(mmHg)	HR(cpm)	RPP(mmHg.cpm/100)
C	121 ± 10	74 ± 8	75 ± 11	93.18 ± 12.08
EH	147 ± 12**	88 ± 9**	80 ± 9	117.59 ± 17.76**
EH E	129 ± 9	80 ± 7	83 ± 11	107.32 ± 15.70
N	127 ± 8	74 ± 6	80 ± 8	102.29 ± 8.54

* P<0.05, ** P<0.01, statistical significance of comparisons with group C

Tab.3: Daytime mean (SD) and night mean (SD) values of rate - pressure product (RPP)

Group	Daytime mean RPP(± SD) 07:00 - 24:00	Night mean RPP(± SD) 01:00 - 06:00
C	101.44 ± 15.06	68.50 ± 3.14
EH	125.44 ± 18.33**	94.16 ± 16.06**
EH E	113.78 ± 14.67	88.00 ± 18.82*
N	109.11 ± 9.76	81.83 ± 4.91

* P<0.05, ** P<0.01, statistical significance of comparisons with group C

The RPP values showed high circadian variability in the control subjects as well as in the EH, EH E and N patients. In EH E patients, enalapril therapy failed to bring the increased RPP to normal values at night.

DISCUSSION

Any increase in sympathetic nervous activity is an important factor contributing not only to hypertrophy of left ventricular mass and arterial smooth muscles but also to the development of myocardial infarction and sudden cardiac death in patients with ischemic heart disease (3, 4, 9). The circadian variation of sympathetic nervous activity is reflected by the increase of the incidence of myocardial infarction and sudden cardiac death in the morning hours (5, 6). The increased incidence of both events begins at 6 a.m. and reaches its peak between 9 a.m. and midday. In the afternoon the incidence of both events is about a half of the peak values. The aim of hypertension chronotherapy is to control blood pressure over the period of 24 hours and to suppress the morning increase in sympathetic activity.

Our data demonstrate that the 24-hour profile of rate-pressure product correlates much better with the incidence of sudden cardiac death and myocardial infarction than with blood pressure curves. This finding can reflect the fact that sympathetic nervous activity is a major determinant of blood pressure. The baroreflex is probably a mechanism increasing the heart rate during nifedipine therapy and this may be the reason for an increase in mortality after nifedipine administration. A morning decrease in blood pressure in our patients during the enalapril treatment was accompanied by a lower increase in heart rate, indicated by the decrease in rate-pressure product, in comparison with that found in non-treated patients with essential hypertension.

A relatively low morning increase in the rate-pressure product in the enalapril treated patients with nephrogenous hypertension is probably due to the fact that hypertension in this group was not caused by increased sympathetic activity. This hypothesis is supported also by our earlier finding of normal baroreflex heart rate sensitivity in the patients with nephrogenous hypertension, which is in contrast to observations in patients with essential hypertension (7, 8, 9).

In conclusion, the noninvasive methods used in this study may facilitate studies of myocardial ischaemia in patients with essential hypertension. The 24-hour measurement

of blood pressure together with evaluation of the RPP may eventually become a useful tool in identification of optimal strategies for different therapies of arterial hypertension and may help to demonstrate a favorable prognostic outcome.

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REFERENCES

1. Robinson BF. Relation of heart rate and systolic blood pressure to onset of pain in angina pectoris. Circulation 1967;65:1037-1045.
2. Roskmann H, Reindel H. Herzkrankheiten. Heidelberg: Springer Verlag, 1989.
3. Julius S, Jamerson K. Sympathetics, insulin resistance and coronary risk in hypertension: The chicken-and egg question. J Hypertens 1994; 12:495-502.
4. Palatini P, Julius S. Association of tachycardia with morbidity and mortality: pathophysiological considerations. J Hum Hypertens 1997;11:S19-27.
5. Willich SN, Levy D, Rocco MB, Tofler GH, Stone PH, Muller JE. Circadian variation in the incidence of sudden cardiac death in Framingham Heart Study population. Am J Cardiol 1987;60:801-6.
6. Muller JE, Ludmer PL, Willich SN et al.Circadian variations in the frequency of sudden cardiac death. Circulation 1987;75:131-138.
7. Siegelová J, Fišer B, Dušek J, Al-Kubati M. Die Baroreflexsensitivitätsmessung bei Patienten mit essentiellen Hypertonie: Einfluss von Enalapril. Nieren und Hochdruckkrankheiten 1995; 24:20-22.
8. Fišer B, Siegelová J, Ševela K, Dušek J. Baroreflex heart rate sinsitivity in patients with renal parenchymal hypertension. J Hypertension 1997;15(Suppl.4):140.
9. Eicher JC, Dobšák P, Wolf J.E. L´échocardiographie -Doppler permet -elle d´evaluer l´effect des thérapeutiques dans la myocardiopthie hypertrophique? Réalités Cardiologiques 1997;106:15-19.
10. Siegelová J., Fišer B., Dušek J., Placheta Z, Cornelissen G., Halberg F. Circadian variability of rate-pressure product in essential hypertension with enalapril therapy Scripta medica 73, 2, 2000, 67-77

BAROREFLEX SENSITIVITY IN CLINICAL PRACTICE

Bořivoj Semrád

Department of Internal Cardiology Medicine, Faculty of Medicine, Masaryk University

Address correspondece to:

Prof. MUDr. Bořivoj Semrád, CSc.
Head
Department of Internal Cardiology Medicine
Faculty of Medicine, Masaryk University
Jihlavská 20
639 00 Brno

Support: MSM 141 100004 (CZ).

The baroreflex is a homeostatic mechanism decreasing the variation of blood pressure. Its physiological function is well described, although its significance for animals and human bodies is unknown. It was suggested that it is important for blood pressure regulation during changes of the position of the body from lying to standying, but patients with orthostatic hypotension have normal baroreflex sensitivity. People with low baroreflex sensitivity (BRS) do not have problems with changes of position of the body. A baroreflex gain similar to that seen in humans was observed in small animals such as cats and rabbits. Where the difference in blood pressure in brain arteries between a horizontal and vertical position is only a few mmHg. The baroreflex probably protects the brain arteries from hypertrophy of the vascular wall. The autoregulatory reaction, which normally returns flow in brain arteries to its original value after a decrease of blood presssure [1], is not seen in subjects with untreated hypertension [2]. During exercise, the baroreflex gain is rapidly diminished [3] or the set point is shifted to higher blood pressures [4]. In this way, the perfusion of the brain is maintained during vasodilatation in working muscles. Surgical elimination of the baroreflex causes an increase of blood pressure, which disappears after several days in experimental animals but elicits hypertension in man. Because of several other control mechanisms, baroreflex elimination is not a threat to life. Several years ago, a low BRS was found to be a risk factor for cardiac death in patients after myocardial infarction. This is why the baroreflex in humans is extensively studied in many laboratories. Research is concentrated into three areas: the search for a convenient method for determination of BRS, the study of baroreflex in patients after myocardial infarction at risk of cardiac death and the study of the baroreflex in other diseases.

The large number of methods for determination of BRS indicates that an ideal method has not been found. The methods can be classified according to several criteria. Classification according to invasiveness of the approach is not up-to-date because the classical intraarterial recording of blood pressure has mostly been replaced by the Penaz volume clamp method of non-invasive recording of blood pressure in digital arteries using the Physiocal criteria of Wesseling. Monitors using this technique include Finapres, Ohmeda or Portapres, TNO-BMI Amsterdam [5].

An analysis of spontaneous baroreflex sensitivity shows the changes of pulse interval (PI) elicited by spontaneous changes of between systolic blood pressure (SBP). Laboratory methods evaluate the changes of PI elicited by artificially produced changes of SBP. Two methods estimate spontaneous BRS, the sequence technique method and the spectral method. Sequences of three or more beats with a high correlation between SBP and PI are used to calculate the average slope of the relation (in ms/mmHg) over several minutes of beat-to-beat blood pressure recording. The disadvatage of this approach is that the slope of the line expressing the relationship between PI and SBP reflects respiratory arrhythmia as well as BRS, the former being mediated by several mechanisms.

Apart from BRS, the direct connection of respiratory and cardio-inhibiting centres play a role in central nervous systém and respiration-induced changes in diastolic filling of the right atrium with its reflex and direct mechanical effect on pacemaker cells in the sinoatrial node. This disturbance by respiration is common for many methods for BRS determination. If the respiratory rate is sufficiently high, a method based on the spectral analysis of several minutes' blood pressure recording is free of this respiratory influence. The method uses a calculation of the BRS spectral peak on SBP and PI power spectra at 0.1 Hz (0.05-0.15 Hz). We use metronome-controlled breathing at 0.33 Hz in our laboratory. Two modifications of the calculation are used (Robe technique and Pagani alpha coefficient); the difference is low if the association between SBP and PI oscillation at 0.1 Hz is high. This association is reflected by the coherence; a high coherence between 0.5 and 1 is a requirement for a reliable BRS estimate. A high coherence also occurs at the respiratory frequency, but the gain in this frequency band reflects not only BRS, but the other respiration induced changes of heart rate mentioned above.

Laboratory methods use the linear increase in blood pressure elicited by the vasoconstrictor drug phenylephrine or by release of occlusion of the venous return at phase IV of the Valsalva manoeuvre, as a stimulus. The slope of the relation between SBP and PI is used for BRS calculation. Increased filling of the right atrium can occur in both cases. A sophisticated technique, using the compensation of venoconstriction by lower body suction, can hold venous pressure constant after phenylephrine administration [6].

The graph showing the open-loop relationship between pressure in the carotid sinus and the PI response is S-shaped, with a central linear part. SBP during BRS measurement generally lies within this linear part of the curve, whose slope is measured by all of the methods described above. We can only obtain the entire curve by a combination of various doses of phenylephrine and vasodilatatory nitroprusside and by a neck-suction, neck-compression technique. The side effects of phenylephrine have been mentioned. Apart from vasodilatation, nitrous oxide, mediating the effect of nitroprusside, has many other effects. It is unknown whether the pressure in the carotid sinus corresponds to the difference between blood pressure and compression pressure or to the sum of blood pressure and suction pressure in the neck-suction/neck-compression technique.

BRS reflects the response of PI and so describes only one branch of the baroreflex.

Baroreflex sensitivity expresses the changes of PI only (and not of systemic peripheral resistance [SPR]). For this reason, "Baroreflex heart rate sensitivity" or "Baroreflex pulse interval sensitivity" would be terms that are more appropriate.

The estimation of baroreflex mediated vasomotor changes (i.e., changes in SPR) is more difficult. The recording of muscle sympathetic nervous activity (MSNA) is a technique that is difficult to standardise for use in different laboratories, and the activity in one nerve can be different from changes in SPR. This technique for the determination of the blood pressure component of the baroreflex is not perfect. A promising method is that of the Wesseling group using the shape of the pulse wave for calculation of stroke volume or SPR beat-to-beat [5]. An alternative approach is an estimation of SPR beat-to-beat changes from the rate of diastolic blood pressure decay [7]. We use the following technique in our laboratory to measure baroreflex sensitivity. Occluding cuffs are placed on both thighs and inflated abruptly on suprasystolic pressure. This ceases circulation in both lower extremities for 5 minutes. Rapid deflation causes a decrease in arterial blood pressure of 10-20 mmHg. The rate of the baroreflex mediated return of systolic and diastolic pressure in mmHg/s gives an estimate of the sensitivity of the blood pressure component of the baroreflex [8].

During recent years, BRS has been studied in varous cardiovascular and non-cardiovascular diseases. In essential hypertension, increased sympathetic activity is associated with a low BRS and normal blood pressure senstitivity [9]. A low BRS is unlikely to be the cause of hypertension but there is evidence that suggests that impairment of the baroreflex may have an important influence on long term arterial pressure [10].

BRS is a marker of the vagal reflexes, which protect the heart against ventricular fibrillation. BRS also suppresses sympathetic nervous activity, which jeopardies the heart during a spontaneous increase of blood pressure.

Stratification of the risk of death in patients after myocardial infarction (post-MI patients) is now more important than in the past because of the possibility of treating the patients with implantable cardioverter-defibrillator devices. Decreased BRS as a risk factor in post-MI patients [11,12]. The risk of sudden cardiac death (SCD) in patients after myocardial infarction is estimated by means of several measures of heart functions.

The majority of these, e.g., ejection fraction, arrhythmias, late potentials and heart rate variability, is usually monitored in these patients. In the last few years, BRS has been evaluated as an independent and additive prognostic value. The ATRAMI multicenter prospective study involving nearly 1300 patients clearly proved that low values of BRS (<3 ms/mmHg, measured as the rate-pressure response to intravenous phenylephrine) carried a significant multivariate risk of cardiac mortality, independent of standard markers [13]. Even the clinical usefulness of BRS measurement was demonstrated in the ATRAMI study. The possibility of introducing the phenylephrine method of BRS determination as a standard clinical method is undergoing much discussion because of the need of drug injection. Similar results were found, however, by using the spectral analysis of resting heart rate and blood pressure variability [14-16]. The critical value for BRS determined by the spectral method, together with seven other standard indexes, was tested [17]. BRS < 3 ms/mmHg was found to be the critical value in this study.

Other studies are comparint the results of the phenylephrine method with different non-invasive methods of BRS determination [18]. The most promising results were found for the Robe spectral analysis technique [14-17]. Less consistent data were found for the

alpha-low frequency and alpha-high frequency spectral technique [18,19] or for the sequence method [19].

Different indexes typically use critical values as a measure of risk. This approach does not distinguish between patients whose values are over, although near the critical value and patients with values far beyond the critical values. The significance of 10 non-invasive idexes, including BRS, relevant for prediction of risk of cardiac death for the individual patients was evaluated quatitatively by regression analysis [20]. This approach does not také into account critical values. Univariate and multivariate regression analyses were used quantitavely to estimate the level of risk. The statistical significance of regressors was calculated for each index. By means of regression equations, the risk was calculated in individual subjects. The value of risk ranged from 0 (patients with no risk of cardiac death) to 1 (patients who are expected to die). The best prediction is obtained by a combination of BRS, SDNN index (the mean standard deviation of RR intervals determined in 5-minute periods in 24 hours) and RR. These results demonstrate the clinical applicability of BRS and its inclusion among standard non-invasive criteria for implantation of cardioverter-defibrillator devices.

A logical next step in following BRS in pos-MI patients, is the evaluation of the therapeutic effects on BRS. A positive effect was proven for thrombolytic therapy [21-23]. It was further proven that a higher BRS is associated with sustained patency of the infarct-related artery, with the restoration of anterograde flow in in, and this effect is independent of infarct size [24].

Some questions remain unanswered. Unpublished studies in yout laboratory revealed that BRS in ms/mmHg has better predictive power than BRSf in Hz/mmHg (Two years mortality in patients after myocardial infarction 60-69 years old: forty-fours survivors versus 15 deceased; 5.12 + 3.9 vers. 2.12 + 1.27 ms/mmHg, $p = 0.0027$; 0.00729 + 0.00588 vers. 0.00388 + 0.00196 Hz/mmHg, $p = 0.056$; Wilcoxon). On the other hand, BRSf is heart rate independent [25], BRSf decreases with age in children [26] as does the compliance of arteries and BRSf correlates with the second value obtained by repeated investigation after 1 year interval in 100 young adults [27]. This is opposite to BRS. It seems that BRS. It seems that BRSf corresponds to the baroreceptor sensitivity and BRS expresses the gain of the heart rate baroreflex component, including sympathetic and parasympathetic nervous tonic activity that is also expressed by markers of heart rate variability. It is also important that BRS is not constant on 24-hour recordings [28]. Introducing a method of continous evalution of BRS by spectral analysis, we observed a large fluctuation of BRS which makes repeated measurements inevitable [29]. It seems that preparation of guidelines for the determination of BRS is a very important task.

REFERENCES

1. Savin D, Siegelova J, Fiser B, Bonnin P. Intra- and extracranial artery blood velocity during a sudden blood pressure decrease in humans. Eur J Appl Physiol 1997;76:289-293.
2. Dusek J, Siegelova J, Fiser B, Savin E. Blood pressure-carotid blood flow relationship in treated and non-treated and non-treated hypertensives. J Hypertens 1997;15:140.
3. Krticka A, Honzikova N, Fiser B. Spectral method of continuous determination of baroreflex sensitivity. Med & Biol Eng & Comp 1997;35:533.
4. Spaak J, Sundblad P, Linnarsson D. Human carotid baroreflex during isometric lower arm contraction and ischemia. Am J Physiol 1998;275:H940-945.
5. Langewouters GJ, Settels JJ, Roelandt R, Wesseling KH. Why use Finapres or Portapres rather than intra-arterial or intermittent non-invasive techniques of blood pressure measurement? J Med Eng Technol 1998;22:37-43.
6. Convertino VA, Baumgartner N. Effects of hypovolemia on aortic baroreflex control of heart rate in humans. Aviat Space Environ Med 1997;68:838-843.
7. Honzikova N, Fiser B, Penaz J, Honzik J. The relationship between spontaneous fluctuations in diastolic pressure and in the rate of blood pressure decrease during diastole. Homeostasis 1995;36:160-164.
8. Honzikova N, Fiser B, Honzik J. Noninvasive determination of baroreflex sensitivity in man by means of spectral analysis. Physiol Res 1992;41:31-37.
9. Mancia G. Bjorn Folkow Award Lecture. The sypmpathetic nervous systém in hypertension. J Hypertens 1991;15:1553-1565.
10. Sleight P. The importance of the autonomic nervous systém in health and disease. Aust NZJ Med 1997;27:467-473.
11. Schwartz PJ. The autonomous nervous systém and sudde death. Eur Heart J 1998;(Suppl F):F72-80.
12. La Rovere MT, Schwartz PJ. Baroreflex sensitivity as a cardiac and arhythmic mortality risk stratifier. Pacing Clin Electrophysiol 1997;20:2602-2613.
13. La Rovere MT, Bigger JT, Marcus FI, Mortara A, Schwartz PJ. Baroreflex sensitivity and heart-rate variability in prediction of total cardiac mortality after myocardial infarction. ATRAMI (Autonomic Tone and Reflexes After Myocardial Infarction) Investigators. Lancet 1998;351:478-484.
14. Semrad B, Fiser B, Honzikova N. Ageing and cardiac autonomic status. In: Malik M, ed. Clinical Guide to Cardiac Autonomic Tests. Dordrecht: Kluwer Academic Publishers, 1998:285-300.
15. Fiser B, Honzikova N, Semrad B. Blood pressure control by baroreflex in patients after myocardial infarction. Arch Physiol & Biochem 1997;105:243.
16. Honzikova N, Fiser B, Semrad B. Autonomic nervous systém activity and myocardial infarction. Fund & Clin Pharmacol 1998;12(Suppl 1):76.
17. Honzikova N, Semrad B, Fiser B, Labrova R. Risk stratification after myocardial infarction. Scripta Medica 1997;70:153-158.
18. Maestri R, Pinna GD, Mortara A, La Rovere MT, Tavazzi L. Assessing baroreflex sensitivity in post-myocardial infarction patients: Comparison of spectral and phenylephrine techniques. J Am Coll Cardiol 1998;31:344-351.
19. Pitzalis MV, Mastropasqua F, Passantino A, Massari F, Liguargo L, Forleo C, Balducci C, Lombardi F, Rizzon P. Comparison between noninvasive idicies of baroreceptor sensitivity and the phenyleprine method in post-myocardial infarction patients. Circulation 199897:1362-1367.
20. Semrad B, Honzikova N, Fiser B, Labrova R. Prediction of the risk for sudden cardiac death. Scripta Medica 1998;71:247-251.
21. Odemuyiwa O, Farrell TG, Staunton A, Sheddon J, Poloniecki J, Bennett D, Malik M, Camm AJ. Influence of thrombolytic therapy on the evolution of baroreflex sensitivity after myocardial infarction. Am Heart J 1993;125:285-297.

22. Honzikova N, Semrad B, Fiser B. Baroreflex sensitivity and heart rate variability in patients after myocardial infarction: Effect of thrombolytic therapy. In: Man IntVeld AJ, van Montfrans GA, Langewouters GJ, Lie K, Mancia GBV, eds. Measurement of Heart Rate and Blood Pressure Variability in Man. Amsterdam: Van Zuiden Communications, 1995:113-117.
23. Honzikova N, Semrad B, Fiser B, Labrova R. Heart rate variability and baroreflex sensitivity in helthy subjects and in postinfarction patients treated by thrombolysis. Exp Clin Cardiol 1997;2:60-72.
24. Mortara A, Specchia G, La Rovere MT, Bigger JT, Marcus FI, Camm JA, Hohnloser SH, Nohara R, Schwartz PJ. Patency of infarct-related artery. Effect of restoration of anterograde flow on vagal reflexes. Circulation 1996;93:1114-1122.
25. Al-Kubati M, Fiser B, Siegelova J. Baroreflex sensitivity during psychological stress. Physiol Res 1997;46:27-33.
26. Fiser B, Honzikova N, Semrad B, Novakova Z. Aging and baroreflexsensitivity. Fund Clin Pharma 1998;12(Suppl 1):74.
27. Fiser B, Honzikova N, Novakova Z, Hrstkova H. Baroreflex sensitivity in children and young adults. J Physiol (London) 1998;511:113P.
28. Parati G, Di Rienzo M, Bertinieri G, Pomidossi G, Cassadei R, Groppelli A, Pedotti A, Zanchetti A, Mancia G. Evaluation of the baroreceptor- Heart rate reflex by 24-hour intra-arterial blood pressure monitoring in humans. Hypertension 1988;12:214-222.
29. Krticka A, Honzikova N, Fiser B. Method for continuous determination of baroreflex sensitivity in man. Med & Biol Eng & Comp 1996;34:105-106.

