HHS Public Access

Author manuscript

Prog Neurobiol. Author manuscript; available in PMC 2020 December 01.

Published in final edited form as:

Prog Neurobiol. 2019 December ; 183: 101682. doi:10.1016/j.pneurobio.2019.101682.

G protein-coupled receptors in acquired epilepsy: Druggability and translatability

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Abstract

As the largest family of membrane proteins in the human genome, G protein-coupled receptors (GPCRs) constitute the targets of more than one-third of all modern medicinal drugs. In the central nervous system (CNS), widely distributed GPCRs in neuronal and nonneuronal cells mediate numerous essential physiological functions via regulating neurotransmission at the synapses. Whereas their abnormalities in expression and activity are involved in various neuropathological processes. CNS conditions thus remain highly represented among the indications of GPCR-targeted agents. Mounting evidence from a large number of animal studies suggests that GPCRs play important roles in the regulation of neuronal excitability associated with epilepsy, a common CNS disease afflicting approximately 1-2% of the population. Surprisingly, none of the US Food and Drug Administration (FDA)-approved (>30) antiepileptic drugs (AEDs) suppresses seizures through acting on GPCRs. This disparity raises concerns about the translatability of these preclinical findings and the druggability of GPCRs for seizure disorders. The currently available AEDs intervene seizures predominantly through targeting ion channels and have considerable limitations, as they often cause unbearable adverse effects, fail to control seizures in over 30% of patients, and merely provide symptomatic relief. Thus, identifying novel molecular targets for epilepsy is highly desired. Herein, we focus on recent progresses in understanding the comprehensive roles of several GPCR families in seizure generation and development of acquired epilepsy. We also dissect current hurdles hindering translational efforts in developing GPCRs as antiepileptic and/or antiepileptogenic targets and discuss the counteracting strategies that might lead to a potential *cure* for this debilitating CNS condition.

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Keywords

AED; cAMP; epilepsy; epileptogenesis; GPCR; seizure

1. Epilepsy

Epilepsy is one of the most common neurological disorders that afflicts approximately 1-2% of the population globally. The disease is symptomized by epileptic seizures that are defined as unprovoked and spontaneous electrical disturbances in the brain due to abnormally excessive and synchronous activities of a group of brain neurons. The process of transforming a normal brain into one that generates epileptic seizures is known as epileptogenesis. Over the course of decades, we have witnessed the remarkable scientific advances in understanding the pathophysiological processes associated with seizure initiation, escalation and dissemination in the brain, leading to the introduction of the third-generation antiepileptic drugs (AEDs, also often called anticonvulsants) to the drug market (Luszczki, 2009). However, there are still about 30-40% of epilepsy patients who are inadequately treated, as they develop epilepsy forms that are refractory to the current frontline treatments (Ben-Menachem, 2014). Moreover, most antiseizure drugs are well-recognized for their broad neurotoxic adverse effects, the most common of which include, but are not limited to, drowsiness, dizziness, nausea, fatigue, headache, blurred vision, tremor, incoordination, and cognitive impairment in young children (Perucca and Gilliam, 2012).

It is another very unfortunate fact that all current antiseizure medications act merely to suppress seizures in patients who have already been diagnosed with epilepsy (Loscher et al., 2013; Varvel et al., 2015). Notwithstanding, nearly 50 clinical trials for antiepileptogenesis and seizure prevention based on a hypothesis that antiseizure drugs could also be antiepileptogenic, to date there is no therapeutic strategy that has been shown to either prevent the development of epilepsy in patients prior to the first seizure or modify the disease outcome in those diagnosed with epilepsy (Abou-Khalil, 2007; Temkin, 2001, 2009). While some preclinical studies have suggested antiepileptogenic effects from certain AEDs (Blumenfeld et al., 2008; Russo et al., 2010) or potential therapeutic agents (Aronica et al., 2017; Klein et al., 2018; Ravizza and Vezzani, 2018) in animal models of epilepsy, they have not been proven in human studies to date (Kaminski et al., 2014). One seminal conclusion that can be drawn from these monumental efforts at both preclinical and clinical levels is that the biology of epileptogenesis is likely quite different from the biology of the epileptic brain itself. Discovery of epilepsy prevention or modification strategies must ultimately hinge on a well understanding of the signaling pathways that govern the pathogenic process of epileptogenesis following the initial precipitating events, which can help to identify novel molecular targets that can be manipulated by small-molecule agents (Dey et al., 2016).

Epilepsy is a disease with great diversity, as there are more than 15 different seizure types and 30 epilepsy syndromes (Berg et al., 2010). Many Mendelian forms of epilepsy often involve ion channel mutations (Di Cristo et al., 2018). In fact, most of the current AEDs interrupt seizures by directly modulating ligand- or voltage-gated ion channels (Rogawski

and Loscher, 2004). However, for the acquired forms of epilepsy we must seek molecular mechanisms that cause the dysfunction of ion channels, which might guide us to identify key epileptogenic mediators that dictate the expression and functional state of ion channel proteins that set the seizure threshold (Varvel et al., 2015). Control of these potential epileptogenic mediators might be the key to interrupting epileptogenesis and could provide prevention and/or modification for acquired epilepsy. Over the past decade, a number of G protein-coupled receptors (GPCRs) have been emerging as candidates for such epileptogenic mediators owing to their essential roles in the regulation of ion channel functions, thereby altering neuronal excitability and setting the seizure threshold. In this review, we highlight our current understanding of GPCRs as potential epileptogenic mediators by focusing on recent preclinical and clinical efforts in seeking novel therapeutic targets to control acute seizures and interrupt acquired epileptogenesis.

