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Review

MicroRNA-mediated regulation of neurotransmitter receptors in epilepsy: A systematic review

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ABSTRACT

Background: Pathogenesis of epilepsy involves dysregulation of the neurotransmitter system contributing to hyper-excitability of neuronal cells. MicroRNA (miRNAs) are small non-coding RNAs known to play a crucial role in post-transcriptional regulation of gene expression.
Methods: The present review was prepared following the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines, employing a comprehensive search strategy to identify and extract data from published research articles. Keywords suchas epilepsy, micro RNA (micro RNAs, miRNA, miRNAs, miR), neurotransmitters (specific names), and neurotransmitter receptors (specific names) were used to construct the query.
Results: A total of 724 articles were identified using the keywords epilepsy, microRNA along with select neurotransmitter and neurotransmitter receptor names. After exclusions, the final selection consisted of 17 studies, most of which centered on glutamate and gamma-aminobutyric acid (GABA) receptors. Singular studies also investigated miRNAs affecting cholinergic, purinergic, and glycine receptors.
Conclusion: This review offers a concise overview of the current knowledge on miRNA-mediated regulation of neurotransmitter receptors in epilepsy and highlights their potential for future clinical application.

1. Introduction

Epilepsy is a chronic neurological disorder characterized by recurrent seizures which are clinical manifestations of abnormal discharges of hyper-excitable neurons [1]. It is a multifactorial disorder that continues to be a challenge for medical sciences. Numerous studies have been made in an effort to unravel the complex mechanism of epileptogenesis. Among the different contributing factors, altered neurotransmitter receptor expression has direct implications for the pathophysiology of epilepsy [2].

The basic principle of brain function is the regulation of neuronal communication via neurotransmitter receptors. The fine balance between excitation and inhibition is meticulously regulated by the ion channels and G-protein coupled receptors. Disruption in this equilibrium has been implicated in the development of epilepsy. Understanding the regulation pattern of these neurotransmitter receptors may help to provide insight into the molecular mechanism of disease development.

In recent years, microRNA (miRNA) has emerged as a key element in the intricate regulatory network governing neurotransmitter receptors and synaptic signaling [3]. For over a decade, the role of miRNAs has been extensively studied in the field of epilepsy [4]. MiRNA, an 18–23 nucleotide short non-coding molecule, is widely distributed in Eukaryotes [5]. Although miRNAs do not encode any protein, they play a very crucial role in the regulation of gene expression involved in various

Abbreviations: AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid; ASM, Antiseizure medicines; CaMKIIγ, Ca2+/calmodulin-dependent protein kinase γ; CREB1, CAMP responsive element binding protein 1; GABA, gamma-aminobutyric acid; GlyR, Glycine Receptor; mAChR, metabotropic muscarinic acetylcholine receptor; miRNA, microRNA; MTLE-HS, mesial temporal lobe epilepsy with hippocampal sclerosis; nAChR, ionotropic nicotinic acetylcholine receptor; NMDA, N-methyl-D-aspartate; P2X7R, Purinergic 2X7 receptor; PRISMA, Preferred Reporting Items for Systematic Reviews and Meta-Analyses; UTR, untranslated region.

