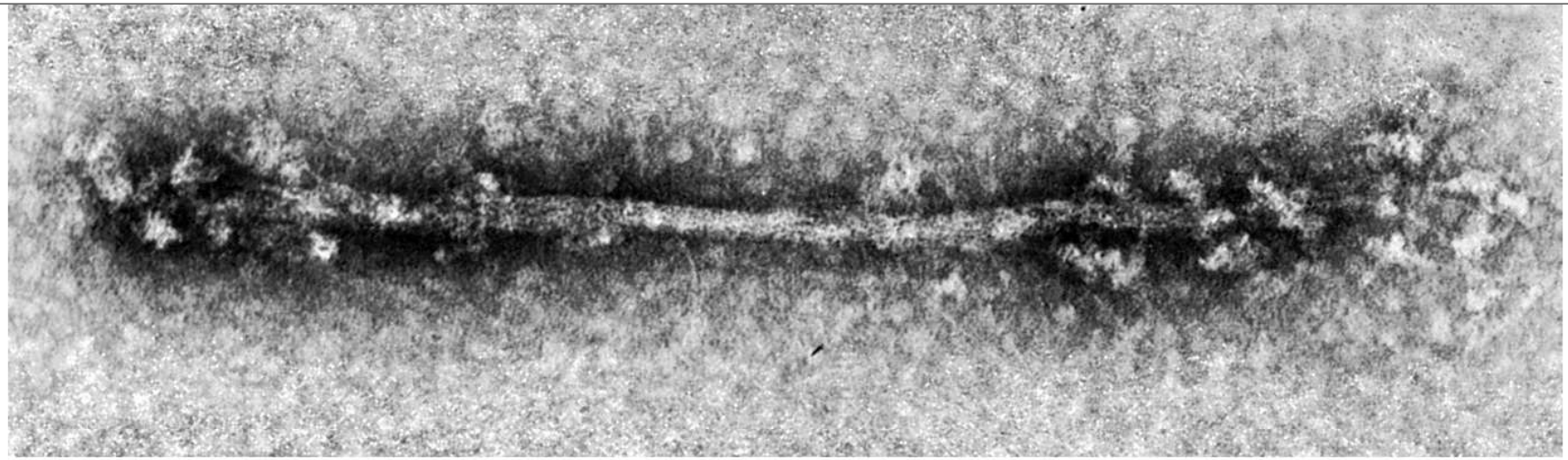
Muscles

Skeletal ~ Cardiac ~ Smooth

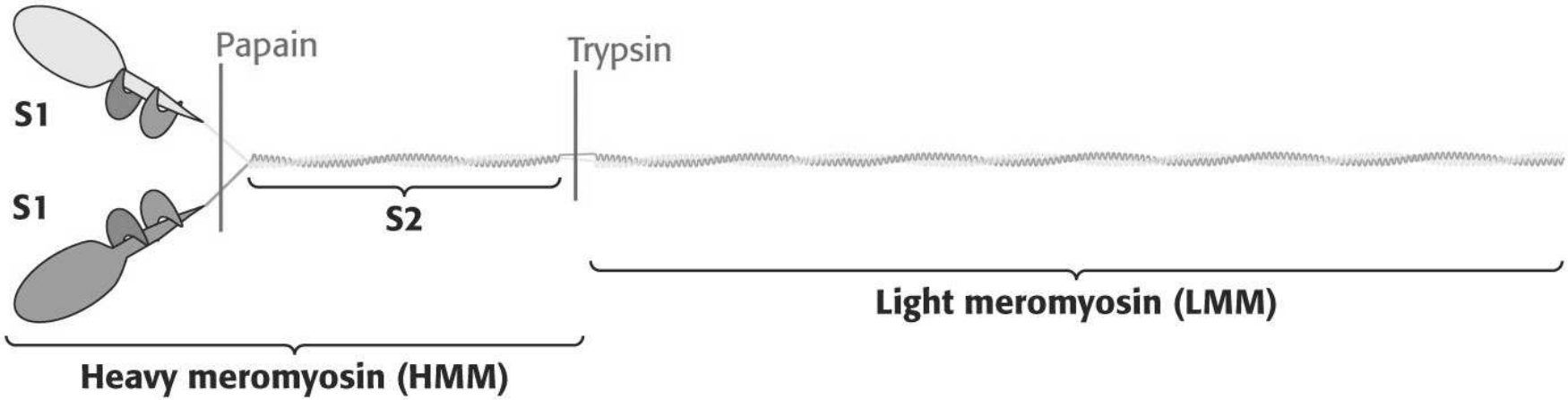
Seminar No. 12

- Chapter 19 -

Thick filament is the myosin aggregate of cca 350 monomers



Myosin monomer



- two heavy chains (they make a double helix)
- four light chains (MLC – myosin light chains)
- *N*-terminal of a heavy chain forms a globular head with ATPase activity ($ATP + H_2O \rightarrow ADP + P_i$)