2. GPCRs as CNS drug targets

2.1 GPCRs – the most studied drug target family

GPCRs, also known as seven-transmembrane (7-TM) receptors, represent the largest super protein family of receptors that detect extracellular molecules and trigger signal transmission inside of the cell. GPCR signaling transduction occurs through coupling to a number of intracellular proteins, such as heterotrimeric G proteins, β-arrestins and kinases, which then activate different downstream effectors and initiate cascades of molecular, cellular and physiological responses. The heterotrimeric G protein complex consists of a G_{α} subunit, of which there exist four major types (i.e., $G_{\alpha s}$, $G_{\alpha i/o}$, $G_{\alpha q/11}$, and $G_{\alpha 12/13}$), coupled to a combination of G_{β} and G_{γ} subunits. In response to the ligand binding, GPCR undergoes conformational change that enables its intracellular portion to function as a guanine nucleotide exchange factor (GEF) to allosterically activate the associated G_{α} protein by replacing the bound GDP with a GTP. The G_{α}-GTP then dissociates from $G_{\beta\gamma}$ dimer to further act on intracellular signaling proteins or target functional proteins directly, depending on the type of G_{α}. GPCR can also mediate pathways that are independent of G protein via recruiting β-arrestins to initiate G protein-independent signaling.

These highly specified membrane receptors are only found in eukaryotes, and there are more than 800 different genes in humans – or approximately 4% of the entire protein-coding genome – that code for GPCRs (Bjarnadottir et al., 2006; Ikeda et al., 2018). Based on their protein sequence and structural similarity, GPCRs are phylogenetically divided into five major classes (GRAFS): Glutamate (15 members to date), Rhodopsin (701 members), Adhesion (24 members), Frizzled/Taste2 (24 members), and Secretin (15 members), with each class displaying distinct ligand binding properties (Bjarnadottir et al., 2006; Stevens et al., 2013). GPCRs are detected in almost all types of tissues and organs, where they are involved in nearly all types of physiological, functional and pathological processes in the body. The ligands that can bind and activate GPCRs include compounds, hormones, odors, pheromones, and neurotransmitters, as they vary in size from small molecules to biological macromolecules, such as nucleic acids, lipids, peptides and proteins, endowing them robust and extensive druggability. As of the year 2018, about 475 drugs targeting 108 unique members of this super receptor family have been approved by the US Food and Drug

Administration (FDA). These GPCR-targeted drugs contribute to approximately 34% of all FDA-certified drugs on the market and a global sales volume of over 180 billion US dollars annually (Hauser et al., 2018).

2.2 GPCRs and CNS conditions

In the CNS, more than 90% of non-sensory GPCRs are widely expressed and involved in numerous essential physiological functions including cognition, sensory, mood, appetite, neurogenesis, and synaptic plasticity via regulating neurotransmission at both presynaptic and postsynaptic sites (Gainetdinov et al., 2004; Huang and Thathiah, 2015). Malfunctions in GPCR-regulated neurotransmission contribute to various neuropathological processes such as pain, seizure, anxiety, depression, neurodegeneration, neuroinflammation, etc., and could lead to multiple neurological and psychiatric conditions, making GPCRs appealing molecular targets for these diseases. Indeed, disorders associated with CNS functions remain highly represented among the indications of GPCR-targeted therapeutic agents and account for 124 (~26%) of all FDA-approved GPCR-targeted drugs to date. Among the most prominent targets for the CNS diseases are dopamine, endocannabinoid, opioid, prostanoid, and serotonin receptors. In addition, a myriad of GPCR-targeted agents are currently in clinical studies for CNS indications, demonstrating a sturdy continual interest (Hauser et al., 2017).

Mounting evidence from a large number of animal studies suggests that GPCRs might play some essential roles in the mediation of neuronal excitability via regulating $G_{\alpha s}$ and $G_{\alpha i}$-controlled cAMP signaling as well as $G_{\alpha q}$-initiated Ca^{2+}-sensitive pathways, suggesting their appealing therapeutic indications in seizure disorders. In contrast, none of the FDA-approved (>30) current AEDs suppresses epileptic seizures by directly acting on GPCRs. This surprising yet disappointing disparity raises concerns about the translatability of these preclinical findings and the druggability of GPCRs for acute seizures and chronic epilepsy. Among the GPCR families that have been intensively studied for their contributions to the pathophysiological processes related to epileptic seizures are prostanoid, endocannabinoid, adenosine, metabotropic glutamate receptors and several others. Recent progresses in understanding the signaling pathways of these receptors regulating neuronal excitability and setting seizure threshold are summarized below; their potential as novel molecular targets for acute seizures and the development of acquired epilepsy is also discussed.