FROM THE FIRST SPECTRAL ANALYSIS OF BLOOD PRESSURE VARIABILITY TO THE PREDICTION OF SUDDEN CARDIAC DEATH: ONE ASPECT OF A SCIENTIFIC CONTRIBUTION OF PROFESSOR FISER

Nataša Honzíková

Department of Physiology, Faculty of Medicine, Masaryk University, Brno

Address correspondence to:

Prof. MUDr. Nataša Honzíková, CSc.
Department of Physiology
Faculty of Medicine, Masaryk University
Komenského nám. 2
662 43 Brno

Support: MSM 141 100004 (CZ).

The contemporary analysis of cardiovascular responses requires a strictly quantitative approach. This appeared a proper field of Fišer´s mathematical talent already 30 years ago. In my contribution I would like to mention one of many facets of Fišer´s activities. His contribution to the quantification of a short-term variability in circulation appears primary. In my recapitulation, I would like to turn to an overview of key studies done by our group with Professor Jan Peňáz at the Department of Physiology, in logical continuity - from their first attempt to perform spectral analysis of heart rate variability and plethysmogram by an analog method to a promising application of spectral analysis of heart rate and blood pressure variability in prediction of sudden cardiac death. The above mentioned pioneer study was done in the sixtieth of the last century and nowadays the spectral analysis of circulatory parameters expands into clinical medicine as a non-invasive diagnostic tool.

The history of research on short-term rhythmic fluctuations of systemic blood pressure or heart rate is over one hundred year old. Fundamental discoveries and classification of respiratory and Traube-Hering-Mayer waves in circulation were vague. However the possibility of analyzing circulatory rhythms exactly using computer methods has led to renewed interest in this phenomenon. The first attempt to quantify spontaneous waves in the plethysmogram and heart rate as an amplitude spectrum was realized by an analog method in our department by Peňáz and Fišer [1]. The first mathematical spectral analyses were realized by Peňáz during his short stay in the Netherlands. These studies

were really "pioneer" studies in this field. Power spectra of plethysmogram, heart rate and respiration were published as first [2], later spectral analysis of blood pressure was added [3].

These first hopeful results stimulated us to perform a wide-ranging quantification of short-term variability in blood pressure, heart rate, plethysmogram and respiration by Fourier transformation in Brno. We had this possibility thanks an unrivalled device at that time - a plethysmomanometr which Peňáz had patented in 1969. Such an analysis was possible only by computer which was not at our Department at that time and so we realized our project in cooperation with Computer Department of the Faculty of Electrical Engineering of Brno Technical University. The results were first presented at the Annual Meeting of the Physiological Society [4], later in extenso [5, 6]. These papers were not only the first papers dealing with spectral analysis of short-term blood pressure variability in the journal, but they included also a comparison of differences in spectra of systolic, diastolic and pulse pressures together with spectra of heart rate, finger blood flow and respiration. Individual differences in spectra were described together with their individual reproducibility within 20 minutes. The cross-correlation and cross-spectral analysis was applied for the determination of coherence and time relationships between variability in various parameters. It is necessary to stress that cross-spectral analysis was later a basis for calculation of transfer function between the variability in systolic blood pressure and heart rate and a non-invasive determination of baroreflex sensitivity. In between, but for a relatively long time, no other laboratory advanced the spectral analysis of blood pressure - untill the American device Finapres based on Peňáz´ principle occured on the market.

We have systematically performed a number of studies of physiology of circulatory rhythms dealing with the individual characteristics of these rhythms [7]. The studies on the relationships between rhythms in respiration and circulation [8, 9] are principal from the point of view of their reproducibility [10] and the determination of baroreflex sensitivity as I will mention later. Our interest to get the information about ontogenesis of the circulatory rhythms was a logical next step. Spectra of heart rate in age-groups of newborns, children (6 years) and two groups of adults (20 and 60 years) showed the increase of respiratory sinus arrhythmia in children and young adults which corresponds to the development of parasympathetic activity, and the predominance of the 0.1 Hz rhythm in young adults. In this study we have also shown the shift in a frequency of the respiratory and 0.1 Hz rhythms with age [11]. Comparing mature, premature newborns, those with respiratory distress syndrome (RDS) and who later died for RDS we compared the presence of 0.1 Hz rhythm. Its amplitude increases with the maturity of the child [12]. In newborns we have followed also 24 hour rhythm in heart rate and we have described a rhythm slower than the circadian one, of 10.5 hours on average [13].

Naturally, we also analysed changes in the circulatory and respiratory power spectra during mental load [14] and during exercise in healthy adults [15].

During the eighties and nineties, dozens of similar physiological studies of blood pressure variability were performed in many laboratories. Next qualitative step in application of spectral analysis of variability in circulation was the calculation of baroreflex sensitivity as a gain of transfer function between variability in systolic blood pressure and pulse intervals. It was Robe [16], who realized such calculation as the first. Till that time, baroreflex sensitivity was determined after an application of a vasoconstrictor drug phenylephrine. Fišer´s merit was that this fully non-invasive method was modified into a physiologically more pure approach: we have conducted our own

approach based on experience with the relationship between respiration and circulation - controlled breathing at higher frequency of respiration (20 per min.) is used and transfer function is calculated at a frequency of 0.1 Hz [17-19].

Calculation of the baroreflex sensitivity opened new possibilities for interpretation of estimation of the role of sympathetic and parasympathetic system in regulation of circulation [20, 21]. It is substantially important for studies of genesis of hypertension [22, 23]. But till now, clinically most important seem to be, the studies concerning the prediction of risk of sudden cardiac death after myocardial infarction. In this respect, we have co-operated with Professor Bořivoj Semrád and the 1st Department of Internal Medicine – Cardiology [24-30]. Our results have clearly showen that the baroreflex sensitivity calculated by spectral analysis gives comparable results with phenylephrine method [31]. Predictive value of non-invasive risk parameters is very high, but still, it is not reliable enougt for clinical decision [32]. Actually, not only new mathematic approaches are needed, but also new studies because of the changes in therapy of myocardial infarction.

The above insight on studies of baroreflex sensitivity, heart rate and blood pressure variability is not in any case a comprehensive review of the scientific studies of professor Fišer, but it shows, how studies all-theoretic at their beginning, contribute to solving of important clinical tasks. Naturally, professor Fiser co-operates also with other clinical departments of our Faculty. Moreover, the spectral analysis of circulatory parameters has developed into a standard methodology at other departments. Thus it is obvious that professor Fišer has a formating part on the introduction of this method into physiology and medicine.

LITERATURE

1. Peňáz J, Fišer B: Amplitudové spektrum spontánních vln plethysmogramu a tepové frekvence u člověka. Čs fysiol 1966; 15: 517.
2. Penaz J, Roukens J, vd Waal HJ: Spectral analysis of some spontaneous rhythms in the circulation. Biokybernetic I, Karl-Marx University Leipzig 1968; 233-236.
3. Penaz J, Roukens J: Spectral analysis of spontaneous fluctuations of blood pressure, heart rate and vascular volume in man. 4th Internat Biophysics Cong., Abstracts, Moskva 1972; 167.
4. Honzíková N, Peňáz J, Fišer B: Spectral analysis of spontaneous fluctuations of some cardiovascular variables. Physiol Bohemoslov 1975; 24: 441-442.
5. Peňáz J, Honzíková N, Fišer B: Spectral analysis of resting variability of some circulatory parameters in man. Physiol Bohemoslov, 1978; 27: 349-357.
6. Fišer B, Honzíková N, Peňáz J: Power spectra of spontaneous variations of indirectly recorded blood pressure, heart rate and acral blood flow. Automedica, 1978; 2: 143-147.
7. Honzíková N, Peňáz J, Fišer B: Interpretation of differences in power spectra of blood pressure, heart rate and respiration in man. In: Chronobiology and chronomedicine (Eds. G Hildebrandt., R Moog, F Raschke), Peter Lang Frankfurt am Main/Bern/New York/Paris 1987; pp. 172-176.
8. Honzíková N, Peňáz J: Influence of modified respiratory rhythm on fluctuations of circulatory parameters. Scripta Medica 1985; 58(6): 323-336.
9. Honzíková N, Peňáz J, Fišer B, Honzík J: The relationship between spontaneous fluctuations in circulation and depth and rate of respiration. Homeostasis 1995; 36: 165-169.
10. Honzíková N, Peňáz J, Fišer B: Individual features of circulatory power spectra in man Eur J Appl Physiol, 1990; 59, 430-434.,

11. Honzíková N, Konvičková E, Fišer B, Fialová Z: Ontogenese der Herzfrequenzvariabilität beim Menschen. Wiss Zeitschrift der Humboldt-Universität zu Berlin, R Med 1990; 39(2) : 93-94.
12. Honzíková N, Fišer B, Konvičková E: Spectral analysis of heart rate variability in premature newborns. In: Chronobiology and chronomedicine. (Eds.: Gutenbrunner Ch, Hildebrandt G, Moog R) Peter Lang Verlag Frankfurt am Main/Berlin/Bern/New York/Paris/Wien 1993; pp. 98-102.
13. Fišer B, Honzíková N, Konvičková E, Kalábová H: Die Herzfrequenzfluktuationen von Neugeborenen in Laufe von 24Stunden. Wiss Zeitschrift der Humboldt-Universität zu Berlin, R Med 1990; 39(2): 132-133.
14. Honzíková N, Peňáz J, Fišer B: Power spectra of blood pressure and heart rate fluctuations during mental load. J. of Interdisciplinary Cycle Research 1988; 19(1), 75-79.
15. Weise F, Fišer B, Honzíková N: Cardiac baroreflex function during static muscular contraction assessed by finger arterial pressure and pulse interval spectral analysis. Homeostasis 1995; 36: 170-176.
16. Robe HWJ, Mulder LMJ, Ruddel H, et a.: Assessment of baroreceptor reflex sensitivity by means of spectral analysis. Hypertension 1987; 10: 538-543.
17. Fišer B, Honzíková N: Spectral analysis of heart rate and blood pressure in man. In: International symposium on hypertension. (Eds. WH Birkenhänger, F Halberg, P Přikryl). Brno 1990; 38-46.
18. Honzíková N, Fišer B: Spectral analysis of circulatory rhythms and baroreflex sensitivity in man. In: Rhythms in physiological systems. Eds. H Haken, HP Koepchen). Springer, Berlin/ Heidelberg/ NewYork/ London/ Paris/ Tokyo/ Hong Kong/ Barcelona/ Budapest 1991; pp. 97-102
19. Honzíková N, Fišer B, Honzík J: Noninvasive determination of baroreflex sensitivity in man by means of spectral analysis. Physiol Res 1992; 41: 31-37.
20. Fišer B, Honzíková N, Peňáz J: Baroreflex heart rate sensitivity studied by spectral analysis during exercise. Wiss Zeitschrift der Humboldt-Universität zu Berlin. R Medizin 1992; 41(4): 71-75.
21. Honzíková N, Fišer B, Kukleta M, Honzík J, Peňáz J: Baroreflex sensitivity assessed by spectral analysis. Wiss Zeitschrift der Humboldt-Universität zu Berlin. R. Medizin 1992; 41(4): 67-70.
22. Fišer B: Škodlivý účinek zvýšené sympatické aktivity u arteriální hypertenze. Cor et Vasa 1999; 41(5): 241-246.
23. Honzikova N, Zavodna E, Novakova Z, Fiser B, Lokaj P, Paderova J, Vaclavkova P, Hrstkova H: Baroreflex sensitivity and blood pressure variability in hypertensive children, children with white coat effect and healthy children. J f Hypertension 2003; 21 (suppl 4), S10.
24. Fišer B, Semrád B, Honzíková N, Lábrová R: Circadian variation of heart rate variability in post-infarction patients. In: Biosignál 94, Rozman J. (ed.), El Eng Soc, Brno 1994; pp.196-198.
25. Semrád B, Honzíková N, Fišer B: Metody určení rizika náhlé srdeční smrti u nemocných po infarktu myokardu. Cor at Vasa 1994; 36(6): 295-298.
26. Honzíková N, Semrád B, Fišer B, Lábrová R: Heart rate variability and baroreflex sensitivity in healthy subjects and in postinfarction patients treated by thrombolysis. Exp Clin Cardiol 1997; 2(1) : 69-72.
27. Semrád B, Fišer B, Honzíková N: Ageing and cardiac autonomic status. In: Clinical Guide to Cardiac Autonomic Tests. Ed. M. Malik. Kluwer Academic Publishers, Dordrecht, Boston, London 1998; 285-300.
28. Semrád B, Fišer B, Honzíková N: Baroreflex sensitivity: Do we have any progress? Cardiac Electrophysiology Review 1999; 3(4): 290-293.
29. Semrád B, Fišer B, Honzíková N: Nonlinear structure analysis of inter-beat interval data in patients after myocardial infarction. Scripta Medica 2000; 73(3): 191-194.
30. Semrád B, Honzíková N, Fišer B: Ejection fraction and baroreflex sensitivity in patients after myocardial infarction. In: Proceedings, Symposium The importance of chronobiology in diagnosing and therapy of internal diseases, eds. Halberg F, Kenner T, Fišer B, Brno, 2002; pp.159-163.
31. Honzikova N, Fiser B, Semrad B: Critical value of baroreflex sensitivity determined by spectral analysis in risk stratification after myocardial infarction. PACE 2000; 23 [Pt. II]: 1965-1967.
32. Honzíková N, Semrád B, Fišer B, Lábrová R: Baroreflex sensitivity determined by spectral method and heart rate variability, and two-years mortality in patients after myocardial infarction. Physiol Res 2000; 49: 643-650.

MEMORY MECHANISMS IN THE FORMATION AND MAINTENANCE OF STRESS STIMULI

Miroslav Kukleta

Department of Physiology, Medical Faculty, Masaryk University, Brno

Address correspondence to:

Prof. MUDr. Miroslav Kukleta, CSc.
Department of Physiology
Faculty of Medicine, Masaryk University
Komenského nám. 2
662 43 Brno

Support: MSM 141 100004 (CZ).

One of the advances of neurosciences with strong impact on clinical practice is the present understanding of how our brains are processing danger. Possibility to react appropriately to threatening events is the function of basic importance for both animals and humans. Much of current knowledge on the neurobiological basis of defense behavior can be traced back to four great developments in the former half of the 20th century:

- Cannon (1925) and Bard's (1928) work on sham rage;
- Papez circuit model of emotion (1937);
- Kluever and Bucy's observations on "psychic blindness" (1937);
- MacLean's conception of the visceral brain or limbic system (1952).

More recent findings have imposed reexamination of the limbic system theory of emotion (LeDoux, 1991), as have imposed reexamination of the anatomical concept of a limbic system (Brodal, 1982; Kotter and Meyer, 1992).

The crucial step in the neurobiological approach to any mental or behavioral function is to identify the neural system that mediates the function in question. At least in animal, the system mediating behavioral defense can be delineated within the brain, and conceptions about its functional organization are also available. Key structures of the system belong to the limbic forebrain and can be listed as follows: olfactory cortices, amygdalar and septal nuclei, hippocampus, gyrus parahippocampalis, gyrus cinguli, and hypothalamic nuclei. Numerous pathways mutually interconnect components of the system, and many other pathways connect them with the brain areas which elaborate sensory information from the outer and inner world.

The system of behavioral defense has the access to all sensory information processed in a given moment in the brain and the evaluation of behavioral value of these stimuli is the first step in its functioning. All the forms of brain memory participate in this evaluation, which leads to the conclusion whether or not the situation comprises threat. When signs of threat are recognized, the system triggers a defense response.

IMPLICIT MEMORY INVOLVEMENT

The behavioral value of a stimulus is determined by associated information embodied in the form of genetically mediated memory or memory resulting from learning. The progress achieved in animal studies on the neural basis of emotional learning has considerably improved our understanding of the brain mechanisms engaged in the decoding the stimulus value, which is based on implicit memory (LeDoux, 1995). A large number of these studies have consistently supported the notion that the amygdala is the key structure in this process (Armony and LeDoux, 2000). While the conditioning of fear reactions to auditory stimuli, damage to the medial geniculate body or inferior colliculus also abolishes this capacity (LeDoux et al., 1990). Removing the auditory cortex does not interfere with the conditioning unless the task is not complicated. However, if the conditioning paradigm involves, for instance, discrimination training, where one stimulus is paired with shock and a different one is not, then lesions of the auditory cortex interfere with conditioning (Jarrell et al., 1987). The hippocampal formation is another structure implicated in the formation of salient stimuli and in their processing. Lesions of the hippocampus have no effect on conditioning fear to the simple auditory stimulus, but they interfere with the conditioning of fear to the context, i.e. to the whole experimental situation (Kim and Fanselow, 1992; Phillips and LeDoux, 1992).

Recent advances in functional neuroimaging techniques have made it possible to study how the human brain processes salient stimuli. Facial expressions that reflect basic emotions, as for instance fear or anger, are conjectured to possess innate value determined by evolutionary selection. Comparing the PET responses associated with processing fearful and happy expressions revealed the activation of the left amygdala and left paraamygdaloid cortex specific to the fear condition (Morris et al., 1996). When the behavioral significance of facial expressions, but not other aspects, was altered through aversive classical conditioning, the right hemisphere activations in the pulvinar, orbitofrontal cortex, superior frontal gyrus, and amygdala were observed in response to conditioned stimuli (Morris et al., 1997). In auditory conditioning, in addition to primary auditory cortex changes, increased activity in a region of the left inferoposterior thalamus that includes medial geniculate nucleus and pulvinar is also evident. This finding suggests that changes in effective connectivity between medial geniculate nucleus and auditory cortex accompany the processing of stimuli with new adaptive value (Friston et al., 1997). Measuring neuronal responses elicited by complex visual conditioned stimuli (neutral faces) by event-related functional magnetic resonance imaging revealed the most robust differential activation in bilateral cingulated and insular cortices and less pronounced differential responses in the amygdala, supplementary motor area and both red nuclei. In conclusion, functional imaging findings from the human brain complement the animal data that stress the importance of thalamoamygdala interactions in decoding stimulus value obtained through conditioning. At the same time, the involvement of other structures in this process is, in humans, also evident.

DECLARATIVE MEMORY INVOLVEMENT

At present, the study of processing the stimuli associated with declarative memory is not systematic. Available data from clinical studies have shown that damage to the amygdala interferes with non-declarative emotional memories but not declarative memories about emotions, whereas damage to the medial temporal lobe interferes with declarative memories about emotions but not with non-declarative emotional memories.

For example, patients with the amygdala lesions do not exhibit conditioned fear responses to the CS but remember that the CS was related to the US (patients with the hippocampal damage exhibit conditioned responses but have no memory of the CS-US pairing experience).

Data from EEG recording studies represent another important source of information relevant to the human brain. The mostly studied response to a behaviorally relevant stimulus is N2/P3a/SW complex, recorded from the human scalp with peaks at approximately 200, 280, and 350 ms after the auditory stimulus. Slightly before the scalp recorded response, peaks with similar waveforms and task correlates are generated in widespread cortical areas, especially in the cingulate gyrus, inferior parietal lobule and dorsolateral prefrontal cortex (Halgren and Marinkovic, 1995). The P3 component of N2/P3a/SW complex has received considerable attention since it has been linked to both attention and memory mechanisms. P3-like potentials have been reported in a variety of mammalian species including rats, cats, and monkeys (Palmer, 1994). Its ubiquitous occurrence across species is interpreted as the possibility that the P3 phenomenon represents activity of the basic neural system involved in the early detection and recognition of a significant sensory stimulus.

Following results illustrate our approach to the problem of how the human brain decodes the stimulus value. In one of our studies we analyzed EEG data recorded in epileptic patients by depth electrodes. During the pre-surgical evaluation of seizure origin, they were invited to participate in experimental session, in which a simple auditory stimulus was used as a warning event signaling the presentation of another stimulus following the first one after a 3 s interval. In the study, only EEG responses following closely the first stimulus were analyzed. Available recording contacts made it possible to investigate the temporal dynamics of event-related potentials recorded in the prefrontal cortex, temporal auditory cortex, and amygdalo-hippocampal complex.

In the prefrontal recordings, the most prominent neural activity observed (65% of cases) was a sharp waveform with two negative peaks at latencies of 108 ms and 285 ms, respectively. In the superior temporal gyrus, the classical form of auditory ERP was found in 58% of cases.

In the amygdala and hippocampus, the prevalent form of recorded ERP was a large solitary wave starting from 0.46 to 0.148 s after the S1 onset in the amygdala and from 78 to 207 ms after the S1 onset in the hippocampus. In several other sites, the elicited ERP was more complex consisting of several components. Mean onset values of potentials from selected areas are presented in Table 1.

Tab. 1. Onset values of evoked potentials elicited in selected brain areas by simple auditory stimulus, which was given behavioral value through verbal instruction.

Group of values	Mean onset value and SD
Superior temporal gyrus	21.0 ± 6.9 ms
Prefrontal cortex – first wave	37.7 ± 8.0 ms
Amygdala	101.4 ± 40.2 ms
Hippocampus	149.0 ± 48.9 ms
Prefrontal cortex – second wave	177.1 ± 15.4 ms

The comparison of these potentials demonstrated the following spatio-temporal sequence of mean onset values of activation during the processing of salient auditory stimulus: the auditory cortex, prefrontal cortex (first wave), amygdala, hippocampus, prefrontal cortex (second wave). Analysis of all available data allowed concluding that the first prefrontal wave could be related to the recognition of value of the first stimulus, and the second prefrontal wave to the activation of selective attention directed to the second stimulus. For more advanced interpretation of the results the data were not representative enough.