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physiological processes. Upon binding to the 3′ untranslated region (UTR) of target mRNA, miRNA either degrades it or inhibits its translation [6]. Understanding the functions and mechanisms of miRNAs provides insights into the complex gene expression regulation and the related cellular pathways. A dysregulation of miRNA is known to be associated with several diseases including cancer, neurodegenerative diseases, metabolic diseases, etc [7]. The unique ability of miRNAs to modulate gene expression by binding to target mRNA plays a pivotal role in neurobiology, where the effect is felt across the neurotransmission. Targeting dysregulated miRNAs implicated in epileptogenesis has also been proposed as a potential therapeutic approach. The most common strategy is the application of an miRNA antisense oligonucleotide (antimiR), which binds to the target miRNA, causing its functional inhibition. Other miRNA inhibition methods include RNA sponges that contain multiple miRNA binding sites or short hairpin RNAs that disrupt specific miRNA-mRNA binding. However, miRNA-targeting drugs face several challenges. First, due to the nonpermeability of the blood–brain barrier, these agents generally need to be applied intrathecally. Second, a single miRNA can have a wide spectrum of target mRNAs with varying binding affinities, making the effects of inhibition hard to predict. The most studied miRNA therapeutic in epilepsy thus far has been the miR-134 antimiR, which reduced seizure frequency in a mouse epilepsy model for two months without noticeable adverse effects. The proposed mechanism of action was derepression of *LIMK1* leading to a change in neuron dendritogenesis [8].

Although this article specifically focuses on miRNA influence on neurotransmitter receptors, it is important to mention that miRNAs also regulate neuronal excitability by modulating ion channels, ion transporters, and synaptic transmission. For instance, miR-324-5p represses *KCND2*, reducing Kv4.2 potassium channel expression, which can lower the threshold for seizures. Conversely, miR-335-5p reduces seizure susceptibility by targeting the sodium channel subunit Scn2a. MiR-499-5p and miR-101 regulate ion channels and transporters Cav1.2 and NKCC1, impacting calcium influx and chloride ion gradients, respectively. These interactions facilitate processes like memory and GABAergic signaling. Additionally, miRNAs influence synaptic transmission. For example, miR-92a and miR-124 modulate AMPA receptor subunits GluA1 and GluA2, affecting synaptic plasticity. MiR-132 and miR-134-5p further regulate synaptic function and dendritic spine dynamics, demonstrating miRNAs' role in shaping neural network excitability through diverse molecular pathways [9].

This systematic review presents an in-depth literature search to elucidate the intricate relationship between miRNAs and neurotransmitter receptors involved in the pathogenesis of epilepsy. The search focuses particularly on neurotransmitter receptors involved in epilepsy such as glutamate, GABA, acetylcholine, glycine, and purinergic receptors, their miRNA-mediated regulation, and unveils the implications of miRNA dysregulation in altering neuronal excitation/inhibition balance and its potential role in seizure development. The ability of miRNA to modulate and restore the crucial equilibrium of neuronal transmission offers the potential to develop a new causative therapy for epileptic patients.

2. Methodology

The present review was prepared in line with the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines [10]. A comprehensive search strategy was employed to identify and extract data from published research articles. The MEDLINE database accessed through PUBMED, Scopus, Web of Science, and ScienceDirect was used for the strategic search of the articles. The search spanned from the date of inception to November 2023.

The eligibility criteria employed for the inclusion of articles in this review were as follows: Based on abstracts, only articles reporting on miRNA post-transcriptional regulation of neurotransmitter receptors in the context of epilepsy pathogenesis were selected for full-text review. The article had to be an original contribution and written in the English language. Original articles written in a language other than English were excluded. Abstracts, reviews, *meta*-analyses, letters, editorials, and commentaries were excluded from the search. There was no restriction on the year of publication of the articles.

The search query was constructed using combinations of the following keywords: epilepsy, micro RNA (micro RNAs, miRNA, miRNAs, miR), neurotransmitters (specific names), and neurotransmitter receptors (specific names). Two independent authors participated in the screening of the articles. The screening process involved a comprehensive evaluation of the titles, abstracts, and full-length articles. Articles meeting the established inclusion criteria were further evaluated and short-listed for inclusion in the review. Any difference of opinion between the authors was resolved by constructive discussion and by unanimity.