3. GPCRs in epileptic seizures

3.1. Prostanoid receptors

Prostanoids are a subclass of eicosanoids consisting of prostaglandin D2 (PGD_2), PGE_2, $PGF_{2\alpha}$, PGI_2 (also known as prostacyclin), and thromboxane A2 (TXA_2). These bioactive lipids have been found in nearly all human tissues and mediate numerous physiological and pathological processes including allergy, immunoregulation, inflammation, mitogenesis, muscle contraction, neurotransmission, pain, thrombosis, vasoconstriction, and vasodilatation (Hirata and Narumiya, 2011). Their biosyntheses in the body are comprised of several enzymatic steps and are highly regulated by various physical, chemical and pathophysiological stimuli. During the first step, arachidonic acid, a 20-carbon unsaturated

fatty acid, is freed from membrane-bound phospholipids by phospholipase A2 (PLA2) and then converted in a dual enzymatic reaction by cyclooxygenase (COX) to an intermediate molecule prostaglandin H2 (PGH_2). Short-lived PGH_2 is further rapidly converted to certain prostanoid types by the tissue-specific prostanoid synthases (Qiu et al., 2017; Rojas et al., 2014b; Smyth et al., 2009) (Fig. 1). Prostanoids exert their physiological and pathological functions through acting on a panel of GPCRs. Two currently known GPCRs (DP1 and DP2) are activated by PGD_2 and four by PGE_2 (EP1, EP2, EP3, and EP4), whereas each of the other three types of prostanoids acts on a single receptor (FP, IP, and TP) (Fig. 1). Hydrophobicity analysis of their protein sequences suggest that they all have a typical GPCR structure, i.e., an extracellular N-terminus, seven membrane-spanning segments, and an intracellular C-terminus, and belong to the rhodopsin-like GPCR receptor family, subfamily A14 (Woodward et al., 2011). Among these nine conventional prostanoid receptor subtypes, EP1, EP2, and FP are the most studied in animal models of epilepsy, yielding extensive evidence that attests to their contributions to epileptic seizures. More studies engaging both pharmacological and genetic strategies are required to clarify whether DP1, EP3, EP4 and TP receptors are also involved in the pathophysiology of seizure disorders (Table 1). To date, no study has been reported about any indication of either DP2 or IP receptor in epileptic seizures.

3.1.1 EP1 receptor—In rodents and guinea pigs, the EP1 receptor is widely distributed in organs and tissues such as kidney, lung, stomach, as well as several CNS sites (Ricciotti and FitzGerald, 2011); whereas the expression of EP1 in higher species like humans is more restricted to a few organs and tissues including colon, mast cells, myometrium, pulmonary veins, and skin (Woodward et al., 2011). The EP1 receptor is coupled to G protein complex that contains $G_{\alpha q/11}$ and $G_{\beta/\gamma}$ dimer (Fig. 1). Upon PGE_2 binding, $G_{\alpha q}$ is released from the complex to activate phospholipase C (PLC), which in turn hydrolyzes phosphatidylinositol 4,5-bisphosphate (PIP2) to inositol trisphosphate (IP3) and diacyl glycerol (DAG) (Jiang et al., 2017; Rojas et al., 2014b). IP3, via binding to IP3 receptors in the endoplasmic reticulum (ER) that function as calcium channels, increases cytosolic Ca^{2+} levels, thereby regulating Ca^{2+}-sensitive signaling pathways; whereas DAG functions as a second messenger that activates certain isoforms of protein kinase C (PKC) (Fig. 1). The EP1 receptor has long been known for its roles in neurotoxicity caused by NMDA receptor overactivation and Ca^{2+} dysregulation after cerebral ischemia (Kawano et al., 2006; Zhou et al., 2008), insinuating the potential involvement of the PGE_2 receptor in the pathophysiology of prolonged seizures, which are also often associated with substantial excitotoxic damage within the brain. Indeed, centrally-administered EP1 receptor antagonist SC-19220 reduced pentylenetetrazol (PTZ)-provoked acute seizures in Wistar rats (Oliveira et al., 2008). Likewise, systemic administration of another EP1 antagonist ONO-8713 attenuated, whereas EP1 agonist ONO-DI-004 exacerbated, PTZ-induced seizures in Swiss mice (Reschke et al., 2018). Systemic pre-treatment with high dose of EP1 antagonist SC-51089 significantly decreased the seizure severity in NMRI mice after amygdala kindling (Fischborn et al., 2010). Moreover, genetic ablation of EP1 receptor increased seizure threshold and showed marked anti-inflammatory and neuroprotective effects in the hippocampus after systemic administration of kainate in mice (Rojas et al., 2014a). These findings from pharmacological

inhibition and genetic inactivation of the EP1 receptor together demonstrate its contribution to increasing the neuronal excitability and lowering the seizure threshold.