CLINICAL EXPERIENCE

Clinical experience about memory participation in the formation and maintenance of threat stimuli is especially abundant among anxiety neurotics, patients suffering from post-traumatic stress disorder, and psychosomatic patients exposed to sustained stress. In one of our studies we were looking for examples of memory participation in neurotic patients. Many stimuli from the patients' surroundings acquired their emotional value through conditioning. In approximately one quarter of cases a change of an originally inoffensive situation into a threatening one after the experience of the threat state was seen. Since that event, this situation or the mere image of it induced the state of threat for which the subject had no explanation. Following case reports demonstrate two of such cases.

Case 1. Male, 28 years, married hospitalized for agoraphobia with panic attacks lasting approximately for 6 months. Within the framework of a desensitization program he went for walks beyond the hospital grounds. During one such outing he came to a place where he experienced an intense state of threat. In the following five outings, he endeavored to reach the place again, but each trial led to an involuntary avoidance impulse preventing him from doing so.

More complex forms of associative learning were also involved in the evolution and maintenance of anxiety disorders. The next reports illustrate the personal experience of a real threat and the mediated confrontation with it at the onset of the development of neurotic symptoms.

Case 2. Male, 28 years, married hospitalized for generalized anxiety disorder lasting for about four months. Neurosis followed an anaphylactic shock developed during Penicillin therapy. Dyspnea and feeling of a threat to his life accompanied the state. Many times there was a spontaneous recall of the event that induced the anxiety. Falling to sleep was another powerful threatening situation. Besides the coincidence in time there was also a logical connection between the event and the form of neurosis.

Case 3. Male, 46 years, married hospitalized for generalized anxiety disorder lasting approximately for five months. Its beginning followed meeting a friend who died of cancer soon after. The remembrance of this event returned many times and emerged even in his dreams. Besides the coincidence in time there was also a logical connection between the event and the form of neurosis.

The knowledge about existence of danger acquired through declarative learning and memory represented the bases for the evolution of efficient threat stimuli and anxiety symptoms in other patients. Two following case reports demonstrate this type of learning.

Case 4. Male, 43 years, married hospitalized for generalized anxiety disorder lasting for about six months. The beginning of neurosis coincided with creating a false belief that repeated anxiety states lead eventually to myocardial infarction. This was based on his

physician's evaluation of his first anxiety attack (" Be careful, this time this was not a coronary, but next time it could easily be...").

Case 5. Male, 34 years, divorced, employed as a security worker in a shopping centre. His functional symptoms appeared after a series of well-organized break-ins in the centre. In one occasion, he was directly confronted with a group of masked robbers armed with sub-machine-guns. During one of his subsequent clinical examinations (in waiting room of neurological department) he red through a leaflet about vascular brain syndrome and he found all his symptoms among manifestations of this disease. Resulting misinterpretation of his anxiety symptoms increased the anxiety to the level necessitating psychiatric hospitalization.

As evident, various forms of learning and memory participate in the formation and maintenance of threat stimuli. From the practical point of view it is important to know that the conditioning and more complex forms of learning often occur simultaneously in the same patient and that therapeutic procedures designed to suppress their pathogenic role have to respect differences in their neural mechanisms. It is not by accident that one of the most efficient psychotherapeutic techniques (i.e. cognitive behavioral therapy) was designed to influence both the stimuli, which gained their pathogenic potency through conditioning and the more complex pathogenic mechanisms, as for instance a wrong interpretation of stimuli.

REFERENCES

J. L. Armony, J. E. LeDoux, How danger is encoded: Toward a systems, cellular, and computational understanding of cognitive-emotional interactions in fear, In: The new cognitive neurosciences, M. S. Gazzaniga, ed., The MIT press, Cambridge, Massachusetts, London, 2000, pp. 1067-1079.

P.A. Bard, A diencephalic mechanism for the expression of rage with special reference to the sympathetic nervous system, Am. J. Physiolol. 84 (1928) 490-515.

A. Brodal, Neurological Anatomy, New York: Oxford University Press, 1982.

W. B. Cannon, S.W. Britton, Pseudoaffective medulliadrenal secretion, Am. J. Physiol. 72 (1925) 283-294.

K. J. Friston, C. Buechal, G. Fink, J. S. Morris, E. T. Rolls, and R. J. Dolan, Psychophysiological and modulatory interactions in neuroimaging, Neuroimage 6 (1997) 218-219.

E. Halgren, and K. Marinkovic, Neurophysiological networks integrating human emotions, In: The cognitive neurosciences, M. S. Gazzaniga, ed., The MIT press, Cambridge, Massachusetts, London, 1995, pp. 1137-1151.

T. W. Jarrell, C. G. Gentile, L. M. Romanski, P. M. McCabe, and N. Schneiderman, Involvement of cortical and thalamic auditory regions in retention of differential bradycardia conditioning to acoustic conditioned stimuli in rabbits, Brain Res. 412 (1987) 285-294.

J. J. Kim, M. S. Fanselow, Modality-specific retrograde amnesia of fear, Science 256 (1992) 675-677.

R, Kotter, N, Meyer, The limbic system: A review of its empirical foundation, Behav. Brain. Res. 52 (1992) 105-127.

H. Kluever, P.C. Bucy, "Psychic blindness" and other symptoms following bilateral temporal lobectomy in rhesus monkeys, Am. J. Physiolol. 119 (1937) 352-353.

P.D. MacLean, Some psychiatric implications of physiological studies on frontotemporal portion of limbic system (visceral brain), Electroencephalogr. Clin. Neurophysiol. 4 (1952) 407-418.

J. Morris, C. D. Frith, D. Perrett, D. Rowland, A. W. Young, A. J. Calder, and R. J. Dolan, A differential neural response in the human amygdala to fearful and happy facial expressions, Nature 383 (1996) 812-815.

J. Morris, K. J. Friston, and R. J. Dolan, Neural responses to salient visual stimuli, Proc. R. Soc. Lond. B 264 (1997) 769-775.

J.E. LeDoux, Emotion and the Limbic System Concept, Concepts Neurosci. 2 (1991) 169-199.

J. E. LeDoux, C. F. Farb, D. A. Ruggiero, Topographic organization of neurons in the acoustic thalamus that projects to the amygdala, J. Neurosci. 10, (1990) 1043-1054.

J. E. LeDoux, In search of an emotional system in the brain: Leaping from fear to emotion and consciousness, In: The cognitive neurosciences, M. S. Gazzaniga, ed., The MIT press, Cambridge, Massachusetts, London, 1995, pp. 1049-1061.

K. A. Palmer, The neural substrates of cognitive event-related potentials: A review of animal models of P3, In: Cognitive Electrophysiology" ERPs in basic and clinical research, H. J. Heinze, T. F. Munte, and G. R. Mangun, eds., Boston: Birkhauser, 1994, pp. 300-333.

J.W. Papez, A proposed mechanism of emotion, Arch. Neurol. Psychiatry 79 (1937) 217-224.

R. G. Phillips, and J. E. LeDoux, Differential contribution of amygdala and hippocampus to cued and contextual fear conditioning, Behav. Neurosci. 106 (1992) 274-285.

ATHEROSCLEROSIS OF PRECEREBRAL ARTERIES IN CERVICAL SPONDYLOTIC MYELOPATHY

Zdeněk Kadaňka*, Stanislav Peška*, René Jura*, Josef Bednařík*, Richard Chaloupka,¶*

*Department of Neurology and the *Department of Orthopaedics, Masaryk University Brno and University Hospital Brno, Brno, Czech Republic.

Addressed for correspondence:

Prof. MUDr. Zdeněk Kadaňka, CSc.
Head, Department of Neurology
University Hospital Brno,
Jihlavská 20,
63900 Brno, Czech Republic.
E-mail: zkadan@fnbrno.cz.

Support: MSM 141 100004 (CZ).

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ABSTRACT

Study design. A prospective ultrasonographic study of precerebral arteries in a group of patients with cervical spondylotic myelopathy (CSM).
Objectives: The aim of the study was to evaluate the prevalence of atherosclerotic changes in precerebral arteries in patients with CSM, in order to assess the degree of the vascular risk, a factor that can significantly influence the outcome of surgery or conservative treatment.

Summary of background data. There are no available data dealing with the status of the precerebral arteries in patients with spondylotic cervical myelopathy.
Methods. The studied group consisted of 62 consecutive patients with CSM resulting from spondylosis. Duplex ultrasonographic assessment of precerebral arteries (carotid and vertebral arteries) was performed using high-resolution duplex ultrasound equipment.
Results. Thickening of the intimal or medial layers of the arterial wall was found in 20/62 (32.2%), stenosis of a. carotis <50% unilaterally or bilaterally in 7/62 (11.2%) and stenosis > 50% bilaterally in 2/62 (3.2%). In one case, vertebral arteries were not detected for anatomical reasons and in another one a signal was not obtainable from the right vertebral artery (aplasia? occlusion?), otherwise the findings were normal in this region.
Conclusion. The distribution of atherosclerotic changes in the precerebral arteries of patients with CSM is similar to that in the general population and is increased in the

presence of vascular risk factors. In spite of the low prevalence of significant stenotic changes in extracranial arteries, we suggest that ultrasonographic exploration should be made part of routine presurgical investigation in CSM, particularly in elderly patients with atherosclerotic risk factors in order to prevent unexpected cerebral events during surgery and in the postoperative period.

Key words: cervical spondylotic myelopathy, ultrasonography, atherosclerosis, precerebral arteries

Precis: The atherosclerotic changes in precerebral arteries were studied prospectively in a group of 62 patients with cervical spondylotic myelopathy. Stenosis of a. carotis > 50% was found in 2/62, stenosis <50% in 7/62 (11.2%). Authors suggest that ultrasonographic exploration of the precerebral arteries should be a part of a routine presurgical investigation.

INTRODUCTION

The relative percentage of patients over 60 years who undergo spinal operations for spondylotic cervical myelopathy (CSM) has increased in recent decades (12), which simultaneously increases the operative risks associated with ageing, including atherosclerosis. This is a reason for being informed precisely about the precerebral vascular system. It can also help substantially to prevent cerebral ischemia during surgery, or to dictate the choice of conservative treatment, a process that can span years. Furthermore, it could be helpful for differential diagnosis, which is often difficult in CSM. Although carotid artery injury has been discussed as a potential complication of cervical surgery with the anterior approach (7,14), it has been reported only twice in actual cases (3,9). The frequency, probability and extent of stenosis in magistral cerebral arteries has not yet been studied systematically in patients with CSM. The distribution of these findings could be different from that in the general population because the vascular factor is mostly included in the pathogenesis of this disorder according to histological findings in the cervical cord and because both are more frequent in elderly.

The aim of this study is to estimate the frequency and degree of atherosclerosis in the precerebral arteries in patients with CSM.

METHODS

Sixty two patients suffering from CSM resulting from spondylosis were studied prospectively. The group included fourteen females, mean age 60.3 ± 6.8 and forty-eight males, mean age 58.3 ± 6.5. The Modified JOA score1 of the whole group was 14.0 ± SD 2.0: in females 14.6 ± 1.7 and in males 13.8 ± 2.0. Clinical, neuroimaging and electrophysiological methods detected clinical or subclinical signs of CSM in all of them. Thirteen underwent cervical spinal surgery and forty-nine were treated conservatively.

Duplex ultrasonographic assessment of the precerebral arteries (carotid and vertebral arteries) was performed using a Phase 2 Biosound high-resolution duplex ultrasound instrument in all subjects. Its system design is specifically tailored to vascular applications. The system incorporates B-mode (grey scale) real-time imaging and Doppler spectral frequency analysis (pulsed/continuous wave Doppler).

A 10-MHz phasar multiple frequency linear array transducer with high-resolution imaging in near field (imaging frequency 3.5-10,0 MHz) and dual mode Doppler 8/6.5 MHz was used, as well as an additional 5-MHz continuous wave Doppler transducer.

Patients were examined in a supine position, with the head slightly turned away from the sonographer. Both the right and left carotid (CCA, ICA, ECA) and vertebral arteries (pre- and intervertebral portion) were carefully scanned in the longitudinal and transverse view by an experienced physician. In addition, periorbital circulation was assessed by means of the CW Doppler transducer.

The measurements were performed under standard conditions. Anatomical position, calibre of the arteries, vessel wall morphological abnormalities, especially intimal changes, and the presence of plaques, including their characteristics, were all noted. The magnitude of the stenosis was calculated using the formula (1-D/N) x 100%, where D is the diameter of the stenotic lumen and N is the normal ICA diameter of the a.carotis interna. This formula was applied according to the method used in the ECST study5. As a standard, the following Doppler measurements were taken: peak systolic velocity, end-diastolic velocity, systolic/diastolic velocity ratio (S/D) and resistance index (RI).

RESULTS

1. The ultrasonographic findings from the carotid territory in a group of 62 patients suffering from cervical spondylotic myelopathy are summarised in Tab. 1. Only 51.6 % of them showed normal arterial wall; in two (3.2%) the stenosis was critical. In one case, vertebral arteries were not detected for anatomical reasons and in another one a signal was not obtainable from the right vertebral artery (aplasia? occlusion?). Otherwise the findings were normal in this region.
2. The presence of vascular risk factors in the studied group is shown in the Table 2. Most frequent are diabetes and hypertension - both 14.5%, smoking in 9.7% and alcohol consumption more than 100 g/day in 6.4%.
3. The relation between the presence of vascular risk factors and presence of the atherosclerotic changes in the precerebral arteries is shown in the table 3.

The correlation is more pronounced with an increasing degree of atherosclerotic change.

DISCUSSION

The need for the investigation of precerebral arteries in patients with CSM, particularly in the presurgical work-up, has not been stressed adequately in literature. After finding critical carotid stenosis in two patients with CSM, we started systematically to investigate the precerebral arteries in this disorder.

The importance of investigation of these vessels is accentuated by the fact that the relative percentage of patients over 60 years who undergo spinal operations has increased in recent decades (12), simultaneously with an increase in operative risks associated with ageing, including atherosclerosis. Furthermore, the surgeon can now decompress the cord more aggressively, which raises the risk of injury to the stenotic precerebral arteries already mentioned.

Thus, information about the vascular system could be interesting in this group of patients from several points of view. Firstly, it is necessary preoperatively to be aware before the proposed operation of eventually significant stenotic processes in the

precerebral arteries because the carotid arteries are situated in the operation field and can be directly or indirectly (by malposition or movement of the head) more easily injured in pre- and postoperative phases, as well as during the operation itself. In performing the anterior approach, it is necessary to go through the alar fascia, which spreads like a wing behind the oesophagus and surrounds the carotid sheath structures laterally (15).

Secondly, the vascular system can be involved in the pathogenesis of CSM since both the occuring cervical degeneration and atherosclerosis accelerate with advancing age. Thirdly, it is important for the differential diagnosis of CSM, which can be difficult, notably in the elderly population. Clinical features suggestive of ischemia in the carotid and vertebrobasilar territory are shared in CSM (sensory loss, clumsiness in the hands, arms, and legs).

A tight stenosis was revealed in 2/62 patients (3.2%) in this study. In both, one carotid artery was completely occluded and the opposite stenotic (90% and 70 %, respectively), the finding exposing a very high operative risk. Another 27/62 patients (43.5%) showed atherosclerotic changes in precerebral arteries of a less extensive nature. It is not easy to compare these data with those in literature because the precerebral arteries have not been systematically studied by ultrasonography in patients with CSM and there are not many studies of the precerebral arteries in the general population. However, the distribution of atherosclerotic changes was similar to that in Finnish men (ages 42, 48, 54, and 60 years), in whom the prevalence of carotid atherosclerosis detected with high resolution B-mode ultrasonography showed in 37% a thickening of the intimal or medial layer of the arterial wall, 10% had plaques, 2% had stenosis in the right or left common artery or in the carotid bifurcation, and only 51% were free of any detectable carotid atherosclerosis (13).

This finding is in correlation with the low relation between atherosclerosis in major vessels and CSM. The role of the vascular factor in the pathogenesis of CSM is still an enigma. The long duration of the disease (several or many years) and its slowly progressive course have not supported the view of its significance. Nor is there any method in current clinical practice for the estimation of its involvement. Distortion and compression of small vessels in the cord may have a pathogenic influence (2,4,6), but there is no correlation with atherosclerosis of major vessels or with obstruction of blood flow in the anterior spinal artery (8,10). Opinions about the influence of the venous stasis also remain controversial.

Patients operated for CSM are at risk of cerebral ischemic event in vertebrobasial territory, too. Visual loss in a group of 37 patients after spinal surgery, in which most deficits were permanent, has been described (11). Speculative intraoperative risk factors considered included patients' positioning, blood loss, intraoperative hypotension, and other known vascular risks such as hypertension, diabetes, smoking, miscellaneous vascular diseases, and increased blood viscosity.

CONCLUSIONS

Stenotic atherosclerotic changes in the precerebral arteries in patients with CSM detected by ultrasonography were revealed in 14.4 % of the sample and in 3.2 % reached more than 50%. A high percentage of patients with stenosis of the carotid arteries displayed some atherosclerotic risk factor. Normal arterial findings were registered in 51.5%. This distribution is similar to that in the general population and does not support the significance of vascular factor in pathogenesis of CSM.

We suggest that ultrasonographic exploration of the precerebral arteries should be a part of routine presurgical investigation to prevent unexpected cerebral events during surgery and in the postoperative period, particularly in elderly patients with atherosclerotic risk factors. As it is important information for differential diagnosis, it is necessary to address the problems of carotid stenosis before spinal surgery in order to take measures to avoid carotid mechanical or positional injury.

REFERENCES

1. Benzel EC, Lancon J, Kesterson, L, Haden T. Cervical laminectomy and dentate ligament section for cervical spondylotic myelopathy. J Spin Dis 1991;4: 286-95.
2. Bohlman HH, Emery SE. The pathophysiology of cervical spondylosis and myelopathy. Spine 1988; 13: 843-7.
3. Chozick BS, Watson P, Greenblatt SH. Internal carotid artery thrombosis after cervical corpectomy. Spine 1994; 19: 2230-2.
4. Cusick JF. Pathophysiology and treatment of cervical spondylotic myelopathy. Clin Neurosurg 1991; 37: 661-81.
5. European Carotid Surgery Trialists´ Collaborative Group. MRC European carotid surgery trial: interim results for symptomatic patients with severe (70-99%) stenosis or with mild (0-29%) stenosis. Lancet 1991; 337: 1235-44.
6. Gooding MR, Wilson CB, Hoff JT. Experimental cervical myelopathy: effects of ischemia and compression of the canine cervical spinal cord. J Neurosurg 1975; 43: 9-17.
7. Graham JJ. Complications of cervical spine surgery. Spine 1989; 14: 1046-50.
8. Hughes JT. Pathology of the spinal cord. London: Loyd Luke, 1978.
9. Lesoin F, Bouasakao N, Clarisse J, Rouseaux M, Jomin M. Results of surgical treatment of radiculopathy caused by cervical arthrosis based on 1000 operations. Surg Neurol 1985; 23: 350-5.
10. Monro P. What has surgery to offer in cervical spondylosis? In: Warlow CH, Garfield J eds. Dilemmas in the management of the neurological patients. Edinburgh:Churchill Livingstone, 1984.
11. Myers MA, Hamilton SR, Bogosian AJ, Smith CH, Wagner TA. Visual loss as a complication of spine surgery. Spine 1997; 22: 1325- 29.
12. Probst C. Neurochirurgie im Alter. Schweiz Runsch MedPrax 1991; 80: 1369-79.
13. Salonen R, Seppanen K, Rauramaa R, Salonen JT, Salonen JT. Ischemic stroke. Arteriosclerosis 1988; 8(6): 788-92.
14. Tew JMJr, Mayfiled FH. Complications of surgery of the anterior cervical spine. Clin Neurosurg 1975; 23: 424-34.
15. Whitecloud III TS, Kelley LA. Anterior and posterior surgical approaches to the cervical spine. In: The adult spine: principles and practice. Sec. Edition. Frymoyer JW ed., Lippincott-Raven, Philadelphia, New York, 1997.

Table 1 . The ultrasonographic findings of precerebral arteries in a group of 62 patients with CSM. In the second column, the number of patients, in the third the per cent from the whole group, and in the forth the mean age are given

Ultrasonographic findings n=62	Number of patients	%	Mean age, years
normal	32	51.6	55.1
thickening of the intimal or medial layer of the arterial wall	20	32.2	63.9
stenosis of a.carotis unilat. or bilat. <50%	7	11.2	64
stenosis of a.carotis bilat. >50%	2	3.2	68

Table 2. Distribution of vascular risk factors in a group of 62 patient with CSM. In the second column the number of patients and in the third the percentage of the whole group are given.

Risk factor	No. of pts	%
Hypertension	9	14.5
Smoking > 10 cig/day	6	9.7
Alcohol intake > 100g/day	4	6.4
Diabetes	9	14.5

p

Table 3. Relation between ultrasonographic findings in precerebral arteries and presence of vascular risk factors (hypertension, diabetes, smoking and high alcohol intake) in a group of 62 patients with CSM. The third column expresses a percentage of the patients with present risk factors relative to the whole group with

Ultrasonographic finding	Risk factor +	Risk factor -	%
normal	6	26	18.7
thickening of the intimal or medial layer of the arterial wall	7	13	35
stenosis of a.carotis unilat. or bilat.<50%	4	3	57.1
stenosis of a.carotis bilat. >50%	2	0	100

SOME CIRCULATORY PARAMETERS IN CHILDREN AFTER ANTITUMOUR THERAPY

Hana Hrstkova, *Nataša Honzikova, *Zuzana Novakova, *Eva Zavodna

Department of Paediatrics, Faculty of Medicine and *Department of Physiology, Masaryk University, Brno, Czech Republic

Address correspondence to:

Prof. MUDr. Hana Hrstkova, CSc.
Department of Paediatrics
Faculty of Medicine
Masaryk University
University Hospital Brno,
Černopolní 9
613 00 Brno, Czech Republic.