3. Results

The initial search retrieved 724 articles, which were subjected to rigorous screening. After the removal of duplicates, the remaining 587 articles were evaluated based on titles and abstracts. Based on obvious inclusion/exclusion criteria, 420 articles were excluded at this stage. 54 articles were short-listed for full-text review. 27 articles were excluded for not being related to neurotransmitter receptors 5 for not being related to epilepsy, 3 were sequencing studies and 2 did not report on miRNA-mediated regulation. The resulting final selection consisted of 17 articles that aligned with the topic of interest (Fig. 1). Relevant information from the selected articles was properly extracted and compiled. The findings were structured based on the neurotransmitters involved in epilepsy and their miRNA-mediated regulation (Table 1, Fig. 2).

4. Discussion

This systematic review identified 17 studies that emphasize the role of miRNAs in the regulation of neurotransmitter receptors in epilepsy. Neurotransmitter receptors, integral to synaptic transmission and neuronal communication, are significantly influenced by miRNAs. Over the last decade, the intricate relationship of miRNA and neurotransmitter receptors has become a focus of epilepsy research. Through this comprehensive review, we have highlighted the critical roles of miRNAs in modulating the neurotransmitter receptors expression. These receptors, including glutamate, GABA, glycine, cholinergic and purinergic receptors play a crucial role in maintaining fine balance in neuronal excitability. Dysregulation of these receptors can lead to abnormal neural firing such as in epilepsy [2]. The objective of this review is to provide a thorough overview of the current knowledge of dysregulated miRNAs and their effects on neurotransmitter receptors implicated in epilepsy. However, it is essential to acknowledge the multifaceated nature of miRNAs where it can target multiple mRNA transcripts that can extend beyond the isolated miRNA-mRNA interactions. This phenomenon adds an additional layer of complexity in understanding the regulatory role of miRNA. Although this review focuses on the specific miRNA-mRNA target interaction related to neurotransmitter receptor, it is important to recognize that miRNA can exert broader effect across neural network involved in synaptic plasticity, neuronal excitability, and seizure susceptibility.

4.1. Glutamate receptors

Glutamate receptors are extensively studied receptors and evidence confirms their dysfunction contributes to the pathogenesis of epilepsy [27]. There are two main classes of glutamate receptors: ionotropic glutamate receptors (iGluRs) [28] and metabotropic glutamate receptors (mGluRs) [29]. iGluRs are integral membrane proteins that form a central ion channel directly allowing the influx of calcium and sodium