Seizures can induce the expression of transporter P-glycoprotein at the blood-brain barrier that might enhance the brain efflux of AEDs, which promotes pharmacoresistance in epilepsy. Among several key signaling pathways that might be involved in seizure-associated transcriptional activation of P-glycoprotein is the COX/PGE_2 cascade (Potschka, 2010). COX-2 inhibition by SC-58236, NS-398, indomethacin, or celecoxib attenuated the increase in P-glycoprotein expression in cortex and hippocampus after status epileptics in rats (van Vliet et al., 2010), which was largely detected in brain capillaries (Zibell et al., 2009). Furthermore, the brain penetration of the antiseizure drug phenytoin was markedly increased by chronic COX-2 inhibition in rats that developed spontaneous recurrent seizures (van Vliet et al., 2010). In line, the EP1-selective antagonist SC-51089 interrupted seizure-promoted expression of efflux transporter P-glycoprotein at the blood-brain barrier following pilocarpine-induced status epilepticus in rats and restored an anticonvulsant effect of a low dose of phenobarbital, a conventional AED and P-glycoprotein substrate (Pekcec et al., 2009). These findings together indicate that the EP1 receptor might be a downstream effector of COX-2 cascade in the upregulation of blood-brain barrier transporter P-glycoprotein following prolonged seizures that mediates brain efflux of AEDs, such as phenytoin and phenobarbital. Thus the EP1 antagonism might represent a promising therapeutic strategy to overcome the pharmacoresistance suffered by more than 30% of epilepsy patients (Potschka, 2010), though the EP1 downstream G_q signaling molecules that are directly responsible for the seizure-driven P-glycoprotein expression remain undetermined.

3.1.2 EP2 receptor—The EP2 receptor is coupled to a G protein that contains $G_{\alpha s}$ and $G_{\beta\gamma}$ complex. Activation of the receptor by PGE_2 results in the dissociation of G_s protein heterotrimeric complex into $G_{\alpha s}$ and $G_{\beta\gamma}$, which in turn regulate several cellular signaling pathways (Fig. 1). Particularly, $G_{\alpha s}$ stimulates adenyl cyclase to elevate cellular levels of cAMP thereby activating protein kinase A (PKA), exchange factor directly activated by cAMP (EPAC), and cAMP response element-binding protein (CREB) regulation of gene transcription. The EP2 receptor appears widely distributed in human body and have been primarily detected in bones, CNS, leukocytes, reproductive system, and smooth muscle (Markovic et al., 2017; Woodward et al., 2011), where it mediates many important normal physiological functions, such as immunoregulation, neuronal plasticity, learning and memory. Furthermore, PGE_2 signaling via the EP2 receptor has shown some protective effects in injury settings such as allograft rejection, bone fracture, gastrointestinal damage, and kidney failure (Jiang and Dingledine, 2013). However, accumulating evidence from numerous CNS studies suggests that EP2 receptor signaling might exacerbate secondary neuronal toxicity in several models of chronic neuronal inflammation and degeneration (Andreasson, 2010; Jiang and Dingledine, 2013; Kang et al., 2017; Liu et al., 2019). The EP2 receptor-mediated neurotoxicity might be associated with brain innate immunity since under these conditions, EP2 activation diminished the beneficial functions of microglia (Johansson et al., 2013; Johansson et al., 2015), which are the resident brain immune cells (Smolders et al., 2019). Intriguingly, EP2 signaling that initially causes the activation of

microglia could facilitate their delayed death later, thereby potentially contributing to the resolution phase of brain inflammation (Fu et al., 2015; Quan et al., 2013). In addition, EP2 receptor expression is highly inducible in neurons by cerebral ischemic injury, and postnatal ablation of the neuronal EP2 receptor in mice is cerebroprotective (Liu et al., 2019). These studies taken together lend support to a translational strategy suppressing the EP2 signaling to reduce inflammation and injury of the brain following excitotoxic insults.

Inspired by the predominant role for the EP2 receptor in the inflammation-associated CNS conditions described above, a series of small-molecular competitive antagonists for the EP2 receptor have been developed for proof-of-concept studies in animal models over the past decade by us and others (af Forselles et al., 2011; Fox et al., 2015; Ganesh et al., 2014a; Ganesh et al., 2013; Ganesh et al., 2014b; Jiang et al., 2012; Qiu et al., 2019). TG4-155, a potent EP2-selective antagonist identified by high-throughput screening (Jiang et al., 2012), diminished the induction of a number of key pro-inflammatory mediators in rat microglia (Quan et al., 2013), and reduced neuronal injury in the hippocampus after pilocarpine-induced status epilepticus in mice (Jiang et al., 2012). TG6-10-1, a second-generation bioavailable EP2 antagonist with improved *in vivo* half-life and brain-permeability, provided much broader benefits in the same mouse model of epilepsy, namely, reduction in delayed mortality, acceleration of recovery from weight loss and functional impairment, prevention of the blood-brain barrier breakdown, and decrease in neuronal inflammation and injury in the hippocampus (Jiang et al., 2013; Jiang et al., 2015). The beneficial effects from these EP2 antagonists in the mouse pilocarpine model were also mostly demonstrated in diisopropyl fluorophosphate (DFP)-treated rats (Rojas et al., 2015; Rojas et al., 2016), and in the mouse kainate model of status epilepticus (Jiang et al., 2019) , suggesting that they are not model or species-specific findings. Surprisingly, these EP2-targeted compounds had no effect on the progression of convulsive seizures after the administration of pilocarpine, DFP or kainate, as they did not alter the seizure duration or intensity (Jiang et al., 2013; Jiang et al., 2019; Rojas et al., 2016). Therefore, these benefits from EP2 inhibition following prolonged seizures were not caused by a direct anticonvulsant effect, but rather likely resulted from an anti-inflammatory action of the compounds. The fact that pharmacological inhibition of the EP2 receptor recapitulated most benefits of the conditional ablation of COX-2 restricted to forebrain neurons after status epileptics indicates that the EP2 receptor might be a primary culprit of COX-2/PGE_2 cascade-mediated detrimental effects in the CNS (Levin et al., 2012; Serrano et al., 2011). However, the question of whether the EP2 receptor activation by PGE_2 also plays a role in the development of spontaneous recurrent seizures following status epilepticus in these animal models remains open.