SUMMARY

Introduction: The aim of the study was the comparison of body and circulatory parameters in healthy subjects, and children and adolescents who were previously treated for a malignant tumour. The evaluation of relationship between plasma lipids and changes in circulatory regulation in subjects after treatment for a malignant tumour was our second task.

Methods: We examined 206 healthy children and adolescents (group C) and 97 subjects after treatment for a malignant tumour (group T) in age 11-21 years with remission in average 3 years. In all subjects we recorded pulse intervals (PI), systolic (SBP) and diastolic (DBP) blood pressures beat-to-beat by non-invasive methods, and we calculated the baroreflex sensitivity (BRS in ms/mmHg). We used standard deviation for variability estimation of PI (PIsd), SBP (SBPsd) and DBP (DBPsd). Plasma lipids were examined: cholesterol, HDL, LDL and triglycerides (TG). Subjects T were divided according to the level of cholesterol into two groups: 84 subjects (group TL) with cholesterol lower than 5 mmol/l and 13 subjects (group TH) with cholesterol higher than 5 mmol/l. The mean values and standard deviation of age, PI, PIsd, SBP, SBPsd, DBP, DBPsd, BRS, cholesterol, HDL, LDL and TG, body weight, height and the body mass index (BMI) were determined.

Results: Children and adolescents in group T had higher BMI ($p<0.01$), prolongation of PI ($p<0.01$), increased TIsd ($p<0.05$), decreased SBP ($p<0.01$), DBP ($p<0.01$) and DBPsd

($p<0.05$), lower BRS ($p<0.01$) vs. C. The subjects TH vs. TL had shorter PI ($p<0.05$). By means of the Spearman correlation coefficients we tested group T and than their subgroups TL and TH. We found negative correlation between BMI and HDL (T, TL, TH: $p<0.01$), positive correlation with TG (T, TH: $p<0.05$). Cholesterol correlated negatively with PI (T: $p<0.05$), HDL negatively with weight (T, TL, TH: $p<0.01$), and height (T, TH: $p<0.01$; TL: $p<0.05$), LDL negatively with PI (T: $p<0.01$), TG positively with age (T, TL: $p<0.05$), and weight (T, TL, TH: $p<0.05$).

Conclusions: The T subjects in comparison to C subject had increased parasympathetic and decreased sympathetic tonic activity with decreased baroreflex sensitivity. The increase of PI variability was neither result of a primary increase of vasomotor activity nor an increase of BRS response to changes in blood pressure. We can explain it by increasing parasympathetic control of heart. The circulatory changes did not bear clearly on obesity, but they were rather specific effect of tumour disease and anti-tumours treatment.
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INTRODUCTION

Chemotherapy has a substantial role in the management of cancers. New antitumour drugs developed in recent years have highly increased the number of patients who survived a cancer. However this successful therapy goes hand in hand with increased number of patients who had side effects after anti-cancer treatment, especially due to their cardio- and neurotoxicity. Neurotoxicity effects may be involved in the cardiotoxicity. Anthracycline antitumour agents are one of the highly effective antineoplastic drugs for the treatment of several human cancers in both adults and children. The risk factors for anthracycline cardiotoxicity are a high cumulative dose of anthracycline, young age of diagnosis, other cardiotoxic treatment (e.g. cyclophosphamids), and previous mediastinal irradiation. The clinical symptoms of acute anthracycline cardiotoxicity include arrhythmias and ECG abnormalities. Chronic cardiotoxicity is primarily due to the development of degenerative cardiomyopathy with congestive heart failure (1). The anthracycline-induced cardiotoxicity causes specific histopathological changes, breakdown of proteosynthesis in the heart and the liver (2). Anthracyclines also interact with the autonomous nervous system: They decrease efficiency of sympathetic part (3, 4) and increase activity of parasympathetic part of the autonomous nervous system (2, 5). Anthracycline are highly neurotoxic, as evident by neurological deficits and pathological changes in the central nervous system parenchyma. The neural lesions are manifested as loci of hemorrhagic necrosis and edema (6, 7). Anthracycline neurotoxicity include loss of neurons, nuclear alterations in peripheral ganglionic cells (8).

It has been suggested that anthracyclines cause cardiomyopathy because of interference with fatty acid metabolism that is connected with significantly increased levels of cholesterol and LDL or TG (9, 10). However, the other studies described another effect of plasma lipids. It was widely reported that cancer patients are usually found to suffer from hypocholesterolemia and hypolipoproteinemia (11). Moreover, the expression level of LDL-receptors on tumour cells was higher than that on normal cells, it could mean that more LDL would be taken up in tumour cells than in normal cells for membrane synthesis. In some other studies, LDL was used as the carrier to deliver the doxorubicin to the target tumour cells. Histological studies showed that doxorubicin carried by LDL

did not cause any heart damage when compared with the control group. In contrast, doxorubicin treatment caused disruption and vacuolisation of myocardial filament (12).

The baroreflex sensitivity is an index of the autonomic control of the heart. In adult subjects, baroreflex sensitivity was shown to decrease with age and a large inter-individual variability was found (13). It was reported that BRS is usually less than 5 ms/mmHg in hypertensive adults. Pathologically low baroreflex sensitivity (BRS < 3 ms/mmHg) was found to be a marker of an increased risk of sudden cardiac death in patients after myocardial infarction (14, 15).

AIM OF THE STUDY

The main task of the study was to compare body and circulatory parameters in healthy subjects, and children and adolescents who were previously treated for a malignant tumour. The evaluation of relationship between plasma lipids and changes in regulation of circulation in subjects after treatment for a malignant tumour was taken into account.

MATERIALS AND METHODS

We examined 303 children and adolescents aged 11-21 years. The control group (C) had 206 healthy children and adolescents, and second group had 97 children and adolescents after the treatment for a malignant tumour (T) by cardiotoxic therapy. Children and adolescents had common therapy according their diagnosis. From cardiotoxic treatment they had anthracyclines, cyclophosphamide or radiotherapy of mediastinum, or their combination. The anti-cancer therapy was terminated in average 3 years before this study, and they had no clinical signs of cardiotoxicity. Group C included 97 boys and 109 girls (mean age SD: 15.2 2.8), and group T consisted of 56 boys and 41 girls (15.8 3.2).

We used laboratory results of plasma lipids: cholesterol, HDL, LDL and triglycerides (TG). Subjects T were divided according to the level of cholesterol into two groups: 84 subjects (TL) with cholesterol lower than 5 mmol/l, and 13 subjects (TH) with cholesterol higher than 5 mmol/l.

Systolic blood pressure (SBP) and pulse intervals (PI) beat-to-beat were recorded for 5 min by the Peňáz non-invasive method (Finapres, OHMEDA) in all subjects. The recordings were taken in a sitting position at rest. Breathing was synchronised by a metronome at 20 breaths per minute (0.33 Hz) and subjects were allowed to adjust the tidal volume according to their own comfort.

The index of baroreflex sensitivity BRS (ms/mmHg) was determined by a spectral method. The gain factor, e.g. modulus H(f) of the transfer function between variations in systolic blood pressure and pulse interlvals, was calculated in the frequency range of 0.1Hz (f):

$$H(f) = \frac{G_{xy}(f)}{G_x(f)}$$

where Gxy(f) corresponds to the cross-spectral density between systolic pressure and pulse intervals, Gx(f) corresponds to the spectral density of systolic pressure. The value of modulus at the frequency of 0.1 Hz was taken as a measure of baroreflex sensitivity - BRS (ms/mm Hg).

We used standard deviation as a marker for a total variability of pulse intervals (PISD),

systolic blood pressure (SBPSD) and diastolic blood pressure (DBPSD).

The Ethics Committee approved of the study and the subjects gave their informed consent.

STATISTICS

The mean values and standard deviation of age, pulse intervals (PI), systolic blood pressure (SBP), diastolic blood pressure (DBP), variability parameters of pulse intervals (PISD), systolic blood pressure (SBPSD) and diastolic blood pressure (DBPSD), baroreflex sensitivity (BRS), body weight, height and the body mass index (BMI), parameters of plasma lipids (cholesterol, HDL, LDL and TG) were determined and Spearman correlation coefficients between all parameters were calculated. Significance of differences in the values between particular groups was tested by Mann-Whitney test.

RESULTS

Children and adolescents in group T had higher BMI, prolongation of PI, increased TISD, decreased SBP, DBP and DBPSD, lower BRS in comparison to group C. The subjects TH in comparison to TL had shorter PI only (Table 1).

Table 1: Body and cardiovascular characteristics of groups and subgroups and differences among them

		C	T	Level of significance	T_L	T_H	Level of significance
Age	**[years]**	15.2±2.8	15.8±3.18	NS.	15.8±3.1	15.7±3.7	NS
Height	**[cm]**	167.2±12.4	166.6±12.1	NS	167.2±11.9	162.6±13.2	NS
weight	**[kg]**	56.8±13.1	60.4±12.1	NS	60.5±15.2	60.6±19.0	NS
BMI	**[kg/m^2]**	20.1±2.9	21.5±3.9	P<0.01	21.4±3.9	22.3±4.3	NS
PI	**[ms]**	741±124	792±142	P<0.01	802.8±142.2	721.2±127.2	P<0.05
SBP	**[mmHg]**	112±12.7	100±16.1	P<0.001	99.8±16.4	101.5±13.9	NS
DBP	**[mmHg]**	68.4±9.1	58.6±8.8	P<0.001	58±8.5	61.9±10.1	NS
PIsd	**[ms]**	44.7±17.9	51.4±23.1	P<0.05	52.6±23.8	43.8±16.8	NS
SBPsd	**[mmHg]**	4.9±1.4	4.9±1.6	NS	5.1±1.6	4.5±1.9	NS
DBPsd	**[mmHg]**	3.1±0.9	2.8±0.7	P<0.05	2.9±0.7	2.8±0.8	NS
BRS	**[ms/mmHg]**	11.2±6.1	9±4	P<0.01	8.9±4.4	9.9±5.3	NS

The following differences in the plasma lipids levels were found between subgroups TL and TH: cholesterol- 3.9 0.5 vs. 5.9 0.7, $p < 0.001$; LDL - 2.2 0.4 vs. 3.6 0.6, $p < 0.001$; HDL - 1.4 0.3 vs. 1.5 0.5, n.s.; TG - 0.9 0.4 vs. 2.2 1.6, $p < 0.001$.

By means of Spearman correlation coefficients there were tested relationships between all the parameters in group T and then their subgroups TL and TH. In group T, negative correlation was found between cholesterol and PI ($p<0.05$), HDL and height ($p<0.01$), HDL and weight ($p<0.001$), HDL and BMI ($p<0.001$); and positive correlation between TG on one side and age, weight, BMI on the other side ($p<0.05$). In group TL, negative correlations were revealed between LDL and SBPSD ($p<0.05$), between HDL and height ($p<0.01$), HDL and weight ($p<0.001$), HDL and BMI ($p<0.01$); positive correlations were seen between TG and age ($p<0.05$), TG and weight ($p<0.05$). In group TH, negative correlations were observed between HDL and height ($p<0.01$), HDL and weight ($p<0.05$), HDL and BMI ($p<0.05$); positive correlations between LDL and PISD ($p<0.05$), TG and BMI ($p<0.05$).

We found significant differences in both, circulatory and body parameters between C and T groups with respect to age.

Children surviving a malignant tumour had only small differences in height according to age in comparison to C, but TH group had grater changes with age in weight and BMI (Fig.1a) compared to C and TL, it means, that in young TH children there is no significant differences in weight and BMI in comparison to other groups, but the TH adolescents (above16 years) had significantly higher ($p<0.01$) weight and BMI in comparison to C and TL group.

Children T had smaller prolongation of PI (Fig.1b) compared with C, but there were no differences with age in children TH. In T subjects, rise of SBP (Fig.1c) with age was lower and rise of DBP greater in comparison to C group, and there were no differences in SBP and DBP age-dependent differences between subgroups TL a TH.

Examining reflex parameters (PISD, SBPSD, DBPSD and BRS) we found these differences: T (and TL) group had smaller fall in PISD (Fig.1e) with respect to age in comparison to C group. TH children had the greater fall in SBPSD (Fig.1f) and DBPSD with respect to age in comparison to C and TL group. TH children had also the greatest increase of BRS (Fig.1d) with age compared with C and TL group.

Table 2: Slopes of correlation between age and the cardiovascular and body parameters, and the differences in these slopes among groups and subgroups

	b (C)	b (T)	b (T_L)	B (T_H)	C vs.T	$T_L$vs.T_H	C vs.T_L	C vs.T_H
Height [cm]	2.79	2,64	2.65	2.56	p<0.01	NS	NS	NS
weight [kg]	2.91	2.87	2.76	3.43	NS	p<0.01	NS	p<0.01
BMI [kg/m^2]	0.40	0.39	0.35	0.61	NS	p<0.05	NS	p<0.05
PI [ms]	17.01	9.06	10.73	0.50	p<0.01	p<0.01	p<0.01	p<0.01
SBP [mmHg]	1.49	0.72	0.68	0.95	p<0.01	NS	p<0.01	p<0.01
DBP [mmHg]	0.27	0.75	0.79	0.59	p<0.01	NS	p<0.01	NS
PIsd [ms]	-0.74	-0.29	-0.31	-0.27	p<0.01	NS	p<0.01	NS
SBPsd [mmHg]	-0.04	-0.01	0.02	-0.19	NS	p<0.01	p<0.05	NS
DBPsd [mmHg]	-0.051	-0.013	0.007	-0.119	NS	p<0.05	p<0.01	NS
BRS [ms/mmHg]	0.089	0.081	0.004	0.464	NS	p<0.01	NS	p<0.01

Legend: **b** – slope defined by the formula $y = a + b\,x$, where **x** is age and **y** is either a cardiovascular or a body parameter.

DISCUSSION

Subjects after antitumour therapy generally had higher weight and BMI than controls. However, detailed analysis disclosed that the biggest differences between groups were thanks to older adolescents after antitumour therapy with high level of cholesterol. Those older adolescents had significantly higher weight and BMI than the other adolescents after antitumour therapy or healthy controls of the same age. Nevertheless there were no differences between all these groups in younger children.

A longer resting mean pulse interval in children and adolescents after antitumour therapy in comparison to controls could be a sign of increased parasympathetic tonic activity. This increase of parasympathetic tonic control of the heart after antitumour therapy need not be a sign of a physical training as in healthy population, but we can

explain it by following pathophysiological mechanism: Proteosynthesis infliction of cholinesterase in the heart could cause an increased action of acetylcholin (2). It is interesting that the prolongation of mean pulse interval with respect to age was found in all younger subjects after antitumorous therapy. Completely different results were found in older adolescents. While mean pulse interval did not differ between controls and older adolescents after antitumour therapy with low level of cholesterol, it was shorter in those with high cholesterol. Additively, mean pulse interval of older adolescents with higher cholesterol was identical with mean pulse interval of treated younger group. Interpretation of such finding is not easy. It is questionable whether younger children could be more sensitive to cardiotoxicity and neurotoxicity of chemotherapy and what kind of linkage is between age-dependent development of mean pulse interval and a plasma level of cholesterol after antitumour therapy.

The significant decrease of systolic and diastolic blood pressure in all children after antitumour therapy in comparison to controls is a sign of decreased sympathetic tonic activity. It could be explained, e.g., by the direct effect of anthracycline on the sympathetic nerves of arteries, because they may cause persistent release of noradrenaline at rest, and than after electrical stimulation of sympathetic nerves is noradrenaline release suppressed (3). The cholesterol had no effect on blood pressure changes in treated children and adolescents.

Baroreflex sensitivity was generally slightly decreased in all subjects after antitumour therapy. Interesting were also differences in variability of pulse intervals. It was identical in treated subjects with high cholesterol and controls, but it was higher in treated subjects with low cholesterol than in controls. It could be in correspondence with their longer mean pulse interval.

We have concluded that the circulatory changes in children and adolescents after antitimour therapy do not bear clearly on obesity, but they are rather specific effect of tumour disease and antitumour treatment. The treated subjects in comparison to controls had increased parasympathetic and decreased sympathetic tonic activity with decreased baroreflex sensitivity. The increase of pulse interval variability was neither result of a primary increase of vasomotor activity nor an increase of BRS response to changes in blood pressure. We can explain it by increasing parasympathetic control of heart.

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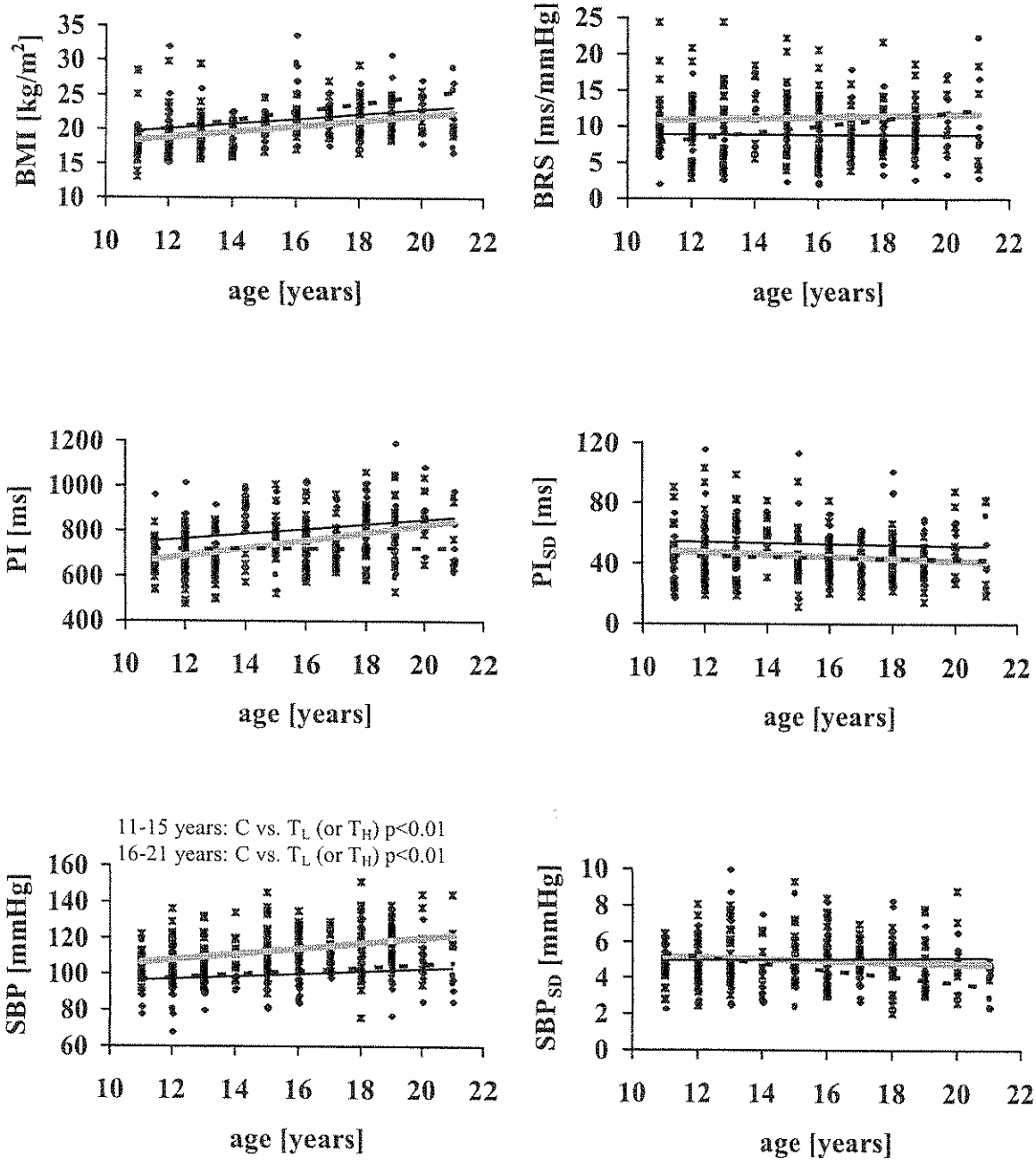
REFERENCES

1. Butera G, Piciacchia D, Chesa M, Condoluci C et al: Evaluation of late cardiotoxicity of anthracycline in childhood. Minerva Pediatr, 50, 111-119, 1998.
2. Geršl V, Bajgar J, Krs O, Hrdina R, Palička V, Mazurová Y.: Changes in cholinesterase activities after daunorubicin administration to rabbits. Human and Experimental Toxicology, 15, 834-838, 1996.
3. Sakai T, Inagki R, Taniguchi T, Shinozuka K, Kunitomo M, Hayashi N, Ishii Y, Muramatsu I: Persistent release of noradrenaline caused by anticancer drug 4´-epidoxorubicin in rat tail artery in vitro. Eur J Pharmacol, 356, 1, 25-30,1998.
4. Lekakis J, Prassopoulos V, Anthnassiadis P, Kostamis P, Moulopoulos S: Doxorubicin-induced cardiac neurotoxicity: study with iodine 123-labeled metiodobenzylguanidine. J Nucl Cardiol, 3 (1), 37-41, 1996
5. Zavodna E, Hrstkova H, Honzikova N, Novakova Z: Autonomous regulation of circulation in children afteer anthracycline therapy. Scripta Medica, 74 (4), 195- 202, 2002
6. Neuwelt EA, Glasberg M, Frenkel E, Barnett P: Neurotoxicity of chemotherapeutic agents after blood-brain barrier modification: neuropathological studies. Ann Neurol, 14 (3), 316-324, 1983
7. Neuwelt EA, Pagel M, Barnett P, Glasberg M, Frenkel EP: Pharmacology and toxicity of intracarotid adriamycin administration following osmotic blood-brain barrier modification. Cancer Res, 41 (11 Pt 1), 4466-4470, 1981
8. Cho ES: Toxic effects of adriamycin on the ganglia of the peripheral nervous system: a neuropathological study. Jneuropathol Exp Neurol, 36, 907-915, 1977
9. Rigatos SK, Stathopoulos GP, Perrea-Kotsarelis D, Couris E, Karayannacos PE, Deliconstatinos G: Investigation of doxorubicin tissure toxicity: does amifostine provide chemoprotection? Anticancer Res, 22(1A), 129-34, 2002.
10. Hong YM, Kim HS, Yoon HR: Serum lipid and fatty acid profiles in adriamycin-treated rats after administration of L-carnitine
11. Alexopoulos CG, Bltsios B, Avgerions A: Serum lipids and lipoprotein disorders in cancer patients. Cancer, 60, 3065-3070, 1987
12. Lo EHK, Ooi VEL, Fung KP: Circumvenion of multidrug resistance and reduction of cardiotoxicity of doxorubicin in vivo vy coupling it with low density lipoprotein. Life Sciences, 72, 677-687, 2002
13. Gribbin B, Pickering EC, Sleight P, Peto R: Effect of age and high pressure on baroreflex sensitivity in man. Cir. Res., 29, 424-431,1971.
14. La Rovere M T, Specchia G, Mortara A et al.: Baroreflex sensitivity, clinical correlates and cardiovascular mortality among patients with a first myocardial infartion. Circulation, 59, 256-262,1987.
15. Honzikova N., Fiser B., Semrad B.: Critical value of baroreflex sensitivity determined by spectral analysis in risk stratification after myocardial infarction. PACE, 23 [Pt. II], 1965-1967, 2000.