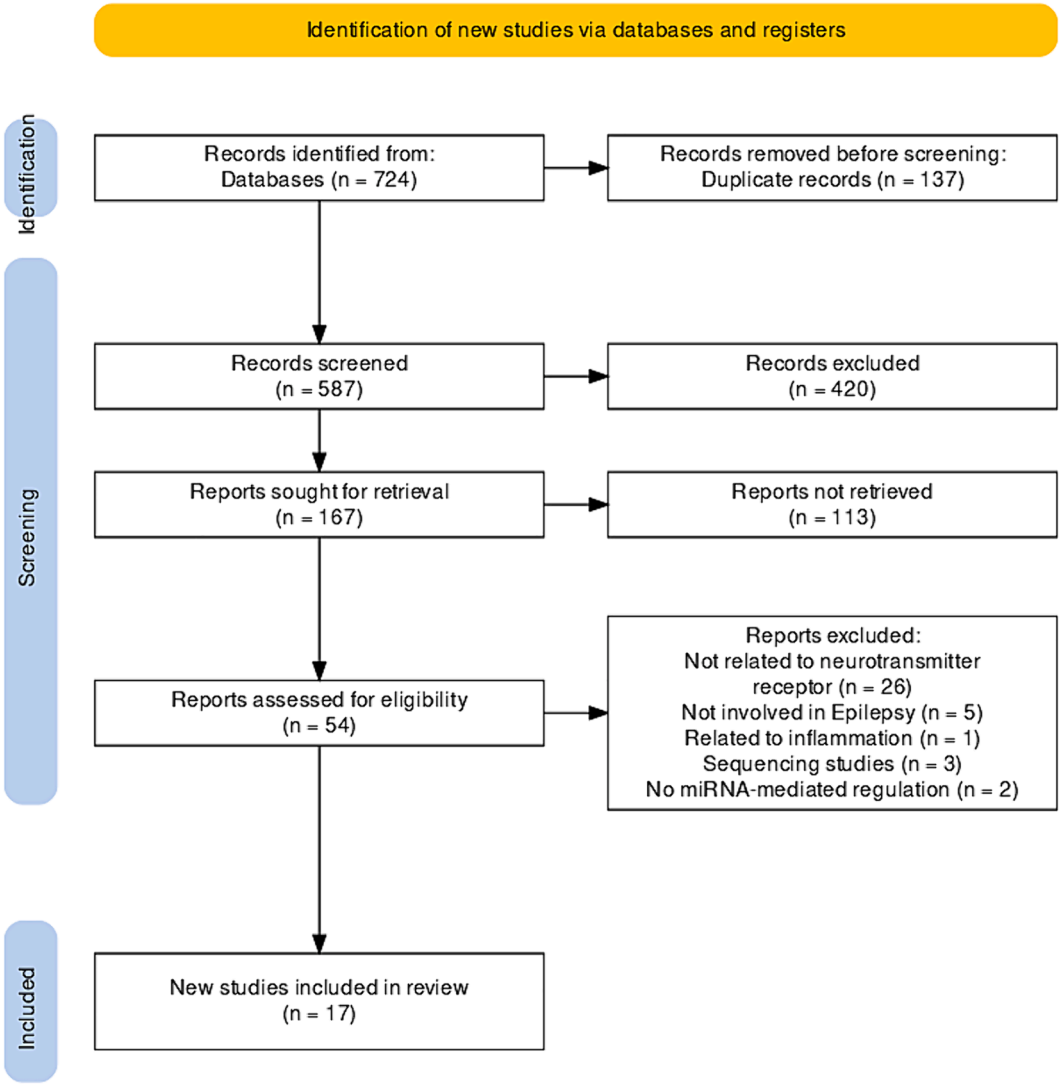

Fig. 1. Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flow diagram [11].

Table 1
Summary of neurotransmitter receptor involved in epilepsy and their miRNA regulators.

Receptor	Gene	miRNA regulator	Reference
Glutamate Rs	*GRIN1*	miR-124	[12]
	GRIN1	miR-219	[13,14,15,]
	GRIN2B	miR-34c	[16]
	GRIN2A	miR-139-5p	[17]
	GRIA2	miR-218	[18]
GABA-A Rs	*GABRA5*	miR-346	[19]
	GABRA3, GABBR2	miR-34c-5p	[20]
	GABRA1	miR-129–2-3p, miR-203	[21,22]
Glycine receptor	*GLRB*	miR-203	[23]
Nicotinic acetylcholine Rs	*CHRNA7*	miR-211	[24]
Purinergic receptor	*P2RX7*	miR-22	[25,26]

ions into the cell [28], while mGluRs are G-protein coupled receptors that modulate the synaptic transmission and neuronal excitability throughout the central nervous system [29]. iGluRs comprise NMDA, AMPA, and kainate receptors [28]. NMDA receptors play a key role in the pathogenesis of epilepsy [30].

NMDA receptor overactivation leads to an excessive calcium ion influx into the cell which can trigger seizures [31] as well as lead to neuronal death via activation of a cascade of intracellular pathways [32]. In humans, both of these pathological mechanisms are evident in patients with anti-NMDAR encephalitis [33]. Several antiseizure medications (ASMs) target iGluRs, including perampanel [34] and topiramate [35], AMPA receptor antagonists, or felbamate [36] and valproate [37], antagonists of the NMDA receptor.