3.1.3 EP3 receptor—The EP3 receptor is widely expressed in the human body, as its mRNA and protein with several splice variants have been detected in the cardiovascular system, CNS, intestinal epithelium, kidney, reproductive system, and urinary bladder, where the receptor has been implicated in a variety of physiological and pathological processes (Woodward et al., 2011). Coupled to G_i protein, EP3 is classified as an inhibitory type of prostanoid receptor owing to its ability, when bound by PGE_2, to inhibit the activity of adenyl cyclase, and thereby to lower cytosol cAMP levels and downregulate the activity of cAMP-dependent signaling pathways (Fig. 1). Intrahippocampal injection of kainate in mice

and rats induced EP3 receptor expression in hippocampal astrocyte feet along with the elevation of COX-2 and microsomal prostaglandin E synthase-1 (mPGES-1) in the brain microvasculature, suggesting that PGE_2 might contribute to neuronal hyperexcitability by regulating glutamate release from astrocytes via activating astrocytic EP3 (Takemiya et al., 2010). Interestingly, central administration of the EP3-selective antagonist L-826266 increased the latency for clonic seizures induced by PTZ in rats (Oliveira et al., 2008). Similarly, systemic administration of EP3 antagonist ONO-AE3-240 attenuated, whereas EP3 agonist ONO-AE-248 potentiated PTZ-induced seizures in Swiss mice (Reschke et al., 2018). It appears that EP3 receptor activation after PTZ treatment in these mice also contributed to the downregulation of Na^+/K^+-ATPase activity, an enzyme responsible for the homeostatic ionic equilibrium and the resting membrane potential (Reschke et al., 2018). The EP3 receptor may represent a novel molecular target for the development of new antiseizure therapeutics; however, future studies using genetic strategies overexpressing or disrupting the EP3 receptor are required to validate these pharmacological findings.

3.1.4 EP4 receptor—Resembling the EP2 receptor in many respects, EP4 is coupled to $G_{\alpha s}$-$G_{\beta\gamma}$ heterotrimeric complex, and upon PGE_2 binding to the receptor, dissociates into $G_{\alpha s}$ and $G_{\beta\gamma}$ that act to regulate cell signaling pathways predominantly in a cAMP/PKA-dependent way (Fig. 1). However, a direct comparison of EP2 and EP4 receptor signaling revealed that the functional coupling to cAMP pathways seems more efficient for the EP2 subtype than for EP4, as EP4 might also mediate other pathways including PI3K/AKT/mTOR, extracellular signal-regulated kinase (ERK), and p38 mitogen-activated protein kinase (MAPK) pathways (Majumder et al., 2016; Woodward et al., 2011). EP4 is widely expressed in the human body, particularly in the brain, dorsal root ganglion, heart, intestine, kidney, thymus, uterus, etc., and has been implicated in a variety of physiological and pathological processes. However, its role in seizure disorders is not well known, except in a study suggesting that intracerebroventricular administration of an EP4 receptor-selective antagonist L-161982 increased the latency for PTZ-evoked acute seizures in Wistar rats (Oliveira et al., 2008). More in-depth studies are needed to validate the role of EP4 receptor in the pathophysiological processes related to epileptic seizures.

3.1.5 FP receptor—As the sole currently known receptor for $PGF_{2\alpha}$, the FP receptor couples to $G_{\alpha q}$-$G_{\beta\gamma}$ complex and is widely distributed in the human body, particularly in the cardiovascular system, CNS, eye, myometrium, and ovarian (Woodward et al., 2011). When bound to $PGF_{2\alpha}$ or other selective agonists, the FP receptor is activated and $G_{\alpha q}$ is released from the G protein heterotrimeric complex to activate PLC that hydrolyzes PIP2 to IP3 and DAG, which in turn initiate Ca^{2+}-sensitive signaling and activates certain PKC-dependent pathways, respectively (Abramovitz et al., 1994; Woodward et al., 2011) (Fig. 1).

Like other types of prostanoids, $PGF_{2\alpha}$ in the brain can be rapidly and robustly induced by seizures, evidenced in both human patients and animal models of epilepsy (Forstermann et al., 1983). $PGF_{2\alpha}$ levels in the cerebrospinal fluid (CSF) were found to increase nearly 5-fold in children who experienced simple febrile seizures (Tamai et al., 1983). Likewise, environmental stress-induced convulsions in gerbil mice elevated the $PGF_{2\alpha}$ levels in the cortex and hippocampus more than 5-fold within 3 minutes (Seregi et al., 1985). $PGF_{2\alpha}$

levels began to increase in the brain within 10 minutes following systemic injection of kainate in rats and peaked in the hippocampus 30 minutes after the administration of the neurotoxin (Baran et al., 1987). Histological examination of rat brain tissues 30 minutes after kainate injection demonstrated a major presence of $PGF_{2\alpha}$ in hippocampal CA3 neurons, hilar neurons, and granule cells of the dentate gyrus, suggesting the hippocampus is the major source of $PGF_{2\alpha}$ in the brain after kainate-induced seizures (Takei et al., 2012). Because these $PGF_{2\alpha}$-generating neurons also express the FP receptor, it is likely that they exert $PGF_{2\alpha}$-mediated functions by activating the FP receptor in an autocrine or paracrine manner following seizures.