- treatment of myosin with proteases affords stable fragments (for research purposes).

Thin filament – Actin

- globular monomer (G-actin) makes a double helix (F-actin)
- F-actin has other accessory proteins attached:
- tropomyosin (double helix)
- troponin C – binds calcium ions
- troponin I – inhibits interaction actin-myosin
- troponin T – binds to tropomyosin and other troponins

Q.

Which signal molecule triggers the contraction of skeletal muscles?

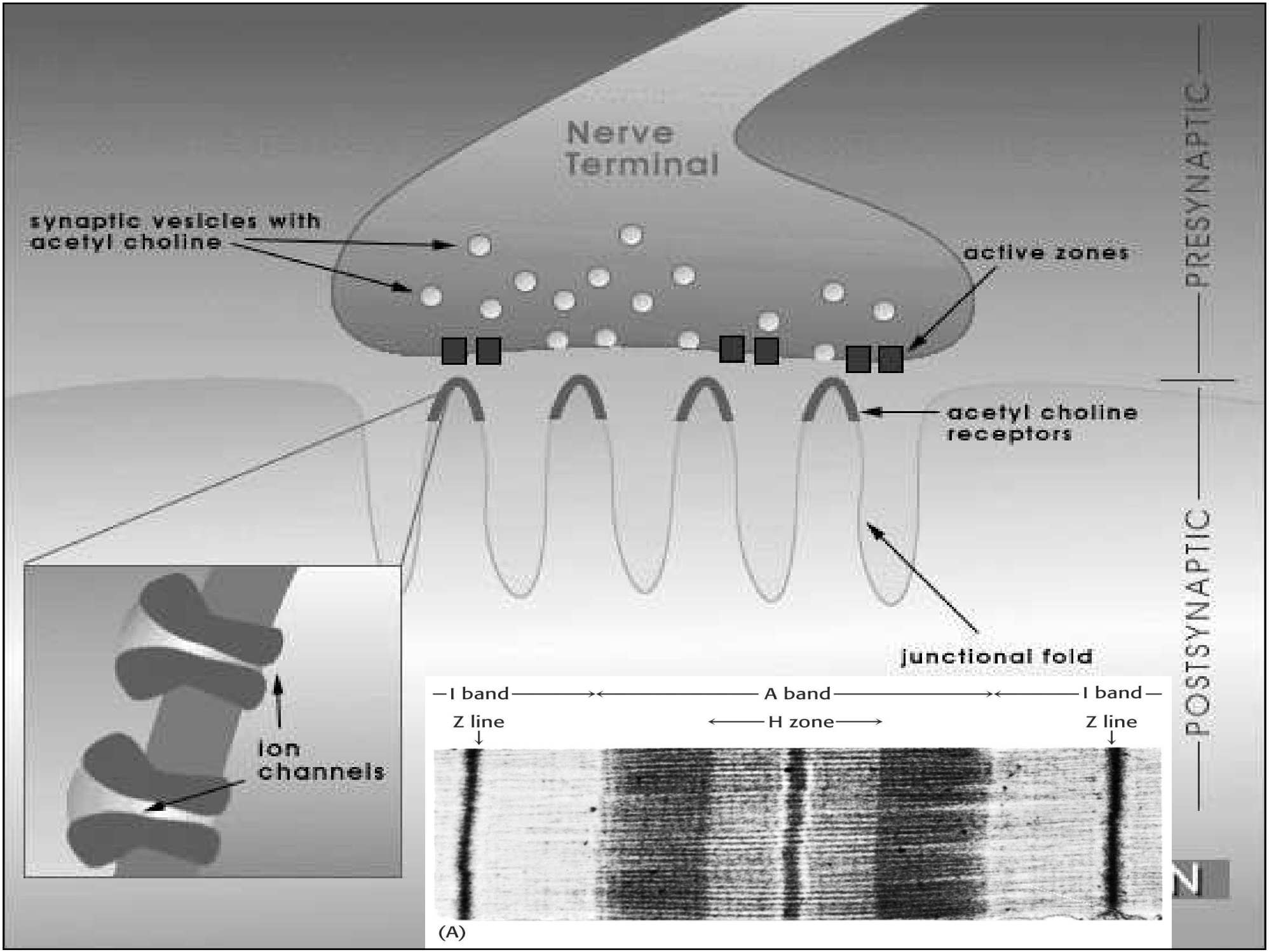
A.