miR-124, one of the most abundant and brain-specific miRNAs, is involved in the regulation of various neurological processes including neuronal differentiation, maturation, and synaptic plasticity. In both patients and rat models of epilepsy, the expression of miR-124 was found to be downregulated [12,38]. Intra-hippocampal administration of a miR-124 mimetic inhibited neuronal firing and excitability as well as susceptibility to seizures [12]. miR-124 has been identified as a direct regulator of CAMP-responsive element binding protein 1 (*CREB1*) mRNA. CREB1 plays an important role in epilepsy. It enhances NMDAR-mediated currents and surface expression. Consistent with this, intra-hippocampal application of a miR-124 mimetic, significantly reduced NMDAR trafficking and localization to the cell surface, thereby affecting the NMDAR's synaptic activity and overall synaptic transmission [12].

miR-219, another brain-specific miRNA expressed in both rodents and human brains, is known to play a critical role in the regulation of neural activity, especially oligodendrocyte differentiation, myelination,

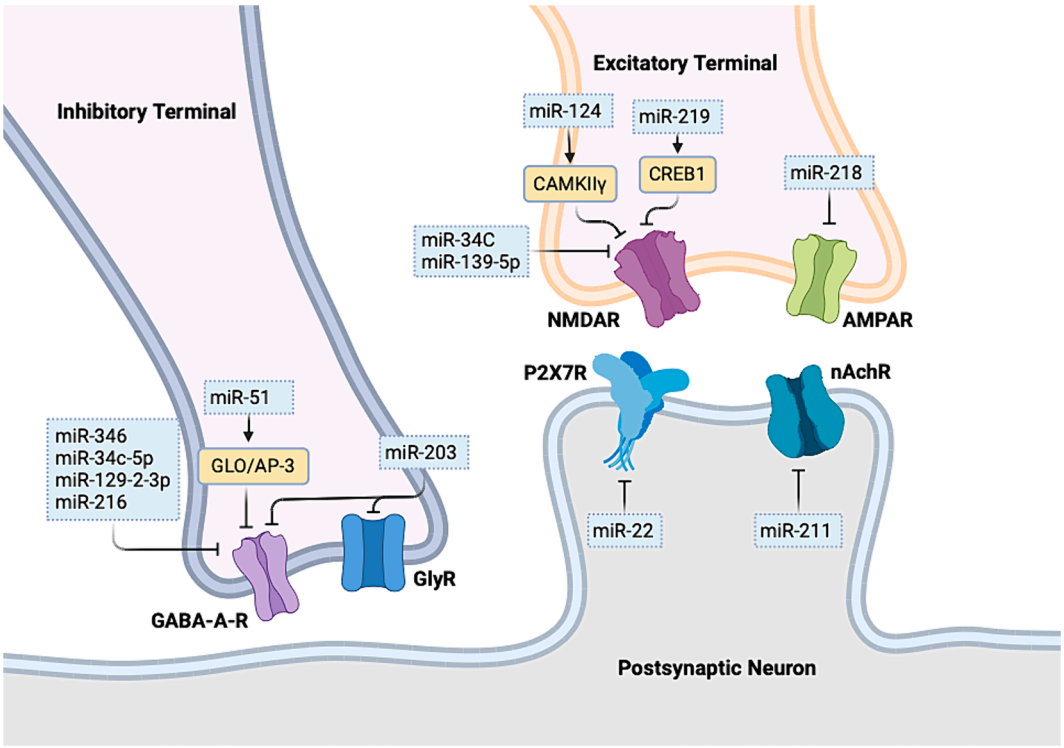

Fig. 2. Overview of miRNA mediated regulation of neurotransmitter receptors (created with BioRender.com) Note that glial cells adjacent to pre- and postsynaptic neurons (not shown in this figure) also contain neurotransmitter receptors whose translation is affected by miRNA regulation and can participate in epileptogenesis.