Fig. 1: Relationship between age and body (BMI) or cardiovascular (pulse interval, systolic blood pressure, baroreflex sensitivity, pulse interval variability, systolic blood pressure variability) parameters in controls (C - full thick grey line), in subjects after antitumour therapy with low level of cholesterol (TL - full thin black line), and in subjects after antitumour therapy with high level of cholesterol (TH - dashed black line). Only significant differences are described.



BAROREFLEX SENSITIVITY IN ESSENTIAL HYPERTENSION AND INCREASE IN PULSE PRESSURE

Siegelová Jarmila, Hofírek Ivo *, Fišer Bohumil, Dušek Jiří, Placheta Zdeněk, Jančík Jiří, Dobšák Petr, Svačinová Hana, Vank Pavel, Vítovec Jiří *

Dept. of Functional Diagnostic and Rehabilitation
*II Dept. of Internal Medicine, St.Anna`s Teaching Hospital, Faculty of Medicine, Masaryk University in Brno, Czech Republic

Address for correspondance:

Prof. MUDr. Jarmila Siegelová, DrSc.
Dept. Functional Diagnostics and Rehabilitation
Faculty of Medicine, Masaryk University
St. Anna Teaching Hospital
Pekařská 53
656 91 Brno, Czech Republic

INTRODUCTION

Increased pulse pressure is one of important cardiovascular risk factors in patients with essential hypertension (1). Another risk factor is an increased sympathetic activity in essential hypertension (2). The measure of increased sympathetic activity is also decreased baroreflex sensitivity (3). The baroreflex (heart rate) sensitivity (BRS) in patients with essential hypertension is generally low (4,5). A slight increase of BRS, but not normalization of values was observed after the monotherapy with Ca antagonists or ACE inhibitors compared with placebo controlled treatment (6). The study was aimed at the analysis of interrelationship between pulse pressure (PP) and total gain of baroreflex in patients with essential hypertension treated with ACE inhibitors or Ca antagonists.

MATERIAL AND METHODS

Thirty patients with mild essential hypertension were examined under treatment with Ca antagonists or ACE inhibitors. The whole set of 30 patients was divided according to PP values being corrected to MAP into two halves: a group with a lower corrected PP (PPL) and that with a higher PP (PPH).

Blood pressure was non-invasively measured beat-by-beat (Finapres Ohmeda, USA, 5 min, 7). Baroreflex heart rate sensitivity was determined by means of spectral analysis

(8). Baroreflex component of blood pressure response was determined by means of an original method developed in our Department (9). We put occlusive cuffs on both thighs and the pressure in them was increased abruptly to the supersystolic value for the time of 5 min. After releasing the occlusion both systolic and diastolic pressure decreased by 10 to 30 mmHg. The curve of the blood pressure return to the original level has a linear middle part. Its slope (velocity of return, mmHg/s) corresponds to the blood pressure component of the baroreflex gain.

The Ethics Committee of the Masaryk University Teaching Hospital approved the study.

STATISTICS

The results are summarized as means SD. The statistical significance of differences was determined by Wilcoxon test. Further analysis was then performed by using the analysis of variance (ANOVA).

RESULTS

The results of age and blood pressure (SBP, DBP, PP, MAP) in both groups of patients with essential hypertension (a group with a lower corrected PP and that with a higher PP) are given in Table 1 (mean SD).

Table 1: Results of age and blood pressure in 30 patients with essential hypertension, divided according PP being corrected to MAP (mean±SD).

	Age	**SBP**	**DBP**	**PP**	**MAP**
	Years	mmHg	mmHg	mmHg	mmHg
PPL	57±9	135±21	75±11	62±14	118±16
PPH	65±9	*146±18	66±11	81±15*	119±13

(p<0.05: * set PPL versus PPH.; Wilcoxon)

The results of baroreflex gain in both groups of patients with essential hypertension PPL and PPH (BRS component and blood pressure component) analysis are seen in Table 2.

Table 2: Results of baroreflex gain in both groups of patients with essential hypertension PPL and PPH (BRS component and blood pressure component)

	BRS	**BP BRS**	
	ms/mmHg	SBP mmHg/s	DBP mmHg/s
PPL	3.73 +3.06	1.05+0.78*	0.71+0.53
PPH	2.14+2.27	0.71+0.50	0.59+0.45

(p<0.05: * set PPL versus PPH.; Wilcoxon)

We have not found any difference between BRS values and a high and low pulse pressure in our treated patients with essential hypertension. On the other hand, the blood pressure

components of baroreflex were significantly increased in the case of a higher pulse pressure.

Our results also showed that treated hypertensives with a higher pulse pressure are significantly older and have a lower blood pressure component of the systolic blood pressure.

DISCUSSION

It has been reported that an increased pulse pressure is a risk factor of cardiovascular morbidity and mortality (10). The magnitude of pulse pressure has been found to depend on left ventricular ejection and the properties of the arterial wall, which determine both the compliance and the transmission characteristic of the arterial system (1). Thus an increase in pulse pressure can occur by means of an increase in the velocity of ventricular ejection, a reduction in the viscoelastic properties of the arterial wall, or a modification in the timing of the reflected waves (11). More recently, the Framingham study has shown that the risk of coronary artery disease was inversely related to diastolic blood pressure at any given systolic blood pressure value of at least 120 mmHg, suggesting that a higher pulse pressure was an important component of risk (1).

Sympathetic overactivity is another important risk of cardiovascular mortality (2). Some results of other laboratories together with the results of the present study can contribute to the elucidation of problems related to two questions, namely why BRS is low in essential hypertension and which changes of baroreflex are responsible for the treatment-induced BRS increase? An attractive hypothesis that a low baroreceptor sensitivity is compensated by the increased responsiveness of smooth muscles of hypertrophic vessel wall (12) is not supported by the available evidence. A long-lasting therapy with ACE-inhibitor enalapril decreased blood pressure and normalized the forearm blood flow after 5 min of ischaemia, which indicated regression of hypertrophy of the resistance vessels (13). BRS, however, remained low in patients treated with enalapril, reaching approximately one half of age-matched normotensive controls (14).

We have not found any difference between BRS and a high and low pulse pressure in our treated patients. On the other hand, the blood pressure components of baroreflex were significantly increased in the case of a higher pulse pressure.

It is concluded that treated hypertensives with a higher pulse pressure are significantly older and have a lower blood pressure component of systolic blood pressure.

Acknowledgement
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REFERENCES

1. Dart AM, Kingwell BA.Pulse pressurė - a review of mechanisms and clinical relevance. J Am Coll Cardiol 2001;37:975s-984s.
2. Mancia G. The sympathetic nervous system in hypertension. J Hypertens 1997;15:1553-65.
3. Grassi G, Seravalle G, Gattaneo BM et al. Sympathetic activation and loss of reflex sympathetic control in mild congestive heart failure. Circulation 1995;92:3206-211.
4. Grassi G, Gattaneo BM, Seravalle G, Lanfranchi A, Mancia G. Baroreflex control of sympathetic nerve activity in essential and secondary hypertension. Hypertension 1998;31:68-72.
5. Siegelová J, Fišer B, Dušek J, AL-Kubati M: Baroreflex-Senstivitaetsmessung bei Patienten mit essentieler Hypertonie: Einfluss von Enalapril. Nieren und Hochdruckkrankheiten 1995;24:20-22.
6. Siegelová J, Fišer B, Dušek J. Baroreflex heart rate sensitivity in essential hypertension: the effect of Isoptin SR 240 therapy. In: Varró V, de Chantel R, eds. 22nd Congress of International Society of Internal Medicine, Bologna: International Proceeding Div. Monduzzi Editore, 1994:99-102.
7. Peňáz J. Photoelectric measurement of blood pressure, volume and flow in the finger. In: Digest of the 10th Int. Conf. Med. Biol. Engineering, Dresden, 1973:104.
8. Robbe HWJ, Mulder LJM, Ruddel H, Langewiz WA, Mulder G. Assessment of baroreceptor reflex sensitivity by means of spectral analysis. Hypertension 1987;10:538-43.
9. Savin E, Siegelová J, Fišer B, Bonnin P. Intra- and extracranial artery blood velocity during a sudden blood pressure decrease in humans. Eur J Appl Physiol 1997;76:289-93.
10. Safar ME. Pulse pressure in essential hypertension: clinical and therapeutical implications (editorial reviw). J Hypertension 1989;7:769s-776s.
11. Jokiniitty JM, Majahalme SK, Kahonen MAP, Tuomisto MT, Turjanmaa VMH. Pulse pressure is best predictor of future left ventricular mass: 10 years of follow-up. J Hypertens 2001;19:2047s-2084s.
12. Zimmerman BG. Adrenergic facilitation by angiotensin: does it serve a physiological function? Clin Sci 1981;60:343-48.
13. Folkow B. The Fourth Volhard Lecture: cardiovascular structural adaptation: its role in the initiation and maintenance of primary hypertension. Clin Sci Mol Med 1978;4:3s-22s.
14. Siegelová J, Fišer B, Al-Kubati M, Dobšák P, Dušek J. Noninvasive determination of baroreflex sensitivity in hypertensives treated five years with enalapril. Scripta medica 70, 1997, 239-244.

VARIABILITY OF BLOOD PRESSURE IN ESSENTIAL HYPERTENSION

Dušek Jiří, Fišer Bohumil, Siegelová Jarmila, Placheta Zdeněk, Svačinová Hana, Svoboda Ladislav, Vohlídalová Ivona, Sosíková Michaela

Dept.of Physiology, Dept. of Functional Diagnostics and Rehabilitation, Medical Faculty, Masaryk University, Brno

Address for correspondence:

MUDr. Jiří Dušek, CSc.
NCO NZO v Brně
Vinařská 6
Brno

Support: MSM 141 100004 (CZ).

INTRODUCTION

The baroreflex heart rate sensitivity (ms/mmHg, BRS) is low in patients with essential hypertension. This fact was demonstrated by classical phenylephrine method (1) and with spectral method (2,3,4,5,6). The mean value of the group of hypertensives is of about one half of the mean value of the group of normotensive subjects. Because of a large scatter of individual values in both groups there are many normotensives with a lower BRS than the mean value of the hypertensive subjects and vice versa.

On the other hand the gain of the blood pressure component of the baroreflex is normal in essential hypertension (7). This is a different finding than in patients with heart rate failure where both the heart rate component and the blood pressure component are decreased (8).

The aim of the present study was to compare BRS in patients with essential hypertension and in healthy young subjects with low BRS. It is an important question whether young normotensives with low BRS can be at risk for development of hypertension later and such a study can contribute to the solution of the problem.

The estimation of the blood pressure component of the baroreflex in the present study is based on the evaluation of the short-time blood pressure variability. It was demonstrated that this variability is decreased in subjects with high BRS (6) because of homeostatic function of the baroreflex in damping the blood pressure oscillations.

METHODS

The blood pressure was non-invasively continuously recorded in finger arteries by Penaz volume-clamp method (9) for 5 minutes (Finapres, Ohmeda) during the metronome-controlled breathing (0.33 Hz) in a group of non-treated subjects with essential hypertension (EH, n=10, mean SD: age - 99 11 years, systolic/diastolic blood pressure - 155 23/99 12 mmHg). The diagnosis of essential hypertension (EH) was established by detecting elevated blood pressure (more then 140/90mmHg) on the basis of sphygmomanometer measurements on three different occasions within one month. The possibility that patients had secondary causes of hypertension was excluded by clinical examination. The other control groups consisted of subjects selected from group C. BRS (ms/mmHg) was determined by spectral analysis of spontaneous fluctuation of systolic blood pressure and cardiac interval. The results were compared with a group of 10 healthy young subjects with low BRS (<6ms/mmHg, LBRS) and with a group of subjects with high BRS (>25 ms/mmHg, HBRS). Both control groups were selected from the population of 100 healthy young adults (age 20-22 years) as subjects with the lowest, respectively the highest baroreflex sensitivity.

The method of BRS determination is described elsewhere(10). At least 30 min before the test the subjects were resting and adjusting to the environment (room temperature 20 degrees centigrade). Meanwhile, a plethysmographic transducer, Finapres Ohmeda was affixed. If the skin of the hands was cool, the subject was asked to put the hands into warm water for several minutes. The blood pressure was monitored for 10 min at rest. Three-minute recording was taken at the end of the resting period for further analysis. Then the subject was instructed to breathe in synchronism with a metronome at 0.33 Hz for 5 min. The last 3 min were recorded and analyzed. The two recordings, one made during spontaneous breathing and the second during metronome- controlled breathing, were evaluated by means of power spectral analysis. From the non-invasive continuous blood pressure recording, beat to beat values of systolic and diastolic blood pressure and pulse intervals were derived and analyzed. The values of all three circulatory variables were linearly interpolated at 2 Hz to ensure equidistant sampling in each time series. The baseline linear trend was removed from all signals. The power spectral densities and cross-spectral densities were calculated from the auto- and cross-correlation functions using Hanning's spectral window. The value of cross-spectral power density of pulse intervals and systolic blood pressure fluctuation [ms*mmHg] was divided by the value of power spectral density of systolic blood pressure fluctuation [mmHg*mmHg] at 0.1 Hz. The obtained value, modulus, was considered to be the measure of BRS [ms/mmHg]. The coherence at 0.1 Hz, i.e. the degree of linear coupling between systolic pressure and pulse intervals fluctuation, was calculated from both recordings, for spontaneous and metronome-controlled breathing, respectively.

The study was approved by the ethics committee of the Masaryk University in Brno and all subjects gave their written informed consent.

RESULTS

The variation of systolic blood pressure at 0.05 Hz (mean SD) was 44.1 35.3 mmHg2/Hz in HBRS and 194.1 141.2 mmHg2/Hz in LBRS.

The results confirmed the former finding that the blood pressure variability is lower in subjects with high BRS and vice versa. On the other hand in hypertensive subjects the blood pressure variability was low despite both low heart rate variability and low BRS.

Variation of systolic blood pressure at 0.05 Hz was 61.8 26.5 mmHg2/Hz (EH versus LBRS: $p<0.05$, Wilcoxon). At 0.1 Hz the variation of systolic blood pressure was higher in EH than in LBRS and HBRS (EH: 52.9 26.5 mmHg2/Hz, HBRS:17.6 15.9 mmHg2/Hz, LBRS: 35.3 13.2 mmHg2/Hz; EH versus HBRS $p<0.05$).

It is concluded that the low variation of blood pressure at 0.05 Hz corresponds to the normal blood pressure component of baroreflex in patients with essential hypertension. The increased blood pressure variation at 0.1 Hz in this group of patients reflects the impaired heart rate component of the baroreflex.

DISCUSSION

The difference in spectra of normotensive subjects and patients with essential hypertension indicates that the low BRS in both groups is caused by different mechanisms. The low BRS in healthy subjects can be caused by low baroreceptor sensitivity because both components of baroreflex - the blood pressure component and the heart rate pressure component - are attenuated. This resembles the situation in heart failure patients, where low baroreceptor sensitivity was proved in animal experiments. The high level of aldosteron stimulating Na/K-ATPase decreases the sensitivity of baroreceptors. This state can be reversed by digoxin, the potent Na/K-ATPase inhibitor (11). In humans, the low baroreflex sensitivity for both heart rate and blood pressure components (12) studied by various methods (13) supports this hypothesis.

In patients with essential hypertension the situation is different. In mild and moderate hypertension the resetting of baroreceptors is responsible for the shift of the set-point to a higher level of blood pressure, but the slope of blood pressure-cardiac interval curve which correspond to BRS and the slope of blood pressure- muscle sympathetic activity curve remain unchanged (14). In severe hypertension the set-point further moves to higher values of blood pressure but the decrease in slope takes place (15). This was not the case of our hypertensive subjects. Most probably the high sympathetic activity observed in essential hypertension accompanied with low parasympathetic activity is responsible for the decrease in BRS at intact baroreceptor sensitivity (16).

It is necessary to notice that the magnitude of fluctuation of blood pressure can be different in hypertensives and this fact can weaken our statement about normal baroreceptor sensitivity in essential hypertension (3,5). On the other hand the results of experiments with vasoactive drugs support this hypothesis (14, 15).

The problem whether the low BRS contributes to the development of hypertensive disease and in which extent remains unsettled. Only a prospective study on large number of subjects can answer this question. The spectral method for BRS determination is non-invasive and in our modification with controlled rate of respiration would be suitable for such testing.

REFERENCES

1. Gribbin B, Pickering EG, Sleight P, Peto R. Effect of age and high blood pressure on baroreflex sensitivity in man. Circ Res. 1971, 29: 424-431.
2. Siegelová J, Fišer B, Dušek J, Al-Kubati M. Die Baroreflex-sensitivitatsmessung bei Patienten mit essentieller Hypertonie: Einfluss von Enalapril. Nieren und Hochdruckkrankheiten. 1995, 24: 20-22.
3. Siegelová J, Fišer B, Dušek J. Baroreflex heart rate sensitivity in essential hypertension: the effect of Isoptin SR 240 therapy. In: 22nd Congress of the Internat. Society of Internal Medicine. Eds. V. Varro, R. de Chatel. International Proceeding, Division Monduzzi Editore, Bologna, Italy, 1994, 99-102.
4. Siegelová J, Hofírek I, Fišer B, Dušek J, Placheta Z, Jančík J, Dobšák P, Svačinová H, Vank P, Toman J. Relationship between pulse pressure and baroreflex sensitivity in patients with essential hypertension. Scripta medica 2002:75(2):101-104
5. Siegelova J, Fiser B, Savin E, Dusek J : Baroreflex mediated vasomotor response in hypertensives. J.Hypertension 1997 ; 15 : 194
6. Grassi G, Cattaneo BM, Seravalle G, Lanfranchi A, Mancia G. Baroreflex control of sympathetic nerve activity in essential and secondary hypertension. Hypertension 1998; 31:68-72.
7. Grassi G, Seravalle G, Cattaneo BM, Lanfranchi A, Vailati S, Giannattasio C et al. Sympathetic activation and loss of reflex sympathetic control in mild congestive heart failure. Circulation 1995, 92: 3206-3211.
8. Mancia G, Parati G, Pomidossi G, et al. Arterial baroreflexes and blood pressure and heart rate variabilities in human. Hypertension 1986, 8: 147-153.
9. Peňáz J. Photoelectric measurement of blood pressure, volume and flow in the finger. In Digest 10th Int Conf Med Biol Eng, Dresden 1973, 104.
10. Al-Kubati MA, Fišer B, Siegelová J. Baroreflex sensitivity during psychological stress. Physiol Res 1997, 46: 27-33.
11. Wang SJ, McClain J, Zucker H. Aldosteron reduced baroreceptor discharge in the dog. Hypertension Dallas 1992, 19:270-277.
12. Semrád B, Fišer B, Honzíková N. Blood pressure control by baroreflex in patients after myocardial infarction. Scripta medica 1997, 70:3-8.
13. Al-Kubati MA. Baroreflex sensitivity during the stimulation of low-pressure receptors. Scripta medica 1996, 69:191-196.
14. Grassi G. Role of the sympathetic nervous system in human hypertension. J Hypertension 1998, 16: 1979 - 1987.
15. Korner PI, West MJ, Shaw J,Uther JB. "Steady state" properties of the baroreceptor-heart rate reflex in essential hypertension in man. Clinical and Experimental Pharmacology and Physiology 1974, 1:65-76.
16. Mancia G. Bjorn Folkow Award Lecture - The sympathetic nervous system in hypertension. J Hypertension 1997, 15:1553-1565.