acetylcholine

(see Harper, Chapter 64)

Events on neuromuscular junctions

- junction consists from nerve terminal separated from postsynaptic region by the synaptic cleft
- **acetylcholine** is released from synaptic vesicles and binds to nicotinic receptors in muscle cell membrane $\Rightarrow$ depolarization of membrane and T-tubules
- T-tubules are connected with sarcoplastic reticulum (SR) $\Rightarrow$ **Ca^{2+} ions** are released from SR (where are associated with calsequestrin protein)
- calcium ions then bind to troponin C $\Rightarrow$ **contraction**


Nerve Terminal
synaptic vesicles with acetyl choline
active zones
PRESYNAPTIC
acetyl choline receptors
POSTSYNAPTIC
junctional fold
ion channels
I band
A band
I band
Z line
H zone
Z line
(A)

Q.

What is the Ca^{2+} concentration?

a) in ICF - sarcoplasm during resting state

b) in ICF - sarcoplasm during contraction

c) in ECF - blood plasma

The concentration of calcium ions in body fluids

Fluid / Condition	mol/l	μmol/l
Sarcoplasm / resting	10^{-8}	0.01
Sarcoplasm / contraction	10^{-5}	10
Blood plasma	2.5×10^{-3}	2 500*

* 2.5 mmol/l

Skeletal muscle: relaxation/contraction cycle

- **Relaxation** (scheme on p. 109)
- troponin I inhibits actin-myosin interaction
- ATP molecule (attached to myosin head) has been hydrolyzed

  $\Rightarrow$ chemical energy is conserved in myosin head conformation
- concentration of calcium ions in sarcoplasm is extremely low

  (10^{-8} M)

Skeletal muscle: relaxation/contraction cycle

- after release of Ca^{2+} from SR $\Rightarrow$ myosin-ADP-P_i complex binds to actin
- ADP and P_i are liberated from myosin head, actin filament is pulled by cca 10 nm towards to sarcomere centre $\Rightarrow$ **chemical energy is transformed to mechanical work**
- new ATP molecule binds to myosin head $\Rightarrow$ dissociation of actin-myosin complex
- the liberation of Ca^{2+} ions from troponin C and hydrolysis of ATP leads to relaxation

Q.

What is the effect of botulinum toxin on the neuromuscular junction?

A. (see scheme on p. 135)

- Botulinum toxin is produced by bacterium *Clostridium botulinum*. The toxin is a two-chain polypeptide with a heavy chain joined by a disulphide bond to a light chain.
- The light chain is a protease that attacks one of the fusion proteins at a neuromuscular junction, preventing vesicles from anchoring to the membrane to release acetylcholine.
  By **inhibiting acetylcholine release**, the toxin interferes with nerve impulses and causes **paralysis of muscles** (botulism).
- no action potential is generated $\Rightarrow$ permanent relaxation

Medical uses of botulinum toxin

- Currently, Botox (= trade name) is finding enormous potential in several therapeutic areas including the treatment of migraine headaches, **cervical dystonia** (a neuromuscular disorder involving the head and neck), blepharospasm (involuntary contraction of the eye muscles), and severe primary axillary hyperhidrosis (excessive sweating).
- Other uses of botulinum toxin include urinary incontinence, anal fissure, **spastic disorders** associated with injury or disease of the central nervous system including trauma, stroke, multiple sclerosis, or cerebral palsy and focal dystonias affecting the limbs, face, jaw etc.