Studies on $PGF_{2\alpha}$ in animal seizure models yielded some controversial results. For instance, intracerebroventricular administration of $PGF_{2\alpha}$ promoted both electrically and chemically induced seizures in mice (Climax and Sewell, 1981); whereas intraamygdaloid administration of $PGF_{2\alpha}$ had no effect on seizure activity in an electrically kindled animal model (Croucher et al., 1991). However, intracisternal administration of $PGF_{2\alpha}$, in the presence of COX inhibitors, completely prevented seizures and decreased neuronal injury in the hippocampus after systemic injection of kainate for seizure induction in ICR mice, though $PGF_{2\alpha}$ alone had no effect on kainate-induced seizures (Kim et al., 2008). In line, AL-8810, a potent and selective FP receptor antagonist, aggravated kainate-induced seizure activity in a dose-dependent manner (Kim et al., 2008; Sharif and Klimko, 2019). Moreover, intracisternal administration of $PGF_{2\alpha}$ in immature mice whose brains have very limited COX-2 expression and induction also reduced kainate-provoked seizure activity and mortality (Chung et al., 2013), suggesting that seizure-induced $PGF_{2\alpha}$ might act as an endogenous anticonvulsant through the FP receptor. Taken together, whether $PGF_{2\alpha}$ plays a pro- or anti-convulsant role in the brain remains elusive; however, the effects of FP receptor activation on seizure activity and neuronal survival are very likely context-dependent, as $PGF_{2\alpha}$ was administered into different brain regions in these studies.

3.1.6 DP1 receptor—As the most abundant lipid metabolite derived from the membrane-released arachidonic acid, PGD_2 is synthesized from PGH_2 by PGD_2 synthase (PGDS), which has two isoforms: hematopoietic PGDS (H-PGDS) and lipocalin PGDS (L-PGDS, or β-trace protein) (Mohri et al., 2003; Post et al., 2018; Urade et al., 2013). PGD_2 binds to two receptors, $G_{\alpha s}$-coupled DP1 and $G_{\alpha i}$-coupled DP2 (Fig. 1), to regulate several physiological and pathological activities, such as inhibition of hair growth, inhibition of platelet aggregation, sleep, smooth muscle contraction and relaxation, vasoconstriction and vasodilation (Garza et al., 2012; Woodward et al., 2011).

Under normal basal conditions, PGD_2 level in the brain is relatively low but is quickly and robustly elevated by seizure activities (Forstermann et al., 1983). Intracerebroventricular administration with PGD_2 or an active analog ZK-118.182 delayed the onset of generalized convulsions and reduced mortality in rats that were treated by PTZ for seizure induction; whereas inhibition of PGD_2 activity or synthesis increased the seizure incidence and intensity (Akarsu et al., 1998). Similarly, genetic ablation of H-PGDS synthase or the DP1 receptor, but not L-PGDS or the DP2 receptor delayed seizure onset, reduced the duration of generalized tonic-clonic seizures, and decreased the number of seizure spikes in mice treated by PTZ. Moreover, the L-PGDS/PGD_2/DP1 axis appears to regulate postictal sleep in these

animals (Kaushik et al., 2014). Therefore, seizure-induced PGD_2 might play a considerable antiepileptic role via activating the $G_{\alpha s}$-coupled DP1, but not the $G_{\alpha i}$-coupled DP2 receptor. It seems that potentiating PGD_2/DP1 signaling in the brain might provide a novel strategy for antiseizure therapeutics. These findings in PTZ seizure model also suggest that targeting specific prostanoid receptors instead of the COX-2 enzyme itself could avoid compromising the PGD_2-mediated potential antiseizure effects. However, it would be critical to determine the cellular sources of the DP1 receptor that accounts for its antiepileptic role using cell type-specific ablation of the receptor and to validate these previous findings in other conventional seizure models.