RATE DEPENDENT CHANGES OF ACTION POTENTIAL WAVEFORM IN RAT VENTRICULAR MYOCYTES

Action potential voltage clamp experiments

Bébarová Markéta, Matejovič Peter, Pásek Michal,
Šimurdová Milena, Šimurda Jiří

Department of Physiology, Faculty of Medicine, Masaryk University, Brno
Komenského nám. 2, 662 43 Brno; mbahnik@med.muni.cz

Address correspondence to:

MUDr. M. Bébarová
Department of Physiology
Faculty of Medicine, Masaryk University
Komenského nám. 2
662 43 Brno

ABSTRACT

Components of the total ionic membrane current responsible for rate-dependent changes of action potential (AP) configuration were analyzed by the AP voltage clamp method in isolated rat ventricular cardiomyocytes. During a sequence of free running APs at 2.5 Hz preceded by a 60 s period of rest, AP duration measured at 75 % of repolarization was gradually prolonged. AP recorded in current clamp mode at the steady state was stored in the computer memory and used as the uniform command signal in a sequence of impulses after rest. Experiments were repeated under the effect of Co2+ (2 mmol/l), 4-aminopyridine (3 mmol/l) and ajmaline (30 mol/l). The results demonstrate that besides Ca2+-mediated currents, cumulative inactivation of the transient outward current Ito significantly participates on the rate-dependent variability of AP configuration. Under the effect of antiarrhythmic drug ajmaline, the fast sodium current INa causes marked changes in the repolarization phase of AP, very likely as a consequence of its use-dependent block.

KEY WORDS
rate dependence, action potential, ionic currents, rat, action potential voltage clamp

INTRODUCTION

The dependence of the course of action potential (AP) on the frequency of stimulation in cardiac cells plays an important role both in the regulation mechanisms participating in the excitation-contraction coupling and in the protective mechanisms preventing initiation of cardiac arrhythmias. The rate dependence shows marked species and tissue

differences. For example, in ventricular myocytes of dog, cat and other species including man, the AP duration (APD) is shortened with increasing frequency of stimulation (Boyett and Jewell 1978; Litovsky and Antzelevitch 1989; Li et al. 1999) whereas it is prolonged in rat (Schouten 1986; Shigematsu et al. 1997). The participation of individual components of total membrane ionic current on the rate dependent changes of the course of AP in rat ventricular myocytes has not been explicitly established so far. Fauconnier et al. (2003) and Bouchard et al. (1995) demonstrated the important role of the L-type calcium current ICa-L and suggested significant contribution of the Na+/Ca2+ exchanger current INaCa relating to their modulation by calcium ions released from the intracellular stores. The effect of the transient outward potassium current Ito was demonstrated indirectly in current clamp experiments (Shigematsu et al. 1997).

With the aim to bring identification of the contributing currents nearer to natural conditions, we investigated the role of individual ionic current components in the rate dependent changes of AP configuration using the AP voltage clamp method. The results of this study demonstrate a significant role of Ito. Under specific conditions, the fast sodium current INa is also involved in the regulation of the repolarization phase of AP.

MATERIALS AND METHODS

Cell isolation

The experiments were performed on 19 enzymatically isolated rat ventricular myocytes (adult male Wistar rats, 250 - 350 g). The animals were sacrificed by cervical dislocation under mild ether anaesthesia. The heart was removed and placed into an ice-cold Krebs-Henseleit solution. Subsequently, the heart was attached to a Langendorff apparatus and retrogradely perfused through the aorta. Perfusion with 0.9 mmol/l CaCl2 Tyrode, lasting 3 to 5 min, was followed by application of a nominally Ca-free Tyrode solution (for up to 4.5 min). Collagenase-Yakult (type S; Yakult Pharmaceutical Ind. Co., LTD., 0.2 mg/ml) and protease (type XIV; Sigma, 0.041 mg/ml) were added to calcium-free Tyrode solution with 44 mol/l EGTA to obtain the first enzyme solution (2.5 min exposure). The second enzyme solution differed from the first one by the absence of protease (22 min exposure). The enzyme solution was then washed out by two low calcium Tyrode solutions (0.09 and 0.18 mmol/l CaCl2). All solutions were oxygenated with 100% O2 at 37 °C.

Right ventricle was then cut into fine pieces in 30 ml of a 0.18 mmol/l CaCl2 Tyrode solution to produce a suspension. This was filtered and 20 ml of the Tyrode solution containing 1.8 mmol/l CaCl2 was added gradually within 20 min to achieve a Ca2+ concentration of 0.9 mmol/l. The whole procedure of myocyte isolation was carried out at 37 °C.

Solutions

The composition of calcium-free Tyrode solution (in mmol/l) was as follows: NaCl, 135; KCl, 5.4; MgCl2, 0.9; HEPES, 10; NaH2PO4, 0.33; glucose, 10 (pH was adjusted to 7.4 with NaOH). The patch electrode filling solution contained (mmol/l): L-aspartic acid, 130 (or L-glutamic acid 130); KCl, 25; MgCl2, 1; Na2ATP, 5; EGTA, 1; HEPES, 5; GTP, 0.1; Na2-phosphocreatine, 3 (pH at 7.25 adjusted with KOH).

The experiments were performed in control conditions and in the presence of Co2+ (2mmol/l) to block ICa, 4-aminopyridine (3 mmol/l) to block Ito. The antiarrhythmic drug ajmaline monoethanol (GilurytmalR10, Solvay Pharmaceuticals) was applied in the concentration of 30 mol/l.

Electrophysiological recordings

All experiments were performed at room temperature (28-33 °C) on single rod-shape cells with a distinct striation. Filled glass electrodes with a low resistance of about 1 M were selected to keep the access resistance as low as possible. The Axopatch 200A equipment (Axon Instruments, Inc.) and pCLAMP program (version 6.0.4) were used for generation of voltage clamp protocols and data acquisition.

The components of total membrane ionic current responsible for the rate-dependent changes in AP configuration were identified in action potential voltage clamp experiments. Steady state AP recorded at 2.5 Hz in the current clamp mode was imposed repeatedly to the same cell in voltage clamp mode in control conditions and in the presence of blocking agents. Thus, changes of ionic current after application of blocking agents and during sequences of pulses after a period of rest could be directly visualized and correlated with the altered AP waveform.

RESULTS

Application of the AP voltage clamp method is illustrated in Fig. 1. Represented are changes of AP configuration (B) and of ionic membrane currents (A, C) induced by ajmaline (30 mol/l), a class Ia antiarrhythmic drug with the high blocking efficacy on INa and Ito (Bahníková et al. 2002). ICa was inhibited by Co2+ (2 mmol/l). At the beginning of each experiment, the cardiomyocyte was stimulated by 1 ms supra-threshold impulses at the frequency of 2.5 Hz in the current clamp mode. After reaching the steady state a representative AP was stored in the computer memory and used as a command signal in the voltage clamp mode at the unaltered frequency of stimulation (2.5 Hz). The control record was subtracted from all the subsequent ones so that the changes in ionic currents in Fig 1A corresponded entirely to the blocking effect of ajmaline.

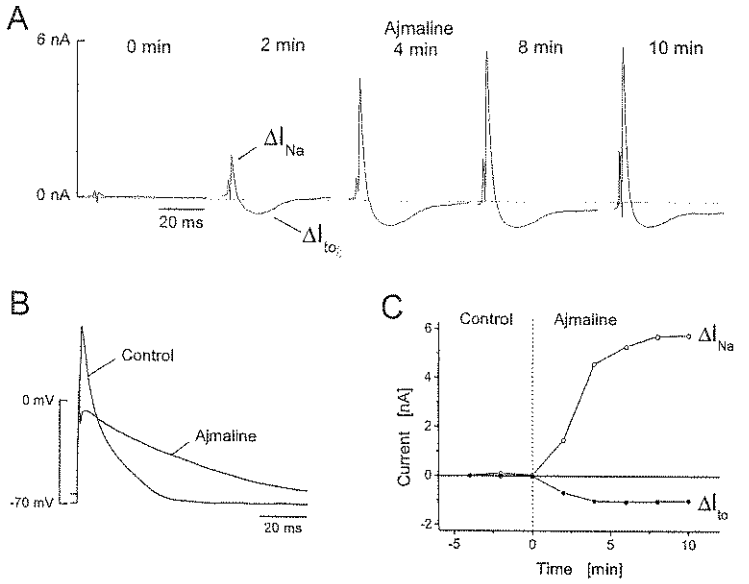


Fig. 1 The effect of ajmaline on action potential configuration and ionic currents studied by action potential voltage clamp method. A: Currents recorded during the first 10 minutes after application of ajmaline (30 μmol/l). I_{Ca} was blocked by Co^{2+} (2 mmol/l). The current recorded in control was subtracted from the current traces so that only the changes caused by ajmaline are visible. B: Action potential recorded in control and 10 min after application of ajmaline. C: Development of I_{Na} and I_{to} block in the presence of ajmaline. Plotted are peak values of ΔI_{Na} and ΔI_{to}.

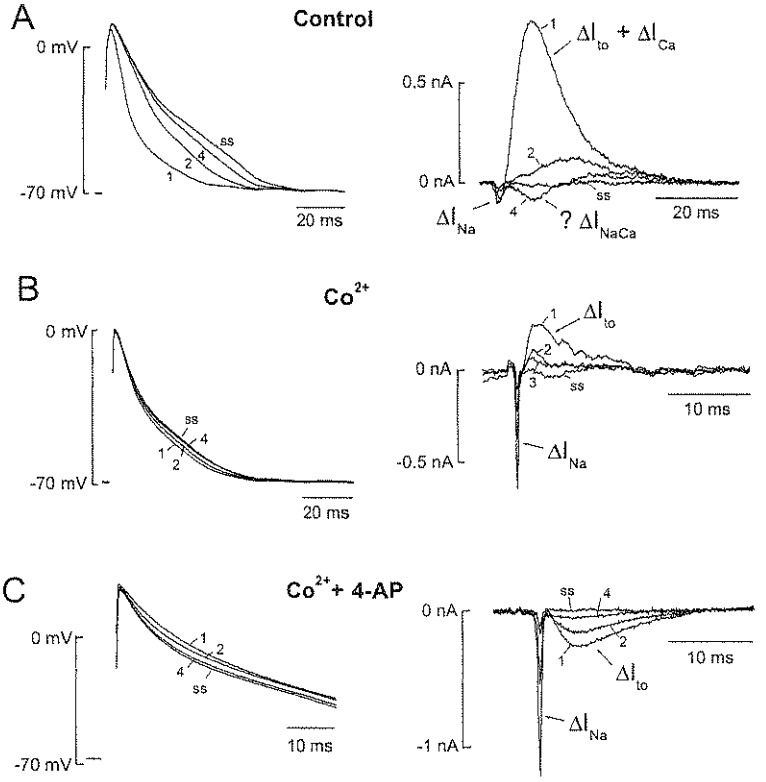
As expected, the gradually inhibited currents INa and Ito were manifest on the current traces. The changes in the course of AP (Fig. 1B) could be explained by the inhibition of these currents. The decreased amplitude of AP was very likely caused by the INa-block, whereas the slowed repolarization was induced by the Ito-block. The blockade of Ito developed about two-fold faster than that of INa (Fig. 1C).

In the next set of experiments cardiomyocytes were first kept at the resting membrane voltage in the current clamp mode for about 60 s and then stimulated at the frequency of 2.5 Hz. APs were recorded untill the steady state (Fig. 2, left). In the voltage clamp mode, the waveform of steady-state AP replaced the stimulating pulses and changes in the components of ionic current responsible for the modulation of AP could be observed. To visualise currents responsible for the differences of free running APs from the steady state pattern the steady state current records were subtracted from all current traces (Fig. 2, right).

In control conditions (Tyrode solution) the first AP induced after a period of rest was markedly shortened and was gradually prolonged during the train of stimuli (Fig. 2A). In the voltage clamp mode, the components of the total membrane ionic current responsible for the observed changes of AP were displayed. INa remained almost unchanged. Prominent changes, however, occurred in the subsequent slow transient current that was probably composed of several components as evidenced by reversed polarity in one of the responses.

In the presence of Co^{2+} (Fig. 2B), an ICa-blocker, the prolongation of APD was less pronounced. Two components of the changed ionic currents could be clearly distinguished.

Their course corresponded to an INa followed by Ito. To confirm that the second component was really Ito, the same experiments were performed in the presence of Ito-blocker 4-aminopyridine (4-AP, 3 mmol/l) in addition to Co2+. We observed inverted changes in APD. The first AP was prolonged, and was gradually shortened during repetitive stimulation (Fig. 2C). Ito of the reversed polarity and gradually diminishing amplitude developed in the records of ionic currents (for explanation see Discussion).



The last measurements (Fig. 2D) were performed in the presence of Co2+ and ajmaline (30 mol/l). This time, the prolongation of APD as well as the causative changes in Ito were negligible. On the contrary, a marked decrease of INa can be seen responsible for the gradual decrease of AP amplitude during the repolarization phase (note different current scales).

DISCUSSION

The present results demonstrate that both ICa and Ito play a prominent role in gradual prolongation of AP in the course of restored stimulation after rest. Both components contribute to the remarkable increase of AP duration in control (Fig. 2A). Their variations are concurrently manifested as outward directed transients on current traces. Ito declines during a sequence of repeated pulses as a result of cumulative inactivation. In contrast to long resting period previous to stimulation sequence, the short intervals between APs at 2.5 Hz are insufficient for Ito to recover (Apkon and Nerbonne 1991; Shimoni et al. 1994). On the other hand, in rat, ICa increases with increasing frequency (Schouten and ter Keurs 1991, Tiaho et al. 1994) as a consequence of the modulatory effect of the Ca2+ transient (Delgado et al. 1999; Fauconnier et al. 2003). Due to opposite direction of ICa and Ito, their variations support each other and lead to AP prolongation. The inwardly directed (negative) current deflection that appears in response

to the fourth imposed AP might correspond to unmasked INaCa after a fast decline of the outward currents. It is absent under the effect of Co2+ (Fig. 2B). Only a gradually decreased slow transient outward component is recorded that is probably caused entirely by the decline of Ito due to cumulative inactivation. It changes direction after addition of 4-AP (Fig. 2C). A full block of Ito should delete the outward going transients completely. The observed reversion of current is explainable as a consequence of the feature of 4-AP-induced Ito-block known as reversed use dependence. This effect was described also in cardiac cells (Šimurda et al. 1989) and gives rise to partial relief of block and, accordingly, to AP shortening during repeated stimulation after rest.

The observation that INa can significantly affect not only the fast depolarization but also the repolarization phase of AP is surprising. These changes were absent in control and appeared under the effect of 30 mol/l ajmaline (Fig. 2D). The explanation was provided by our so far unpublished measurements of time course of INa-recovery after the forgoing excitation. At a frequency of 2.5 Hz, the Na-channel was able to recover from inactivation in control but not under the effect of ajmaline. The recovery from inactivation and a much slower recovery from block proceeded at resting voltage simultaneously. Thus, the cumulative INa block is likely to be responsible for the gradual decline of AP amplitude.

In contrast to voltage clamp experiments using rectangular pulses, currents are tested by natural AP waveform. In addition, only currents responsible for the changes of AP configuration are directly displayed. Thus, for the analysis of rate-dependent changes in AP configuration, the approach applied in this work proved to be more suitable.

Acknowledgement
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REFERENCES

Apkon M, Nerbonne JM. *Characterization of two distinct depolarisation-activated K+ currents in isolated adult rat ventricular myocytes.* J Gen Physiol 1991; 97: 973-1011.

Bahníková M, Matejovič P, Pásek M, Šimurdová M, Šimurda J. *The effect of ajmaline on ionic currents of rat ventricular myocytes.* Physiol Res 2002; 51(4): 1P.

Bouchard RA, Clark RB, Giles WR. Effects of action potential duration on excitation-contraction coupling in rat ventricular myocytes. *Circ Res 1995; 76: 790-801.*

Boyett MR, Jewell BR. A study of the factors responsible for rate-dependent shortening of the action potential in mammalian ventricular muscle. J Physiol 1978;285: 359-80.

Delgado C, Artiles A, Gómez AM, Vassort G. *Frequency-dependent increase in cardiac Ca2+ current is due to reduced Ca2+ release by the sarcoplasmic reticulum.* J Mol Cell Cardiol 1999; 31: 1783-93.

Fauconnier J, Bedut S, Le Guennec JY, Babuty D, Richard S. Ca2+ *current-mediated regulation of action potential by pacing rate in rat ventricular myocytes.* Cardiovasc Res 2003; 57: 670-80.

Li GR, Yang B, Feng J, Bosch RF, Carrier M, Nattel S. *Transmembrane ICa contributes to rate-dependent changes of action potentials in human ventricular myocytes.* Am J Physiol 1999; 276: H98-H106.

Litovsky SH, Antzelevitch CH. *Rate dependence of action potential duration and refractoriness in canine ventricular endocardium differs from that of epicardium: role of the transient outward current.* J Am Coll Cardiol 1989; 14: 1053-66.

Schouten VJ. *The negative correlation between action potential duration and force of contraction during restitution in rat myocyrdium.* J Mol Cell Cardiol 1986; 18(10): 1033-45.

Schouten VJ, ter Keurs HE. *Role of ICa and Na+/Ca2+ exchange in the force-frequency relationship of rat heart muscle.* J Mol Cell Cardiol 1991; 23(9): 1039-50.

Shigematsu S, Kiyosue T, Sato T, Arita M. *Rate-dependent prolongation of action potential duration in isolated rat ventricular myocytes.* Basic Res Cardiol 1997; 92(3): 123-28.

Shimoni Y, Firek L, Severson D, Giles W. *Short-term diabetes alters K+ currents in rat ventricular myocytes.* Circ Res 1994; 74: 620-28.

Šimurda J, Šimurdová M, Christé G. *Use-dependent effects of 4-aminopyridine on transient outward current in dog ventricular muscle.* Pflügers Arch 1989; 415: 244-46.

Tiaho F, Piot C, Nargeot J, Richard S. *Regulation of the frequency-dependent facilitation of L-type Ca2+ currents in rat ventricular myocytes.* J Physiol 1994; 477(Pt 2): 237-51.

INFLUENCE OF 1-YEAR PHYSICAL TRAINING ON BAROREFLEX SENSITIVITY IN YOUNG SWIMMERS

Zuzana Nováková, Bohumil Fišer, Eva Závodná, Nataša Honzíková, Jan Novotný[1], Mohamed Al-Kubati

Department of Physiology, Faculty of Medicine, [1]Department of Sports Medicine and Physical Culture, Faculty of Sports Studies, Masaryk University in Brno, Czech Republic

Address correspondence to:

MUDr. Zuzana Nováková
Department of Physiology
Faculty of Medicine, Masaryk University
Komenského nám. 2
662 43 Brno

Support: MSM 141 100004 (CZ).

ABSTRACT

Increased heart rate and decreased baroreflex sensitivity are correlated with higher cardiovascular mortality and morbidity. Heart rate can be decreased by physical training, evaluation of the changes of baroreflex were the aim of the present study.

Baroreflex heart rate sensitivity (ms/mmHg:BRS, Hz/mmHg:BRSf) was determined in a group of sportsmen (swimmers: 9 girls and 10 boys, age 13-15 years) before and after 1 year of training (10 MET) by spectral analysis of spontaneous fluctuations of pulse intervals and blood pressure (Finapres Ohmeda, 5 min recording, metronome controlled breathing 0.33 Hz). The study was approved by the local ethics committee.

The mean values (SD) of BRS and BRSf for the whole group were 9.57 3.12 ms/mmHg and 0.0153 0.005 Hz/mmHg before, and 10.13 4.76 ms/mmHg and 0.0142 0.006 Hz/mmHg after 1 year. Significant differences between boys and girls as well as between the values before and after were not observed. Significant increase of pulse intervals (before:788 88ms, after:889 105ms) was observed in boys only. The correlations between the first and second values were observed in pulse intervals (boys: r = 0.798, p 0.01, girls: r = 0.842, p 0.01) and in systolic blood pressure (boys: r = 0.639, p 0.05) but not in BRS (boys: r = 0.357, girls: r = 0.252) and in BRSf (boys: r = 0.109, girls: r = 0.057).

It is concluded that a 1-year physical training decreased heart rate in boys and did not change BRS and BRSf in adolescents of either sex. Significant correlation of pulse interval and systolic blood pressure in the first and the second measurement reveals their individual characteristic. On the other hand similar correlation was not found neither for BRS nor for BRSf.

Key words
Baroreflex sensitivity, exercise, swimmers, spectral analysis

INTRODUCTION

The baroreflex system is the most important negative feedback control system functioning physiologically to attenuate the effects of rapid perturbation in arterial pressure.

Physical activity or exercise is perhaps the most profound challenge to circulatory homeostasis. It is well established that exercise is accompanied by sympathoexcitation. Several neural mechanisms have been postulated to mediate the sympathoexcitatory response, including central command and baroreceptor reflexes mechanisms. These mechanisms provide the central nervous system with critical inputs that establish the pattern of cardiovascular motor responses during exercise. A host of peripheral receptors are also activated during exercise; their role of the arterial baroreflex has been challenged, owing to the prevailing sympathoexcitation during exercise. Recently, however, it was reported that human carotid baroreceptor reflex is reset and its overall sensitivity is retained during exercise. But the mechanism of this alteration in baroreflex function is unknown (1).

Increased heart rate and decreased baroreflex sensitivity are correlated with higher cardiovascular mortality and morbidity. On the other hand, the dynamic exercise training has been recommended as an antihypertensive therapy and as a way to modify the effects of many cardiovascular risk factors (2).