Botulinum toxin injections are applied in cosmetics to vanish facial wrinklers

Q.

What are ATP sources for maximal work:

a) during the first 10 sec

b) after 1 min

c) after 10 min

ATP sources for muscle contraction

see page 95

- **During the first 10 sec** – ATP itself and creatine phosphate present in muscle cell

- **After 1 min** – mainly anaerobic glycolysis
  glucose → 2 lactate + **2 ATP**

- **After 10 min** – aerobic oxidation of glc + FA
  glucose → 2 pyruvate → 2 acetyl-CoA → **38 ATP**
  stearic acid → 9 acetyl-CoA → **146 ATP**

Skeletal muscles contain red (slow) and white (fast) fibers

Feature	Red fibers	White fibers
Colour	red	white
Myoglobin	yes	no
Mitochondria	many	few
Contraction rate	slow	fast
Duration	prolonged	short
ATP source	**?**	**?**

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Feature	Red fibers	White fibers
Colour	red	white
Myoglobin	yes	no
Mitochondria	many	few
Contraction rate	slow	fast
Duration	prolonged	short
ATP source	FA, Glc/aerob	Glc/anaerob

Maximal intesity of muscle work (scheme on p. 94)

- anaerobic phase
- 30 sec – 2 min
- working muscles use glucose $\Rightarrow$ metabolized to lactate
- lactate goes to liver $\Rightarrow$ substrate of gluconeogenesis
- small portion of lactate becomes metabolic fuel for resting muscles and myocard

Prolonged muscle work/exercise (scheme, p. 94)

- working muscles are adapted to aerobic metabolism of glucose and FA
- resting muscles utilize FA and KB
- glycerol from lipolysis is the substrate for liver gluconeogenesis

Q.

What is the yield of ATP during:

a) aerobic glycolysis

b) anaerobic glycolysis

A.

Type of glycolysis	ATP / Glc
Aerobic	36 – 38*
Anaerobic	2

* Depends on the type of transport of cytosolic NADH to mitochondria.

Q.

Explain the cause of *rigor mortis*.

A.

Rigor mortis is a recognizable sign of death (L. *mors*, *mortis*, *f.*) that is caused by a chemical change in the muscles, causing the limbs of the corpse to become stiff (L. *rigor*, *oris*, *m.*) and difficult to move or manipulate.

Assuming mild temperatures, rigor usually sets in about 3-4 hours after clinical death, with full rigor being in effect at about 12 hours.

ATP supply from metabolic reactions is exhausted, the muscles remain contracted for ever.

Cardiac muscles - Contraction

- three different sources of Ca^{2+}: ECF, SR, mitochondria
- extracellular calcium enters muscle cells via VOC (voltage operated channels)

⇒ troponin C ⇒ **contraction**

- Ca^{2+} bind to:

⇒ calmodulin ⇒ autoregul. **- relaxation**

Cardiac muscles - Relaxation

- Ca^{2+} ions are liberated from troponin C and removed from sarcoplasm
- there are **four systems** how to vanish Ca^{2+} in sarcoplasm

1. Ca^{2+}-ATPase in SR
2. Ca^{2+}-ATPase in sarcolemma
3. Na^{+}/Ca^{2+}-exchanger (antiport) in sarcolemma
4. Ca^{2+} re-entry to mitochondria

Autoregulation in cardiac muscle

- see scheme on page 110
- intracellular calcium is in the complex with protein calmodulin: Ca^{2+}-CM
- Ca^{2+}-CM stimulates **all** Ca^{2+}-pumps which decrease $[Ca^{2+}]$ in sarcoplasm
- **the increase of intracellular $[Ca^{2+}]$ triggers contraction but, at the same time, stimulates relaxation processes**