3.1.7 TP receptor—Thromboxane A2 (TXA_2) is an essential eicosanoid that is traditionally known for its functions in cardiovascular homeostasis through acting on G_q protein-coupled receptor TP. However, recent studies reveal that TXA_2 can be induced by, and play important roles in, some CNS conditions including epileptic seizures. TXA_2 levels were found to increase approximately 10-fold in epileptic neocortex specimens from drug-resistant epileptic patients, along with PGE_2 and PGI_2 that increased about 5-fold compared to their levels in normal tissues (Rumia et al., 2012). Likewise, TXA_2 in the brain was elevated by PTZ-induced seizures in mice within 10 minutes after PTZ injection (Kaushik et al., 2014). TP-selective agonist I-BOP impaired synaptic transmission and reduced neuronal excitability in the CA1 pyramidal neurons of rat hippocampal slices (Hsu and Kan, 1996); I-BOP and another TP agonist U-46619 suppressed high-voltage-activated calcium channels in rat hippocampal CA1 pyramidal neurons, decreasing Ca^{2+} currents and neuronal excitability (Hsu et al., 1996). Therefore, the TP receptor activation might lead to a decrease of neuronal excitability. Indeed, TP activation by U-46619 increased the latency for PTZ-induced myoclonic jerks and tonic-clonic seizures in mice. However, a TP antagonist, SQ-29548, did not modify PTZ-induced seizures, nor did it blunt the anticonvulsant effect of the agonist U-46619 in the same seizure model (Freitas et al., 2018). The reason for these conflicting findings remains elusive but could be related to the selectivity of the pharmacological drugs used in these studies. Hence, it would be important to follow up on these results using genetic inactivation of receptor and other animal seizure models to better understand the role of TXA_2/TP signaling in seizure disorders.

3.2. Cannabinoid receptors

The endocannabinoid system is a biological system that consists of endocannabinoids, cannabinoid receptors, transporters, and enzymes for endocannabinoid synthesis and metabolism. Endocannabinoids are endogenous bioactive lipid metabolites derived from membrane-bound phospholipids, and the two most prominent types of endocannabinoid ligands are 2-arachidonoylglycerol (2-AG) and anandamide (also known as *N*-arachidonoylethanolamine, or AEA). Both AEA and 2-AG are synthesized from membrane lipid precursors by multiple biosynthetic pathways upon the physiological and pathological stimuli (Fig. 1). As the first identified and the most frequently studied endocannabinoid to date, AEA in brain neurons is primarily produced via the hydrolytic cleavage of a cell membrane-bound *N*-arachidonoyl-phosphatidylethanolamine (NAPE) by *N*-acyl phosphatidylethanolamine-specific phospholipase D (NAPE-PLD) in a single-step reaction (Di Marzo et al., 1994). The phospholipid precursor NAPE can be formed by transferring

arachidonic acid (AA) from phosphatidylcholine (PC) to phosphatidylethanolamine (PE), a reaction catalyzed by *N*-acyltransferase (NAT). 2-AG, on the other hand, is synthesized via a two-step process in neurons. First, PIP2 in the cell membrane is hydrolyzed by phospholipase C-β (PLC-β) to create DAG. The DAG is then further hydrolyzed to 2-AG by diacylglycerol lipase (DAGL) (Murataeva et al., 2014). To date, two primary membrane GPCRs for endocannabinoids have been identified – cannabinoid receptor type 1 (CB1) and cannabinoid receptor type 2 (CB2), which are preferably bound and activated by AEA and 2-AG, respectively. The actions of endocannabinoids are terminated through hydrolysis by fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MAGL). Specifically, AEA is metabolized into AA and ethanolamine (EA) by FAAH; 2-AG is mainly hydrolyzed into AA and glycerol by MAGL (Maccarrone et al., 2015) (Fig. 1). In addition, COX can metabolize endocannabinoids to prostaglandin analogs, namely, PG(D_2/E_2/$F_{2\alpha}$/I_2)-ethanolamide (PG-EA) from AEA and PG(D_2/E_2/$F_{2\alpha}$/I_2)-glycerol ester (PG-G) from 2-AG (Fig. 1). PGE_2-EA can activate all EP1-EP4 receptors with lower binding affinity and potency than PGE_2, whereas $PGF_{2\alpha}$-EA is a ligand of FP receptor, but their functions remain unclear (Alhouayek and Muccioli, 2014).

Both CB receptors are coupled to $G_{\alpha i/o}$ and inhibit the activity of adenylyl cyclase (Fig. 1), thereby mediating a variety of physiological and pathological processes in nearly all organs and systems of the human body. In the CNS, endocannabinoids via CB receptors play important roles in emotion, learning and memory, motility, neurogenesis, neuroinflammation, neuroplasticity, neuroprotection, nociception, stress, sleeping, and addiction (Aizpurua-Olaizola et al., 2017; Chiurchiu et al., 2018). The broad contributions of endocannabinoid signaling to so many important physiological and pathological processes in the body make the CB receptors as promising potential targets for many conditions in the periphery as well as the CNS (Aizpurua-Olaizola et al., 2017; Maccarrone et al., 2015). For instance, both CB1 and CB2 receptors can reduce neuronal activity and excitability of the brain, thereby generating tremendous interests and efforts in modulating these two inhibitory GPCRs for potential anticonvulsant therapies (Table 1) (Blair et al., 2015).