One of the most striking effects of exercise training on the cardiovascular system is the presence of resting bradykardia. Depending on the mode, intensity, and duration of exercise training, and also on the animal species studied, the mechanisms responsible for the bradycardic effect vary according to the relative importance of three major factors: the increase in vagal tone, the decrease in sympathetic tone, and the altered sensitivity of the cardiac pacemacker cells to neurotransmitters (3).

However, the mechanisms underlying the blood pressure lowering effect of chronic exercise are still poorly understood.

AIM OF STUDY

Heart rate can be decreased by physical training, evaluation of the changes of basic circulatory parameters (systolic blood pressure - SBP, pulse interval - PI) and baroreflex sensitivity (BRS) were the aim of the present study.

MATERIALS AND METHODS

Subjects

We examined a group of sportsmen (swimmers: 9 girls and 10 boys, age 13-15 years) before and after 1 year of training.

We estimated the intensity of the swimmer´s training according the Compendium of

Physical Activities at the level 10 METs (4). One MET is defined as the energy expenditure for sitting quietly, which for the average adult is approximately 3.5 ml of oxygen . kg body weight^{-1} . min^{-1}.

The basic characteristics of study group and its division to gender are shown in Table 1.

Protocol

We recorded PI, SBP and diastolic blood pressure (DBP), beat-to-beat on finger arteries by the Peňáz non-invasive method (Finapres, OHMEDA, USA) in all adolescents. The recordings were taken in a sitting resting position during a 5-minute period. Breathing was synchronised by a metronome at 20 breaths per minute (0.33 Hz) and the subjects were allowed to adjust the tidal volume according to their own comfort.

Baroreflex sensitivity determination

The baroreflex sensitivity, assessed on the basis of spectral analysis (5), was expressed in ms/mmHg and in Hz/mmHg (6). The gain factor, e.g. modulus H(f) of the transfer function among variations in systolic blood pressure and pulse intervals, was calculated at a frequency of 0.1 Hz according to the formula: H(f) = Gxy(f)/Gx(f) where Gxy(f) corresponds to the cross-spectral density between systolic pressure and pulse intervals and Gx(f) corresponds to the spectral density of systolic pressure. The value of modulus at a frequency of 0.1Hz was taken as a measure of baroreflex sensitivity, BRS (ms/mmHg). Using the same formula, the modulus at a frequency of 0.1 Hz was also calculated for the instantaneous value of the heart rate and systolic pressure as the second index of baroreflex sensitivity (BRSf, expressed in Hz/mmHg).

Statistical analysis

The mean values and standard deviations of pulse intervals, SBP and DBP, baroreflex sensitivity (BRS and BRSf) and body growth parameters (body weight, height, BMI) were determined. Differences among the mean values were tested by the Mann-Whitney test, correlations among the parameters were evaluated by Spearman's correlation coefficients.

Table 1. Characteristics of groups

		Body weight (kg)	**Body height (cm)**	**BMI (kg.m^{-2})**
Whole group	2001	58.4±7.1	170.2±6.5	20.2±1.8
	2002	60.6±6.7*	172.8±7.2**	20.3±1.7
Boys	2001	58.6±6.9	168.5±6.7	20.6±1.7
	2002	59.9±7.4	171.0±6.8*	20.5±1.9
Girls	2001	58.2±7.7	172.0±6.1	19.6±2.0
	2002	61.3±6.2*	174.9±7.5*	20.1±1.6

Values are presented as means standard deviation (SD).
BMI - body mass index.
Statistical evaluation: * $p<0.05$; ** $p<0.01$ between years 2001-2002

RESULTS

The mean values (SD) of circulatory parameters (PI, SBP) and of baroreflex sensitivity for the whole group before (2001) and after 1-year training (2002) are shown in Table 2. The mean values of baroreflex sensitivity expressed in ms/mmHg (BRS - as a pulse interval dependent parameter) and expressed in Hz/mmHg (BRSf - as a pulse interval independent) for the whole group were not different significantly. Significant differences between boys and girls as well as between the values before and after training were observed neither. Significant increase of pulse intervals was seen only in boys.

The correlations between the first and second values for the subgroups according to sex were observed in pulse intervals and systolic blood pressure but not in BRS and BRSf (Table 3).

Table 2. Characteristics of circulatory parameters and baroreflex sensitivity

		PI	SBP	BRS	BRSf
Whole group	2001	798.9±118.8	103.7±9.9	9.6±3.1	0.0153±0.005
	2002	843.4±121.0	103.3±10.6	10.1±4.8	0.0142±0.006
Boys	2001	788.4±88.1	100.6±7.1	9.8±3.4	0.0200±0.010
	2002	889.6±104.5**	97.7±9.3	9.9±5.1	0.0200±0.010
Girls	2001	810.6±150.8	107.1±11.8	9.3±3.0	0.0141±0.004
	2002	792.1±122.7	109.4±8.6	10.4±4.7	0.0131±0.010

Values are presented as means standard deviation (SD).
PI - pulse interval (ms), SBP - systolic blood pressure (mmHg), BRS a BRSf - baroreflex sensitivity expressed in ms/mmHg and in Hz/mmHg.
Statistical evaluation: ** $p < 0.01$ between years 2001-2002

Table 3. Correlations between the first (2001) and second (2002) values of each selected parameters in subgroups according to sex

	Boys	Girls
Pulse interval	0.798 **	0.842 **
Systolic blood pressure	0.639 *	0.328
Baroreflex sensitivity in ms/mmHg (BRS)	0.357	0.252
Baroreflex sensitivity in Hz/mmHg (BRSf)	0.109	0.057

Values are presented as Spearman's correlation coefficient.
Statistical evaluation: * $p < 0.05$; ** $p < 0.01$ between years 2001-2002

CONCLUSION AND DISCUSSION

It is concluded that a 1-year physical training decreased heart rate in boys and did not change BRS and BRSf in adolescents of either sex. Significant correlation of pulse interval and systolic blood pressure in the first and the second measurement reveals their individual characteristic. On the other hand, similar correlation was not found neither for BRS nor for BRSf. These findings have supported the results of our previous study (7) that baroreflex sensitivity in the age group of 13-15 years old are not stabile.

The prolongation of a mean pulse interval in boys' group may be the first signal, which initiates the reconstruction period to sports heart. This is a general view on the regular physical training.

REFFERENCES

1. Potts J: Exercise and Sensory Integration. Role of the Nucleus Tractus Solitarius. Annals NewYork Academy of Sciences 2001; 940: 221-236
2. Arakawa K: Antihypertensive mechanism of exercise. J Hypertens 1993; 11: 223-229
3. Krieger EM, Da Silva GJJ, Negrao CE: Effects of exercise training on baroreflex control of the cardiovascular system. Annals NewYork Academy of Sciences 2001; 940: 339-347
4. Ainsworth BE, Haskell WL, Leon AS et al.: Compendium of physical activities: classification of energy costs of human physical activities. Med Sci Sports Exerc 1993; 25(1): 71-80
5. Honzíková N, Fišer B, Honzík J: Noninvasive determination of baroreflex sensitivity in man by means of spectral analysis. Physiol Res 1992; 41: 31-37
6. Al-Kubati MAA, Fišer B, Siegelová J: Baroreflex sensitivity during psychological stress. Physiol Res 1997, 46: 27-33
7. Nováková Z, Honzíková N, Fišer B: Baroreflex sensitivity in children. Physiol. Res. 1998; 47: 2P.

ROLE OF SIGMA SIGNALLING IN MAMMALIAN MYOCARDIUM

Marie Nováková

Department of Physiology, Faculty of Medicine, Masaryk University in Brno, Czech Republic

Address correspondence to:

MUDr. Marie Nováková, Ph.D.
Department of Physiology
Faculty of Medicine, Masaryk University
Komenského nám. 2
662 43 Brno

Support: MSM 141 100004 (CZ).

INTRODUCTION

Sigma receptors are defined as saturable, non-opioid, non-dopaminergic binding sites exhibiting high affinity for several important classes of psychotropic drugs. They are well defined by binding studies and distinct in pharmacological profile, tissue distribution and subcellular localisation from any known neurotransmitter or hormonal receptor. The subclassification of sigma receptors is based on differences in binding: ***sigma-1*** binding site or high affinity sigma receptor and ***sigma-2*** binding site or low affinity sigma receptor. Recently, the existence of ***sigma-3*** binding site has been proven. This novel subtype of sigma receptor closely resembles histamine H(1)-type receptor.

Although the first report on sigma receptors was released in 1976, mammalian sigma-1 site was purified, cloned and functionally expressed in different tissues and species only three decades later [1],[2],[3]. Its predicted amino acid sequence suggests two transmembrane domains, indicating that it does not correspond to any traditional G-protein coupled receptor. These receptors undergo a desensitisation process, which was reported in brain as well as in the heart muscle [6], [7].

The presence of sigma binding sites was demonstrated for the first time in the central nervous system. Since then, numerous peripheral tissues have been reported to contain sigma receptors in high densities - immune system, digestive tract, endocrine and reproductive systems, and excretory system. Some authors suggested that sigma receptors represent the putative links between nervous, endocrine and immune systems. Interestingly, these receptors are expressed in high densities also in many neural as well as nonneural tumours. One of the peripheral tissues containing considerable amount of sigma receptors is myocardium. According to our findings, majority of sigma sites in the rat heart is of the subtype-1 [4], [6].

Neither the functional role(s) nor the exact mechanism(s) of action of the sigma receptors are completely understood at present. It is very difficult to define a true sigma effect mediated by a receptor in some bioassays since the relative potencies of used sigma ligands vary from one study to another and there are also many discrepancies in corresponding binding data. This might be caused by the fact that until now the endogenous ligand(s) for sigma site(s) remains obscure. Among hot candidates are mainly neurosteroids.

Many functions are inscribed to sigma receptors in the **central nervous system** (antipsychotic, antiamnesic, cognitive enhancement, antiemetic/emetic, antitussive, anxiolytic or antidepressive, induction of dystonia, blockade of tonic potassium channels, modulation of acetylcholine release, neuroprotective effects potentiation of analgesic activity of opioid receptors, an increase in dopamine synthesis, its metabolism and modulation of its release, psychotomimetic effects of certain drugs and modulation of mood). They have also been implicated in many functions in **non-neural tissues** (in agreement with their distribution there).

The effects of sigma receptor ligands in rat myocardium were studied by our group [5],[6],[8]. Based on numerous experimental approaches, we concluded that:

1) Sigma receptors are definitely present in the membranes of adult rat cardiac ventricular myocytes. The majority (over 80%) belong to the sigma-1 subtype.
2) The highly specific sigma receptor ligands exhibit positive inotropic effect in adult rat ventricular cardiomyocytes. Contractility measured by video-motion analyser increases by 150% on average with respect to control contractions.
3) The increase of contractility is accompanied by an increase of cytoplasmic calcium concentration measured by indo-1 Ca2+ transients.
4) The above-described effects of specific sigma ligands on cardiac contractility are caused by nanomolar concentrations, which are close to Kd values. This finding is of particular importance since all the effects previously reported in the literature were caused by concentrations by two to three orders of magnitude higher. Thus, we have demonstrated that the effects are mediated by specific binding of sigma ligands to sigma receptors.
5) The increase of intracellular calcium concentration due to sigma binding is caused by the release of calcium from the sarcoplasmic reticulum. The trigger for this release is IP3, which is formed after sigma ligand - receptor binding.
6) Cardiac sigma receptors in rat undergo a desensitisation process.
7) The physiological significance of sigma signalling in the rat heart muscle is very likely a fine modulation of cardiac contractility.

Our findings were later confirmed and discussed by other experimental groups. Figure 1 summarises the up-to-date knowledge of the role of sigma receptors in cardiovascular system.

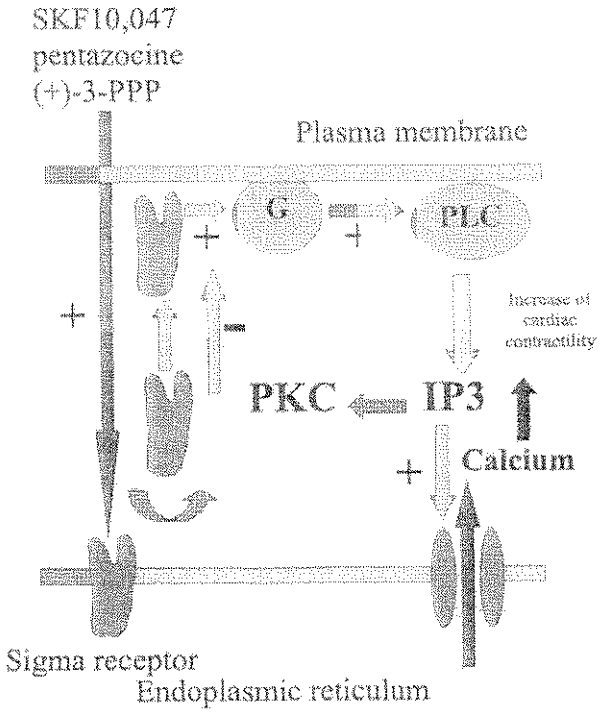

Fig. 1: The proposed diagram of the sigma-1 receptor operation in the heart. The stimulated sigma-1 receptor at the reticular membrane translocates to the plasma membrane. Next, it activates the G protein, which in turn activates the phospholipase C (PLC). The triphosphate inositol (IP3) opens the reticular calcium channel (ryanodine receptor). The release of calcium into the cytoplasm accounts for the positive inotropic effect. By the same token, IP3 stimulates the protein kinase C, which translocates to the cell membrane, phosphorylates and hence desensitizes the sigma-1 receptor.
(Reprinted from: Monassier and Bousquet: Sigma receptors: from discovery to highlights of their implications in the cardiovascular system, Fundamental and Clinical Pharmacology, 2002).

AIM OF THE STUDY

The cardiac effects of sigma ligands have been studied so far exclusively on rat heart, which is rather exceptional among the mammalian species. The aim of this study was to investigate the effect of haloperidol in guinea pig multicellular heart preparations. This model was chosen since this experimental set-up is reliable and the experimental conditions can be strictly controlled.

MATERIALS AND METHODS

In the study, 19 left atria and 15 papillary muscles from the right ventricle of male guinea pigs were included. The body weight of animals varied from 230 to 360 (average, 295±28) grams. The experiments were restricted to males to exclude possible effects of progesterone on female myocardium.

The animals under deep ether anaesthesia were sacrificed by cervical dislocation. The chest was quickly opened, the heart immediately removed and placed in a preparation bowl with a cold (5 °C) Krebs-Henseleit solution of the following composition: NaCl, 118

mM; $NaHCO_3$, 24 mM; KCl, 4.2 mM; KH2PO4, 1.2 mM; MgCl2, 1.2 mM; CaCl2, 1.2 mM; glucose, 5.5m M and Taurine, 10 mM. The right ventricle was opened and all suitable papillary muscles removed. Then, the left auricle strip was cut off and placed together with the right ventricle papillary muscle in the perfusion bath (Figure 2).

We employed a horizontal, plastic, double-walled, thermostatically controlled bath for pharmacological studies. It contains a pair of stimulation electrodes placed on the bottom, one for stimulation (under the clips that held the preparations) and one for grounding. The bath was filled with 10 ml Krebs-Henseleit solution aerated with mixture of O2 and CO2 (95:5%). Both muscles were fixed by a clip at one end and attached to a mechano-electric transducer by a thread bound to its opposite end. Simultaneous recording of the tension generated by the preparations was performed under isometric conditions.

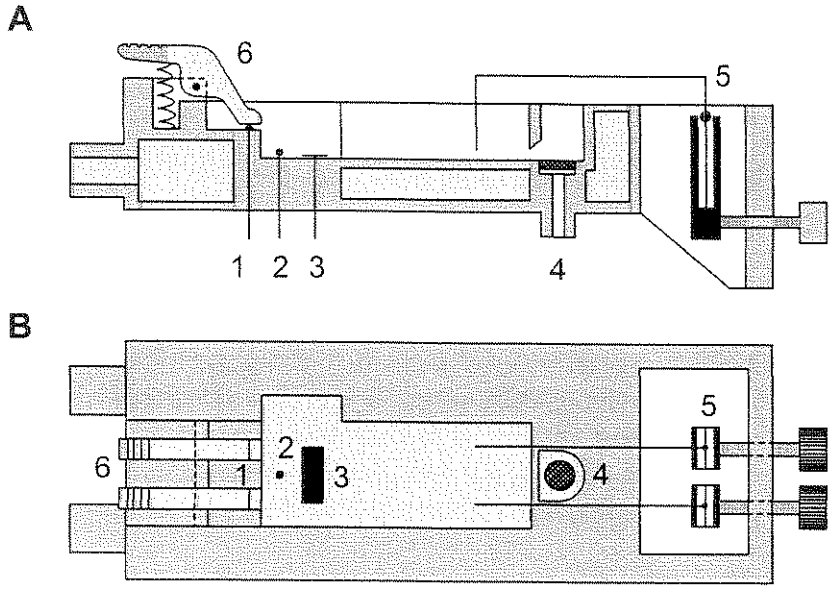

Figure 2: Horizontal perfusion bath for heart multicellular preparations (A, side view; B, from above). 1, stimulation electrodes; 2, thermometer; 3, grounding electrode; 4, bubbling; 5, tensometers; 6, clips.

The recorded signals were amplified and digitised at sampling rate of 250 Hz by a one-channel bipolar 16-bit A/D converter. The specialised software allowed simultaneous 30-minute record of two trends - maximal and minimal tension of the preparations after stimulus and up to ten 15-second snap-shots with detailed (sampling period of 4 ms) recording of stimulated twitches. The recorded data were provided with identification labels, saved in archive files, exported into ASCII and worked out in Excel.

The effects of specific sigma receptor ligand haloperidol on the amplitude of twitch and on the restitution of contractility in both atrial and ventricular guinea pig heart preparations were investigated. The experiments were carried out at 30°C and fluctuations in temperature did not exceed 0.5°C. The preparations were stimulated with 1ms pulses of twice the current threshold. Basic stimulation frequency was 1 Hz throughout the experiments.

Initially, the preparations were stimulated for 30 - 45 min. At the end of this period "control" contractions were recorded. Then the contractions of variable prematurity were recorded according to the protocol (Figure 3) and the mechanical restitution curve was constructed. Next, sigma receptor ligand haloperidol at a concentration 10nM was

administered to the bath for 30 minutes. Snap-shots of 15 contractions at basal rate (1Hz) were recorded after 1, 3, 5, 10, 15, 20, 25 and 30 minutes. Then, the stimulation protocol for assessment of drug affected restitution curve was repeated. This protocol was employed once again after 15 minutes washout with Krebs-Henseleit solution.

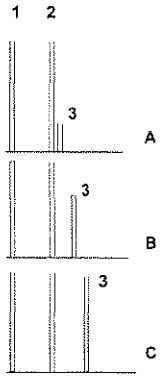

Figure 3: Schematic diagram of constructing the mechanical restitution from discrete data (1, 2, contractions at basal rate; 3, contractions at variable interval)

RESULTS

We observed marked positive inotropic effect previously described in rat ventricular cardiomyocytes in all experiments: in atria, an increase of the twitch amplitude by 36% to 123% in comparison with the control period (average of control twitches represents 100%), with the maximum effect lasting for the first 5 minutes of perfusion. In ventricular preparations, the increase of contraction by 9% to 46% peaked during the first minute of perfusion (Figure 4).

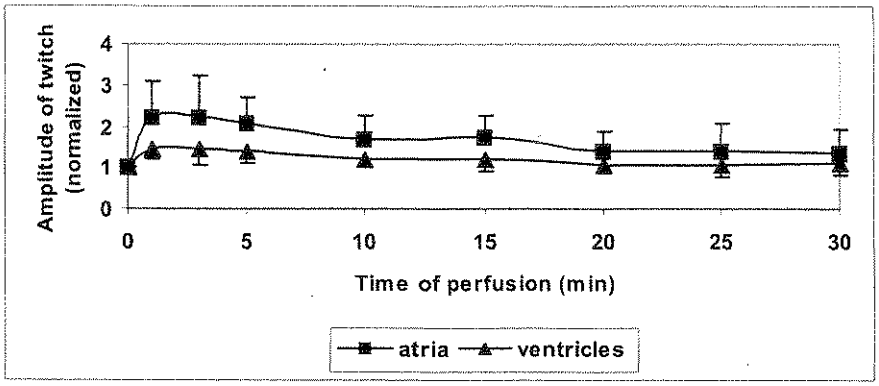

Figure 4: Time course of the effect of sigma ligand haloperidol on normalized contraction amplitudes of guinea pig atria and papillary muscles. Temperature, 30°C; stimulation frequency; 1 Hz. Mean ± SD of 15 experiments. Washout not shown.

The pattern of mechanical restitution was not altered in either preparation under the effect of haloperidol (Figures 5, 6).

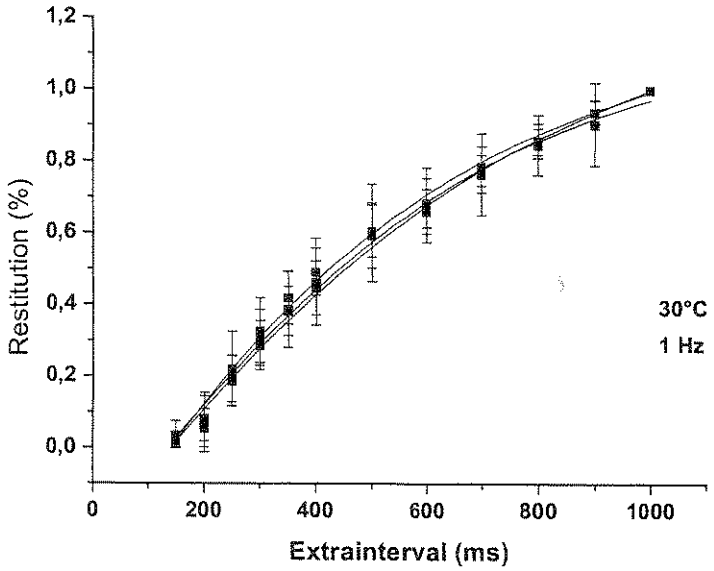

Figure 5: The normalized mechanical restitution of left atrial muscles under control conditions, after 30 minutes of perfusion with haloperidol and after 15 minutes of wash-out. Abscissa, extrainterval, period between a regular and a premature stimulation pulse. Temperature, 30°C; basal stimulation frequency, 1 Hz. Mean ± SD from 15 experiments.