and synaptic plasticity [15,39,40]. The level of miR-219 is found to be downregulated in the brains of status epilepticus rat models and cerebrospinal fluid samples of patients with epilepsy, indicating its role in the pathogenesis of epilepsy [13]. miR-219 negatively regulates NMDA receptors by targeting Ca2+/calmodulin-dependent protein kinase γ) (CaMKIIγ). CaMKIIγ is a component of CaMKII enzymes which is crucial for neuronal signaling. Expression of both CaMKII and NMDAR has been identified to be altered in epilepsy. Zheng et. al. demonstrated that the treatment with a miR-219 mimetic in kainic acid rat models alleviates seizure severity, abnormal EEG patterns, and increased CAMKIIγ and NR1 (subunit of the NMDAR) levels [13]. When studied in brain samples from MTLE patients, an increased level of NMDA-NR1 expression with a decreased level of miR-219 is observed in the amygdala and vice versa was observed in the hippocampus, thus, indicating the involvement of miR-219 in the regulation of NMDA-NR1 receptor subunit expression [14].

MiR-34c plays a crucial role in numerous cognitive disorders [41,42]. In TLE, approximately 30–40 % of patients develop memory impairment, attention deficits, and other cognitive disorders [43]. Huang, et. al in their study demonstrated that the overexpression of miR-34c in epileptic rats caused cognitive impairment [16]. miR-34c was shown to negatively regulate the expression of NR2b, p-NR1, and p-GluR1 genes, which are associated with long-term potentiation (LTP) in the hippocampus. LTP is crucial for memory formation. It is affected by calcium influx caused by NMDAR activation. NR2B, a primary regulatory subunit of NMDAR, is known to play a key role in learning and memory formation [44]. Overexpression of miR-34c significantly reduced the expression of NR2B, p-NR1, and p-GluR1, possibly impairing LTP and cognitive functions in epileptic rats [16]. Targeting these regulatory genes involved in LTP could serve as a therapeutic strategy aimed at alleviating cognitive impairments in epilepsy.

The role of miR-139-5p in epilepsy emerges as a crucial regulatory element. It negatively regulates the NMDAR subunits, particularly NR2A and NR2B. By restoring the levels of miR-139-5p using its mimetic, a corresponding change was observed in the NR2A expression. This interplay suggests that miR-139-5p exerts a regulatory effect on NMDAR activity, contributing to neuronal hyper-excitation and changes in synaptic plasticity implicated in epilepsy [17].

Neuronal projections are highly enriched with miR-218 and its precursor pre-miR-218–1 [18]. During the chronic suppression of neuronal activity, the expression of miR-218 increases, and vice versa, suggesting its crucial role in regulating the homeostasis of synaptic strength. GluA2, an AMPA receptor subunit, is one of the several targets of miR-218. GluA2 is a key player in determining AMPA receptor properties, including calcium permeability, single-channel conductance, and rectification. MiR-218 positively regulates the expression of GluA2, thereby increasing glutamatergic synaptic transmission [18].

In addition, Zhang et. al, through their study verified a negative correlation between several miRNAs and mRNAs associated with the glutamatergic system in post-traumatic epilepsy rats models (PTE). Among them are miR-98-5p–Slc17a6, miR-335-5p-Slc17a6, miR-30e-5p-Slc17a6, miR-1224-Slc25a22 and miR-211-5p-Slc25a22 [45].

4.2. GABA

GABA, the primary inhibitory neurotransmitter in the central nervous system, plays a significant role in epilepsy. It exerts its effects via two primary receptor types: the ionotropic $GABA_A$ receptors ($GABA_A$ Rs), which mediate chloride influx into cells, and the metabotropic $GABA_B$ receptors ($GABA_B$ Rs), which are G protein-linked channels exhibiting a slower inhibitory effect by reducing intracellular calcium influx [46]. Sequence variants in genes that encode GABA receptor subunits have been implicated as causative factors in multiple epileptic syndromes. Several anti-seizure medications exert their effects by enhancing GABA activity, such as benzodiazepines, which act as allosteric agonists of GABA receptors, tiagabine, which blocks the reuptake of GABA into neurons, and more recently, cenobamate, which acts as a positive allosteric modulator of $GABA_A$ Rs [47].