Modulatory effect of cAMP on cardiac muscles

- cAMP is the second messenger produced after the activation of G_s-protein-linked-receptors (β-adrenergic receptors)
- such receptors are activated by catecholamines – nor/adrenaline
- cAMP activates protein kinase A
- protein kinase A catalyzes the phosphorylation of:

calciductin of VOC $\Rightarrow$ influx of Ca^{2+} $\Rightarrow$ **contraction**

Ca^{2+}-ATPase in sarcolemma $\Rightarrow$ eflux of Ca^{2+} $\Rightarrow$ **relaxation**

Ca^{2+}-ATPase in SR $\Rightarrow$ eflux of Ca^{2+} $\Rightarrow$ **relaxation**

cAMP as the second messenger (compare p. 136)

Feature	Adrenergic Receptors			
	α_1	α_2	β_1	β_2
Hormone	adrenaline	adrenaline	adrenaline	adrenaline
G-protein	G_p	G_i	G_s	G_s
2nd messenger	DG, IP_3	cAMP ↓	cAMP ↑	cAMP ↑
Occurence*	smooth muscle	brain	myocard	smooth m.

* Example of occurence

Q.

Which parameters are used as the best markers of myocardial infarction (MI)?

Markers of MI (updated table from p. 25)

Marker	Onset (hours)	Maximum (hours)	Multiple of elevation
CK-MB	3 - 6	16 - 32	up 30
cTnT	3 - 4	12 - 18	up 300
Myoglobin*	0.5 - 3	6 - 12	up 10

* The most sensitive indicator, but not specific for myocard.

Metabolic background of MI

- ischemia (lack of oxygen in tissues) leads to anaerobic metabolism $\Rightarrow$ Glc is converted to lactate
- lactate accumulates in ICF and alters intracellular environment $\Rightarrow$ prolonged acidosis causes irreversible cell damage (necrosis)
- permeability of cell membrane increases $\Rightarrow$ cytoplasmatic/mitochondrial/contractile proteins are released into ECF
- the best markers of MI are: myoglobin, CK-MB, cardial troponins (T or I) – a triple combination is recommended
- LD isoforms are no longer used

Creatine kinase (CK) – see p. 23

- Dimer, two different chains (M – muscle, B – brain)
- Three isoenzymes: **MM** (muscle), **MB** (heart), **BB** (brain)
- Major isoenzyme in blood is MM (95 %)
- **MB form in blood: 0 – 6 %**
- BB in blood: traces (BB cannot pass across blood-brain barrier)
- MB isoenzyme is a marker of myocardial infarction

Smooth muscles - Contraction

- source of Ca^{2+}: ECF (VOC, ROC), SR
- there is no troponine C, but two other regulatory proteins binding calcium – calmodulin + caldesmon
- calcium-calmodulin complex (Ca^{2+}-CM) activates MLCK (myosin light chain kinase)
- activated MLCK catalyzes the phosphorylation of myosin
- phosphorylated myosin is capable to make complex with actin $\Rightarrow$ **contraction**

Smooth muscles - Relaxation

- MLC-phosphatase catalyzes the hydrolysis of phosphorylated myosin:

  $$\text{MLC-P} + H_2O \rightarrow P_i + \text{MLC}$$

- MLC **<u>does not</u>** bind to actin $\Rightarrow$ relaxation

The influence of cAMP on smooth muscles

- **cAMP** activates protein kinase A (PK-A)
- PK-A phosphorylates MLC-kinase:

  MLCK $\rightarrow$ MLCK-P
- MLCK-P is inactive, does not phosphorylates MLC $\Rightarrow$

  no interaction between actin and myosin $\Rightarrow$ **relaxation**

The influence of NO on smooth muscles

- nitric oxide (NO) is a relaxant of smooth muscles

  (e.g. arterial myocytes)
- activates guanylate cyclase in cytosol: GTP $\rightarrow$ cGMP + PP_i
- cGMP activates protein kinase G (PK-G)
- PK-G phosphorylates MLC-kinase: MLCK $\rightarrow$ MLCK-P
- MLCK-P is inactive, does not phosphorylate MLC $\Rightarrow$

  no interaction between actin and myosin $\Rightarrow$ **relaxation**

NO releasing compounds

- **Endogenous**:

  L-arginine (the imino nitrogen of guanidine part)

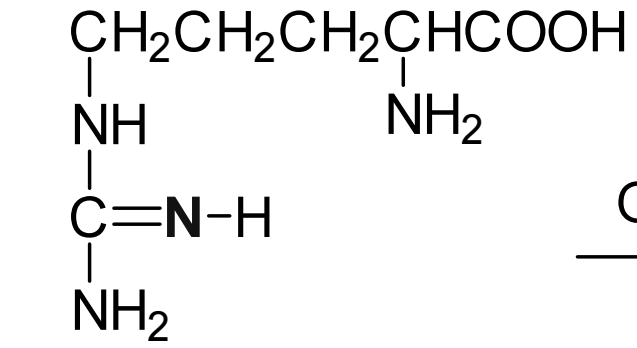
- **Exogenous:**

  organic nitrates = esters of nitric acid (R-O-NO_2)

  organic nitrites = esters of nitrous acid (R-O-N=O)

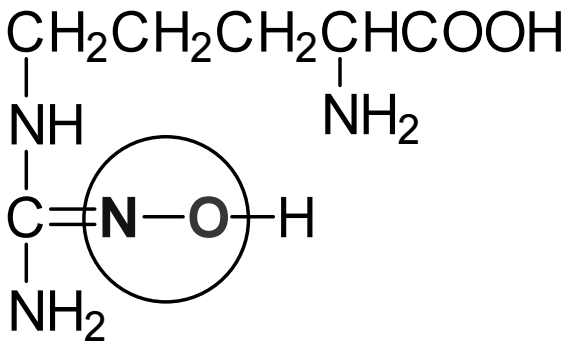
  sodium nitroprusside = a complex of Fe^{3+} with CN^- and NO