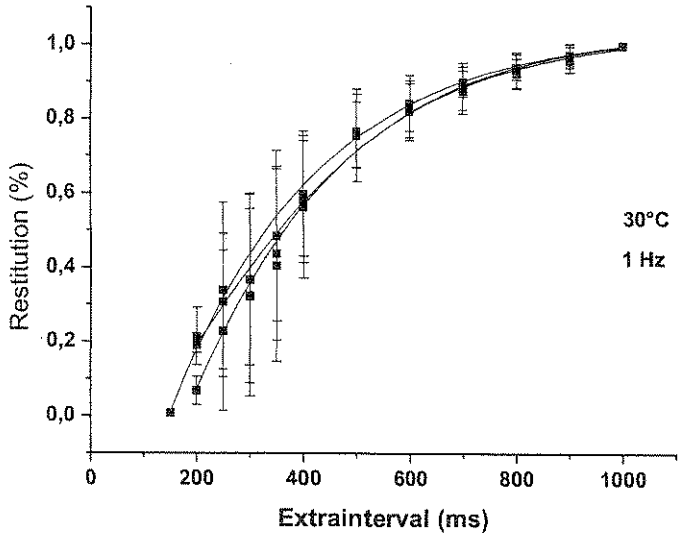

Figure 6: Mechanical restitution of right heart papillary muscles. For a legend see Figure 5.

DISCUSSION

In our study, the effect of haloperidol, a prototypical sigma receptor ligand in guinea pig heart muscle has been investigated. Haloperidol is a psychotropic drug used in everyday clinical practice for treatment of psychoses like schizophrenia or severe agitated delirium, with marked cardiovascular side effects (arrhythmias, and even cardiac arrest). The concentration used in our experiments was 10nM. It was chosen on the basis of our

previous experiments [6] where binding studies revealed that Ki for sigma receptors in rat cardiac myocytes is 6.1±1.3nM. Since many drugs in clinical use bind to sigma receptors with nanomolar affinities (certain neuroleptics, monoamine oxidase inhibitors and several antihistamines - [9], we consider this concentration as relevant.

The effect of sigma binding on the contractility of guinea pig heart multicellular preparations was examined. Such a positive inotropic effect has been first described in neonatal rat cultured cardiomyocytes [5], later in adult rat isolated ventricular myocytes [6]. In all above-mentioned experiments fluctuations in response of cardiac tissue to sigma ligands were observed. This typical pattern can be observed also in the time course of the effect of haloperidol in guinea pig heart preparations (Figure 4).

The mechanical restitution curve is a sensitive tool for estimation of heart preparation viability. Its course is not changed under the effect of haloperidol and does not change even during long lasting experiments. We can assume that positive inotropic effect of sigma ligand is mediated via increased cytoplasmatic Ca2+concentration as previously reported in rat myocardium.

CONCLUSION

The positive inotropic effect of sigma receptor ligand haloperidol was confirmed also in guinea pig atrial and right ventricular heart preparations.

According to the effect of haloperidol on the mechanical restitution we can assume that it is mediated via increased cytoplasmatic Ca2+concentration.

Regardless the effort of many research groups, there is a lot of questions concerning the role of sigma receptors in mammalian myocardium which still remain unanswered.

REFERENCES

[1] Hanner M, Moebius FF, Flandorfer A. et al. Purification, molecular cloning and expression of the mammalian sigma1-binding site. Proc Nat Acad Scien USA 1996; 93, pp.8072-8078.

[2] Kekuda R, Prasad PD, Fei YJ, Leibach FH, Ganapathy V. Cloning and functional expression of the human type 1 sigma receptor (hSigmaR1). Bioch Biophys Res Com 1996; 229, pp.553-558.

[3] Seth P, Fei YJ, Li HW, Huang W, Leibach FH, Ganapathy V. Cloning and functional characterization of a sigma receptor from rat brain. J Neurochem 1998; 70, pp.922-931.

[4] Dumont M, Lemaire S. Interaction of 1,3-di(2-[5-3H]tolyl) guanidine with sigma2 binding sites in rat heart membrane preparations. Eur J Pharm 1991; vol. 209, pp.245-248.

[5] C. Ela, J. Barg, Z. Vogel, Y. Hasin, and Y. Eilam, "Sigma receptor ligands modulate contractility, Ca++ influx and beating rate in cultured cardiac myocytes", Journal of Pharmacology and Experimental Therapeutics 1994, vol. 269, pp. 1300-1309.

[6] Novakova M., Ela C. et al."Inotropic action of sigma receptor ligands in isolated cardiac myocytes from adult rats", Eur. J. Pharm. 1995, vol. 286, pp.19-30.

[7] Ela C., Hasin Y. and Eilam Y. (1996):"Apparent desensitization of a sigma receptor sub-population in neonatal rat cardiac myocytes by pre-treatment with sigma receptor ligands." Eur. J. Pharmacol., vol. 295, pp.275-280.

[8] Novakova M., Ela C. et al. Highly selective sigma receptor ligands elevate inositol 1,4,5-trisphosphate production in rat cardiac myocytes", Eur. J. Pharm. 1998, vol. 353, pp.315-327.

[9] Vilner, BJ., John, CS., and Bowen, WD. Sigma-1 and sigma-2 receptors are expressed in a wide variety of human and rodent tumour cell lines. Cancer Research, January 1995a, vol. 55, no. 2, pp. 408-413.

GASTRODUODENAL PEPTIC DISEASE AND LOW CONCENTRATION OF MG^{2+} IN ERYTHROCYTES

Zdeněk Wilhelm[1], Peter Hegyi[2], Alena Pechová[3]

[1]Department of Physiology, Medical Faculty, Masaryk University, Brno, Czech Republic
[2]Military Hospital, Brno, Czech Republic
[3]Clinic for the Disease of Ruminants, Veterinary and Pharmaceutic University, Brno, Czech Republic

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Address correspondence to:

MUDr. Zdeněk Wilhelm, CSc.
Department of Physiology
Medical Faculty, Masaryk University
Komenskeho nam. 2
662 43 Brno, Czech Republic

Support: MSM 141 100004 (CZ).

SUMMARY

Background and Aim

Magnesium is not only an important element in metabolism, but also a natural antagonist of calcium ions in the process of muscle contraction. Gastroduodenal peptic disease (GDPD) is an illness accompanied by several symptoms indicating the loss of muscle motility expressed as increased muscle tonus. The aim of our work was to determine the extent to which magnesium ion concentrations in serum and erythrocytes differ in samples from patients with GDPD as compared to a control group of healthy individuals.

Material and methods

A group of patients diagnosed with GDPD by the means of endoscopic biopsy (n = 85; age 47 ± 16) was compared to a group of individuals not suffering from the disease (n = 67; age 29 ± 7). The concentration of magnesium in erythrocytes was calculated from hematocrite values, concentration of magnesium in the serum and concentration of magnesium in hemolysed whole blood. Mg^{2+} ions were determined by atomic absorption spectrometry. The results were evaluated by the Wilcoxon test and correlation coefficients were determined for regression of the magnesium data against other haematologic values measured in the study.

RESULTS

We have not found differences in serum magnesium concentration between the two experimental groups. However, we found significantly lower values of magnesium in erythrocytes of patients with the GDPD as compared to healthy individuals (2.00 ± 0.16 vs 1.92 ± 0.23, $p < 0.01$). We also found a significant correlation between magnesium concentration in erythrocytes and mean corpuscular haemoglobin concentration (MCHC)($r = 0.42$, $p < 0.01$).

CONCLUSIONS

1. Measurements of low magnesium concentrations in erythrocytes implicate GDPD associated with muscle motility disorders.
2. Measurements of low magnesium concentrations in erythrocytes of patients with GDPD do not explain the cause for the decrease in magnesium levels.
3. Correlation between magnesium concentrations in erythrocytes and MCHC indicates a mild alteration in erythropoesis in patients with GDPD.

INTRODUCTION

Patients with GDPD usually display an array of accompanying symptoms that are all covered by the term dyspepsy. This term includes abdominal bloating and distension, epigastric pain, regurgitation, postprandial fullness, nausea, vomiting as well as early satiety (1). All these symptoms are probably related to motility disorders of the smooth muscles in the upper gastrointestinal tract.

Increased tonus of the smooth muscles in the gastrointestinal tract is controlled by calcium ions, which have a central role in muscle contraction (2). Magnesium ions are a natural antagonist of calcium ions, therefore they can cause relaxation in these muscles (3). Magnesium action not only improves motility of the smooth muscles in the gastrointestinal tract, but also reduces the occurrence of the above-mentioned accompanying symptoms (4).

The aim of our work was to determine the levels of magnesium in the serum and erythrocytes of patients with GDPD. These values were compared with values from a group of healthy individuals. We also studied additional biochemical and haematological parameters and compared these in the two groups as well.

MATERIAL AND METHODS

A group of patients of both sexes diagnosed with GDPD ($n = 85$, age 47 ± 16) by means of endoscopic biopsy (urease test, histology, culture) was compared to a group of healthy individuals ($n = 67$, age 29 ± 7) of both sexes.

Serum magnesium levels were determined by atomic absorption spectrometry. Levels of magnesium in erythrocytes (EMg) were calculated from hematocrite values, concentration of magnesium in the serum and concentration of magnesium in hemolysed whole blood. Concentration of magnesium in hemolysed whole blood was determined by atomic absorption spectrometry. The results were statistically evaluated using the Statgraphics software package. More specifically, the software was used to perform the Wilcoxon test and to calculate correlation coefficients (r) for regressions of magnesium data against other observed haematological, biochemical and immunological parameters.

RESULTS

Table 1 lists selected biochemical and haematological parameters that show significant differences between the group of patients with GDPD and the control group

Results (x ± SE), ns – not signifacant

	Control group	GDPD patients	p <
Mg^{2+}(mmol/l)	0.91 ± 0.05	0.89 ± 0.06	ns
EMg (mmol/l)	2.00 ± 0.16	1.92 ± 0.23	0.01
Ca^{2+}(mmol/l)	2.38 ± 0.10	2.28 ± 0.13	0.001
Total protein (g/l)	84.5 ± 4.6	77.7 ± 4.7	0.001
Albumine(g/l)	48.8 ± 3.4	44.0 ± 3.6	0.001
Gama-globulin (g/l)	13.7 ± 2.2	12.0 ± 2.9	0.001
Erytrocytes	5.68 ± 0.56	5.15 ± 0.49	0.001
MCV (fl)	86.2 ± 4.4	95.7 ± 6.6	0.001
MCH (pg)	25.7 ± 1.7	28.9 ± 2.3	0.001

MCV - mean corpuscular volume

MCH - mean corpusular haemoglobin

Other observed parameters, such as serum Na+, K+, phosphates, AST, ALT, billirubin, IgG, IgM, TAG, total cholesterol, HLDL and LDL were within normal physiological ranges for healthy individuals. No significant differences between the group of GDPD patients and the control group were found. We calculated the correlation coefficients for the data using the Statgraphics statistical software package to identify possible relationships between serum and magnesium concentrations in erythrocytes on one hand and the rest of the studied parameters on the other hand.

We found only one significant correlation in the group of patients suffering from GDPD, namely between their erythrocyte magnesium levels and the mean corpuscular haemoglobin concentration (MCHC) ($r = 0.42$, $p < 0.01$)

DISCUSSION

GDPD is a relatively wide-spread illness, present even in industrially developed countries with lower concentration of Helicobacter pylori than in most developing countries. Infection of the stomach wall or duodenum is often accompanied by motility disorders of the muscles in the gastrointestinal tract. As a result of these problems, patients display a range of symptoms such as abdominal bloating and distension, epigastric pain, regurgitation, postprandial fullness, nausea, vomiting as well as early satiety. This creates a serious practical problem. Patients who could benefit from improved intake, digestion and resorption of substrates to fight their primary illness (GDPD) do not

cope with the disease well, partly because they have disorders in all the mentioned areas (5,6).

Gastrointestinal tract motility disorders are often associated with increased tonus of smooth muscles. Increased tonus of the smooth muscles in the gastrointestinal tract depends on the calcium ion, magnesium being a natural antagonist of calcium. Therefore, magnesium has the ability to cause relaxation of the smooth muscles and improve the motility of the gastrointestinal tract. This, in turn, can relieve patients from many of the accompanying symptoms, which are often difficult to determine and confirm.

Our group of GDPD patients had significantly lower concentrations of protein and albumin than the control group of healthy individuals. At the same time, both groups displayed values within normal physiological range. Decreased protein and albumin concentrations in patients with physiologically normal levels of liver enzymes could be a result of lower availability of substrates necessary for serum protein and albumin synthesis in otherwise normally functioning liver. On the other hand, the low values could also reflect the lack of factors stimulating such synthesis. Magnesium, one such factor, is necessary for DNA replication.

Findings of lower concentration of protein and albumin in patients suffering from GDPD are associated with lower levels of IgG. It is unclear why IgG levels in these patients are lower than in healthy individuals. The presence of magnesium ions also directly controls cellular immunity, since cytotoxic lymphocytes Tc do not function properly in the absence of the ions. In our experimental groups, we did not study cellular and humoral immunity in detail. General leukocyte values did not differ between the GDPD group and the healthy control group.

Magnesium concentrations in the serum were similar in both studied groups, even though magnesium is known to be partial bound to blood proteins. However, this result is not surprising, since only less than 1% of the total magnesium is present in serum. Compared to other tissues, serum also has the lowest magnesium concentration in the whole organism. The serum concentrations are often 7-10x lower than concentrations found in metabolically active tissues. This means that changes in serum magnesium concentrations remain low even in extreme cases. Our data confirm this observation.

The physiological concentration of magnesium in erythrocytes is approximately 3x higher than serum concentrations (7). Therefore, the differences in erythrocyte magnesium concentration between patients with GDPD and the control group are also higher. Most of the magnesium present in erythrocytes originates from stem cells and becomes bound to cellular proteins, ATP and 2,3-diphosphoglycerate. The dynamics of magnesium levels in the erythrocyte is slower than that in the serum, because the erythrocyte plasma membrane is not physiologically permeable to magnesium ions (8). During the average erythrocyte life time of 100 - 120 days, the changes of magnesium concentration in the erythrocyte do not reflect the rapid changes in serum magnesium concentration, which can be influenced by per oraly or parenteraly administered magnesium.

Our results support these theories. We did not find any differences in serum concentration of magnesium between patients with GDPD and the control group. As for haematological parameters, in samples from GDPD patients we observed a lower number of erythrocytes, larger in size and containing more haemoglobin. The only parameter that correlated with erythrocyte magnesium concentration was the mean corpuscular haemoglobin concentracion (MCHC). This value, like magnesium, depends on the amount of haemoglobin originating in the stem cell. Correlation between EMg and MCHC

suggests a close relationship between Emg and a mild alteration in blood formation in patients suffering from GDPD.

From the clinical point of view, erythrocyte magnesium concentrations are subject to smaller errors from perturbations such as irregular per oral or parenteral intake of magnesium.

Unfortunately, we often hear explanations that physiological or increased levels of magnesium in serum reflect increased levels in tissues, including erythrocytes. In many clinical situations, such interpretation may even postpone treatment.

Our study on the group of patients suffering from GDPD confirmed that erythrocyte magnesium levels were lower, while serum levels remained within the normal physiological range. We think that because of the physiological role of magnesium in muscle contraction, decreased concentrations of magnesium could be responsible for the increased tonus of the smooth muscles in the gastrointestinal tract, motility disorders and the resulting symptoms.

Patients with continuous subjective complaints (such as abdominal bloating and distension, epigastric pain, regurgitation, postprandial fullness, nausea, vomiting as well as early satiety) have lower levels of magnesium in erythrocytes. At the same time, serum levels are similar, compared to a healthy control group. In patients with the illness, magnesium can act not only as the antagonist of calcium ions causing relaxation of smooth muscles, but also as a supporting factor in proteosynthesis, humoral and cellular immunity. Measurements of erythrocyte magnesium concentrations, which can be easily carried out in clinical conditions, can help to direct treatment of patients displaying a broad spectrum of symptoms caused by motility disorders.

CONCLUSIONS

1. Measurements of low magnesium concentrations in erythrocytes implicate GDPD associated with muscle motility disorders.
2. Measurements of low magnesium concentrations in erythrocytes of patients with GDPD do not explain the cause for the decrease in magnesium levels.
3. Correlation between magnesium concentrations in erythrocytes and MCHC indicates a mild alteration in erythropoesis in patients with GDPD.

LITERATURE

1. Epstein O., Perkin G. D., et al.: Abdomen. In: Bild-Lehrbuch der klinischen Untersuchung, Stuttgart, Thieme, 1994, 8.1 - 8.39.
2. Guyton A.C, Hall J. E.: General Principles of gastrointestinal Function - Motility, Nervous Control, and Blood Circulation. In: Textbook of Medical Physiology, Philadelphia, W.B. Saunders Company, 2000, 718 - 727.
3. Holtmeier H.J.: Das Magnesiummangelsyndrom beim Menschen. In: Magnesium und Calcium. Stuttgrart, Wissenschaftliche Verlagsgesellschaft mbH, 1995, 3 - 31.
4. Nakatsuka K., Inaba M., Ishimura E.: Hyper- and Hypomagnesaemia. In: Calcium in Internal Medicine. London, Springer, 2002, 191 - 198.
5. Schmidt-Wilcke H.A.: Magen. In: Klinische Pathophysiologie, Stuttgart, Thieme, 2001, 797 - 810.
6. Schulzke J.D., Riecken E.O.: Dündarm. In: Klinische Pathophysiologie, Stuttgart, Thieme, 2001, 812 - 827.
7. Golf S.: Biochemie und Physiologie von Magnesium im Menschen. In: Magnesium und Calcium. Stuttgrart, Wissenschaftliche Verlagsgesellschaft mbH, 1995, 39 - 49.
8. Hespel P., Lijnen P., Fiocchi R., Lissens W., Amery A.: Effect of calcium antagonism on intracellular concentrations and transmembrane fluxes of cations in erythrocytes of men at rest and during exercise. J Hypertens 1986, 4 (6): 767 - 72.

ERPS TO TARGET AND FREQUENT STIMULI IN VISUAL ODDBALL PARADIGM (SEEG STUDY)

R. Roman[1], M. Brázdil[2], P. Jurák[3], I. Rektor[2], M. Kukleta[1]

[1]Department of Physiology, Medical Faculty, Masaryk University, Brno, Czech Republic
[2]Department of Neurology, St. Anne's Hospital, Masaryk University, Brno, Czech Republic
[3]Institute of Scientific Instruments, Academy of Sciences, Brno, Czech Republic

Address correspondence to:

MUDr. Robert Roman
Department of Physiology
Medical Faculty, Masaryk University
Komenskeho nam. 2
662 43 Brno, Czech Republic

Support: MSM 141 100004 (CZ).

In humans as well as in cats, alpha responses (8-15Hz oscillations) to sensory stimuli returning to baseline 200-300 ms after stimulus were described. Intracerebrally recorded alpha responses in cats were found not only in specific primary sensory areas but also in hippocampus where they were present independently on stimulus modality.

Event related potentials (ERPs) obtained in visual oddball paradigm are composed of early sensory and late "cognitive" components. In some contacts of intracerebral electrodes both components can be present and they can be superimposed on each other.

The aim of the present study was to investigate the intracerebral distribution and morphology of separated early ERP components.

A total of 268 sites of the frontal, parietal and temporal lobes were investigated with depth electrodes in 7 patients with medically intractable epilepsies. Visual oddball paradigm was performed. Patients were instructed to respond only to target stimulus as quickly as possible by pressing a button and to count target stimuli mentally. To separate early ERPs components and to reject long-latency cognitive potentials the digital filter of 5.5-15 Hz was used. For each subject, approximately 50 trials to target and 100 trials to frequent stimuli were averaged. The sites with prominent ERPs components were investigated.

The early ERP components were identified in 98 contacts (6 patients). After both target and frequent stimuli they were present identically in phase in several successive peaks varied in range from 1 to 5, with the latency 60±10 ms, 102±12 ms, 156±11 ms, 213±13 ms, and 289±17 ms. The earliest peaks, usually followed by two or three others were found in the hippocampus, fusiform gyrus and dorsolateral prefrontal cortex. More frequently,

the first phase identical peaks followed by one or two others occurred around 102 ms in the cingulate gyrus, parahippocampal gyrus, superior, middle and inferior temporal gyri, and frontoorbital cortex.

Using the proper digital filter enables one to separate early ERP components from non-filtered signal. The resulting morphology of the components in some contacts seems to be very similar to alpha responses recorded in cats. Each deflection could probably represent and/or at least contribute to the corresponding component of the non-filtered ERP, usually described as P1, N1, P2, N2 and possibly P3.

Simultaneous occurrence of identical components after target and frequent stimuli in different brain structures probably reflects the basic level of processing visual stimuli through diffusely distributed neuronal networks independently on stimulus significance.

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