In a rat model of temporal lobe epilepsy induced by status epilepticus, miR-346 was found to be significantly downregulated in

extracellular vesicles extracted from forebrain structures. This miRNA's predicted target includes *GABRA5*, a gene encoding the alpha 5 subunit of the $GABA_A$ R [19]. Upregulation of *GABRA5*, in this case, could represent a reaction to the epileptic discharges and a potential arresting mechanism.

In human mesial temporal lobe epilepsy patient hippocampal samples, significantly higher expression of hsa-miR-34c-5p was identified by Haenisch et al. Among the predicted targets of this miRNA are *GABRA3* and *GABBR2* [20]. In both rat models of status epilepticus and human TLE, miR-129–2-3p was found to be significantly upregulated [21,48]. In silico modeling identified *GABRA1* as a target of this miRNA, which was confirmed by luciferase assay. Knockdown of miR-129–2-3p by an antagomir in vivo resulted in reduced seizure-like activity on EEG in the rat model [21].

miR-51 was found to regulate the amount of $GABA_A$ Rs on synaptic surfaces. In a C. elegans model, loss of miR-51 led to a decrease in GABAergic synapses and a lower density of $GABA_A$ Rs. The GLO/AP-3 pathway, which is involved in lysosomal trafficking, was found to mediate this effect [49] Additionally, a significant number of miRNAs were predicted to target *GABRA1* in silico in a study by Zhao et al. Verification with a luciferase assay revealed that only miR-181, miR-203, and miR-216 significantly affected the mRNA levels in a cell culture model[50].

4.3. Glycine receptor

Glycine receptors (GlyRs) are ligand-gated chloride ion channels that mediate fast inhibitory neurotransmission. GlyRs are primarily found in the spinal cord and brainstem, with their presence also noted in the human limbic system, including the hippocampus, and the cerebral cortex, although in lesser amounts [51]. Low levels of extracellular glycine activate presynaptic GlyRs, thereby lowering the seizure threshold, while higher levels bind to extrasynaptic GlyRs, reducing excitability. In samples from rat and human temporal lobe epilepsy hippocampi, dysfunctional glycine signaling has been observed. This dysfunction is characterized by increased expression of the reuptake glycine transporter 1 (GlyT1), leading to decreased intra-synaptic glycine levels [52]. Overexpression of miR-203 in hippocampi of epileptic mice models and human epileptic brains led to inhibition of the glycine receptor-β gene (*GLRB*), thus potentially reducing the seizure threshold. An antagomir of this miRNA restored hippocampal GLRB levels and decreased the number of seizures in the mice model [23]. Interestingly, miR-203 also targets *GABRA1* [50], further amplifying its potential role in epileptogenesis.

4.4. Cholinergic receptor

Cholinergic receptors, also called acetylcholine receptors due to their binding of acetylcholine, are classified into two groups, the metabotropic muscarinic acetylcholine receptors (mAChRs) and the ionotropic nicotinic acetylcholine receptors (nAChRs). Dysfunction of both of these receptor groups has been implicated in epileptogenesis and seizure propagation via an increase in neuronal excitability [53]. In a mouse model, hippocampal miR-211 was shown to be downregulated in the acute setting of an induced epileptic seizure by a pilocarpine injection. One of the targets of this miRNA in both mice and humans is the gene for neuronal acetylcholine receptor subunit alpha-7 (nAChRα7). Due to a disrupted acetylcholine signaling in the brain, a decrease of miR-211 may lower the seizure threshold. Indeed, a reduction of miR-211 levels in mice engineered to overexpress miR-211 led to a manifestation of electrographically recorded seizures [24].