NO originates from imino nitrogen of L-arginine



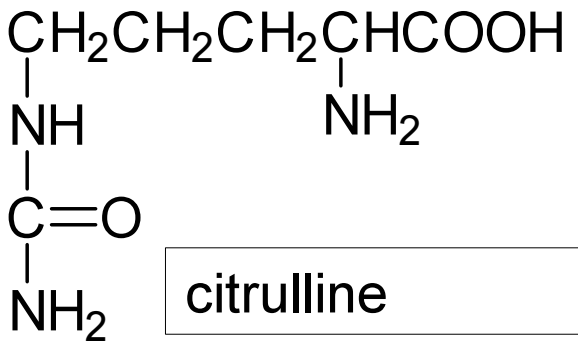
L-arginine

O_2, NADPH



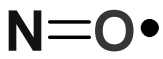
N-hydroxy-L-arginine

O_2, NADPH



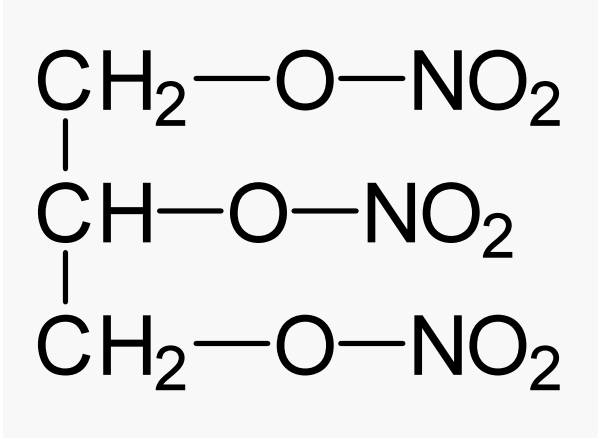
citrulline

+

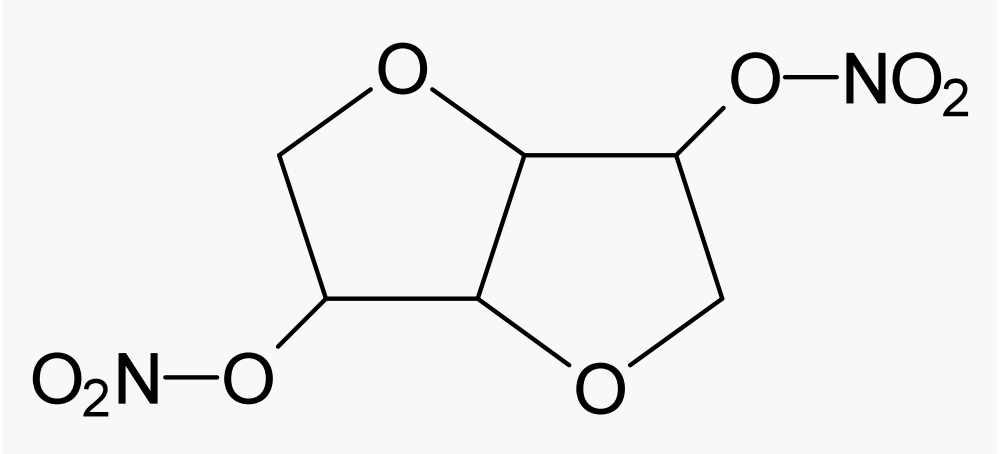


nitric oxide radical

Organic nitrates (alkyl nitrates)



glycerol trinitrate
(glyceroli trinitras)

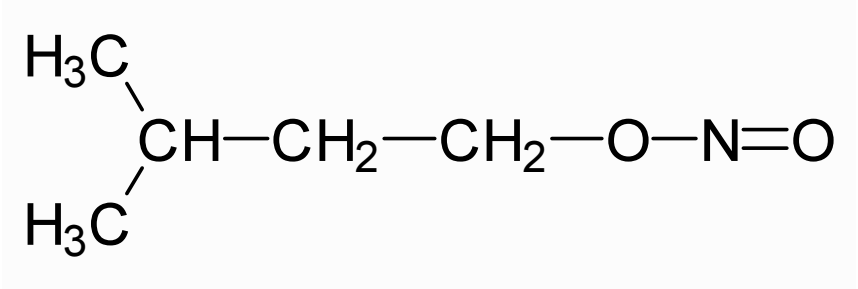


isosorbide dinitrate (isosorbidi dinitras)

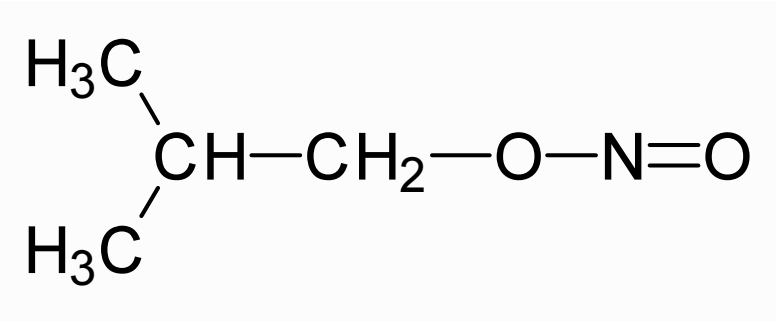
In myocytes, they are reduced by glutathion

and subsequently release NO - **vasodilators**

Organic nitrites (alkyl nitrites)



isoamyl nitrite
(amylis nitris)



isobutyl nitrite
volatile liquid, new drug
(poppers, rush, liquid aroma ...)

Alkyl nitrites as well as inorganic nitrites ($NaNO_2$) have oxidation properties $\Rightarrow$ oxidize Fe^{2+} in hemoglobin to Fe^{3+} $\Rightarrow$ they cause **methemoglobinemia**

Other NO releasing compounds

$Na_2[Fe(CN)_5NO]$

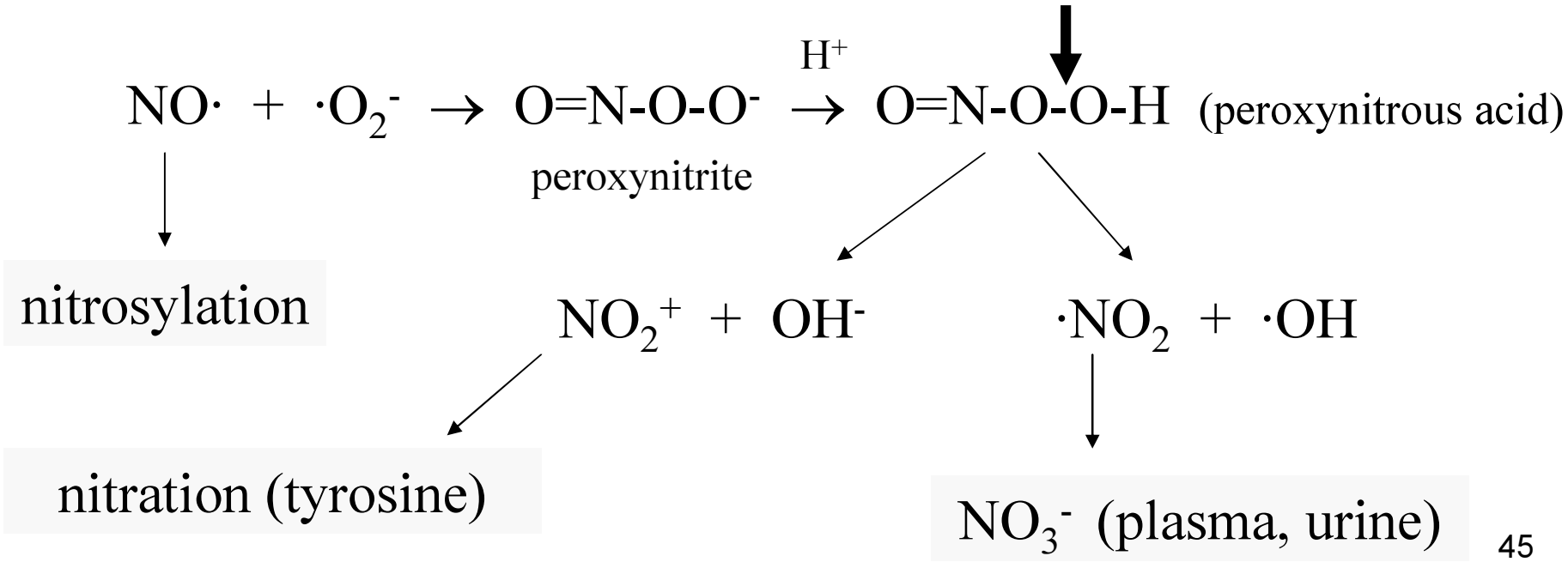
sodium nitroprusside (natrii nitroprussias)

sodium pentacyanonitrosylferrate(III)

extremely potent vasodilator

Other metabolic pathways of NO

- nitric oxide is a radical (·N=O)
- reacts with superoxide to yield peroxynitrite
- the cleavage of peroxy bond (O-O) can occur in two ways



Q.

What effect on **<u>smooth muscle</u>** contractility is caused

by a signal molecule acting through:

α_1-adrenergic receptors

α_2-adrenergic receptors

β-adrenergic receptors

A.

Effects on **<u>smooth muscle</u>** contractility through:

α_1-adrenergic receptors $\Rightarrow$ **contraction**

α_2-adrenergic receptors $\Rightarrow$ **contraction**

β-adrenergic receptors $\Rightarrow$ **relaxation**

Actions mediated through adrenergic receptors (Harper, Ch. 49)

Feature	**Adrenergic Receptors**			
	α_1	**α_2**	**β_1**	**β_2**
Hormone	adrenaline	adrenaline	adrenaline	adrenaline
G-protein	G_p	G_i	G_s	G_s
2nd messenger	$DG/IP_3/$**Ca^{2+}**	**cAMP ↓**	cAMP ↑	**cAMP ↑**
Muscle action	**contraction**	**contraction**	↑ contractility	**relaxation**
Muscle type	**smooth**	**smooth**	myocard	**smooth**