4.5. Purinergic receptor

Purinergic receptors are plasma membrane receptors that play a crucial role in cellular signaling processes ranging from neurotransmission to immune responses [54]. This class of receptors is broad and encompasses receptors that respond to purines including adenosine, adenosine triphosphate and other purine nucleotides. Purinergic receptors are classified into two main types: P1 receptors which are activated by adenosine and P2 receptors which are activated by adenosine triphosphate (ATP). P1 receptors includes adenosine A_1, A_{2A}, A_{2B} and A_3 receptors. A_1 receptors exert inhibitory effect on neural activity and A_{2A} receptors are often stimulatory in nature. Whereas A_{2B} and A_3 receptors have varied effects. On other hand, P2 receptors are further divided into two subtypes: P2X receptor (P2XR) and P2Y receptor (P2YR). P2XR are ligand-gated ion channels allowing the passage to various cations such as Ca2+, Na+, and K+ [55]. These receptors are crucial for fast signaling events such as synaptic transmission. They also serve as a key regulator of neuroinflammation. On the other hand, P2YR are G-protein coupled receptors that influence cascade cellular processes involved in neurotransmitter release, immune cell activation, and vasodilation [54].

In recent years, purinergic receptors have gained increasing attention due to their involvement in neurological disorders. P2X7R, a member of the P2XR family, showed pathologically increased expression inMTLE-HS patients and experimental TLE animal model [26].. Activation of P2X7Rs triggers the activation and proliferation of microglia and the release of pro-inflammatory cytokines. In addition, P2X7R located at synapses are suggested to directly regulate neurotransmitter release. Given this function, modulating the expression of these receptors could serve as a potential target.

miR-22 emerges as a regulator of *P2RX7* gene expression, controlled by Sp1 transcription factor [25,26]. Sp1 binding to the P2X7 promoter increases during neuronal activity regardless of the strength of the stimulus. Sp1-induced miR-22 transcription occurs only during mild neuronal stimulation, thereby attenuating P2X7R expression [25]. During strong neural activity, the increased calcium influx serves as a significant factor regulating the fine balance between miR-22 and *P2RX7* gene expression. This identified feed-forward loop regulating P2X7R expression provides a novel therapeutic approach to treating neurological disorders [25]. In addition to this, Leal, B. G. et. al, in their study demonstrated a inverse relationship between serum miR-22 levels and P2X7R expression in the hippocampus and temporal neocortex of human MTLE-HS patients. Thus, the circulating level of miR-22 in serum could serve as a indicator of P2X7R brain expression in MTLE-HS patients and potential sensitive predictor of drug refractoriness [26].

5. Conclusion

In conclusion, the complex regulatory role of miRNAs in the regulation of neurotransmitter receptors in epilepsy offers an intriguing avenue for comprehending the underlying mechanism in complex neurological disorders. Owing to their ability, miRNAs have become a crucial factor in defining the expression level of neurotransmitter receptos. Specific miRNAs such as miR-124, miR-219, miR-34c, miR-139-5p, miR-218, miR-346, miR-34c-5p, miR-129–2-3p, miR-203, miR-51, miR-211, and miR-22 have been identified as a key regulators of neurotransmitter receptor expression in epilepsy. By targeting gene involved in glutamatergic, GABAergic, glycinergic, cholinergic, and purinergic signaling pathways, these miRNAs exert a significant impact on synaptic plasticity, excitability and seizure susceptibility.

Thus, the dysregulation of miRNAs and their target neurotransmitter receptors constitutes a critical aspect of epileptogenesis. Understanding the complex interaction of miRNAs and their target mRNA and their related downstream signaling pathway holds a significant promise in advancing our knowledge of pathophysiology of epilepsy and develop treatment approaches for epilepsy patients.

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CRediT authorship contribution statement

Shivani Sonawane: Writing – original draft, Methodology, Investigation. **Vít Všianský:** Writing – review & editing, Writing – original draft. **Milan Brázdil:** Writing – review & editing, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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