3.2.1. CB1 receptor—As the primary molecular target of endocannabinoid ligand AEA, the CB1 receptor is predominantly found in nervous systems, particularly in the brain, where the CB1 receptor is considered the most abundant GPCR. Of note, the CB1 receptor is expressed presynaptically at both GABAergic and glutamatergic interneurons of the hippocampus and, when activated, acts as a neuromodulator to inhibit the release of GABA and glutamate, thereby regulating the balance between the inhibitory and excitatory neurotransmission (Chiarlone et al., 2014; Chiodi et al., 2012). Mice lacking CB1 displayed hypoactivity, hypoalgesia, and increased mortality (Zimmer et al., 1999), and showed decreased seizure threshold in kainate model of temporal lobe epilepsy (TLE) (Marsicano et al., 2003). WIN 55212-2, a non-selective CB agonist showed dose-dependent anticonvulsant effects in hippocampal neuronal culture models of status epilepticus and acquired epilepsy; whereas the anticonvulsant action of WIN 55212-2 in these models was specifically blocked by a CB1 receptor-selective antagonist SR141716 (Blair et al., 2006). Moreover, genetic ablation of the CB1 receptor or pre-treatment with the CB1-selective antagonist SR141716 increased seizure severity without altering seizure-promoted cell proliferation and neuronal

death after pilocarpine injection in mice; however, pre-treatment with CB1 agonist CP55940 did not show any noticeable effect on pilocarpine-induced seizures (Kow et al., 2014). Further, pre-treatment with the CB1 selective inverse agonist AM251 reduced the latency to onset of generalized tonic-clonic seizures and increased mortality in systemic kainate-treated mice (Sugaya et al., 2016). It appears that the majority of these studies on the CB1 receptor in various *in vitro* and *in vivo* models indicate that CB1 receptor activation causes anticonvulsant effects, while inhibiting CB1 might aggravate convulsive seizures.

3.2.2. CB2 receptor—Compared to CB1 receptor, the expression of CB2 in the body is more controversial, inasmuch as the CB2 receptor was previously reported to be present solely in organs of the immune system, where it contributes to the regulation of immune responses and mediates the anti-inflammatory effects of cannabis (Buckley et al., 2000). However, it is now well-known that CB2 is also expressed in the CNS with restricted distribution. Of note, the CB2 receptor is strongly expressed by microglial cells, where its function is associated with the regulation of innate immunity and neuroinflammation in the CNS (Maresz et al., 2005; Palazuelos et al., 2009; Zoppi et al., 2014). More recently the CB2 receptor was also detected in neurons and astrocytes responding to brain insults or inflammatory stimuli (Chiurchiu et al., 2018). These findings together with the fact that CB2 is expressed by principal neurons of the hippocampus make the receptor an appealing target for seizure control despite its relatively low density in the CNS (Stempel et al., 2016).

Although CB2-lacking mice have not been reported to show a seizure phenotype, activation of the receptor has been revealed to induce a chronic membrane potential hyperpolarization in hippocampal pyramidal cells that was not associated with the CB1 type receptor (Stempel et al., 2016), and to antagonize epileptic seizures in mice after kainate-induced status epileptics (Sugaya et al., 2016). Intriguingly, CB2 inverse agonist AM630, when co-administered with non-selective CB receptor agonist WIN 55212-2, but not by itself alone, showed considerable antiepileptic effects in a rat model of partial epilepsy (Rizzo et al., 2014). Moreover, about one-third of CB1 and CB2 double-knockout mice, but not the CB1 or CB2 single-knockout mice, showed spontaneous seizures or handling-induced seizures (Rowley et al., 2017), suggesting that a significant portion of CB double-knockout mice developed epilepsy. Therefore, CB1 and CB2 receptors likely work synergistically to regulate neuronal excitability and set the seizure threshold.

3.2.3. COX-2 and CB1/2 signaling—Endocannabinoids and prostaglandins are both derived from cell membrane-bound phospholipids; endocannabinoid metabolites can be the precursors of prostaglandins, and *vice versa* (Fig. 1), suggesting that these two biolipid signaling systems might interact with each other in the initiation of G protein-dependent signaling. Indeed, CB1 inhibition by selective antagonists SR141716 or AM251 enhanced synaptic responses in CA1 pyramidal neurons upon stimulation of the Schaffer collaterals in a GABA-independent manner, and this effect was largely prevented by the treatment with COX-2 inhibitors meloxicam or NS-398. These interesting findings suggest that COX cascade might regulate the formation of endogenous CB1 ligands that downregulates the excitatory neurotransmission in the hippocampus (Slanina and Schweitzer, 2005). Moreover, COX-2 oxidative metabolites of endocannabinoids promoted hippocampal long-term

potentiation (LTP), an effect opposite to that of their precursors 2-AG and AEA (Yang et al., 2008), reinforcing the notion that COX-2 is at the interface of the endocannabinoid and prostanoid systems (Alhouayek and Muccioli, 2014). An interesting pharmacological strategy involving substrate-selective COX-2 inhibition augmented endocannabinoid production without altering the synthesis of prostanoids or other related non-endocannabinoid lipids, indicating the importance of COX cascade in the regulation of CB1/2 signaling (Hermanson et al., 2013). Taken together, COX-2-mediated endocannabinoid oxygenation appears to represent an important mechanism for terminating CB signaling and, thus COX-2 inhibition might provide a strategy to positively modulate the CB1/2 signaling for therapeutic potential (Hermanson et al., 2014). Whether this substrate-selective COX-2 inhibition strategy has any effect on CB1/2-mediated G_i signaling pathways during seizures and therefore alters the neuronal excitability in the epileptic brain remains to be determined.

3.3. Adenosine receptors

Endogenous adenosine has long been known for its role in the regulation of neuronal excitability during convulsive states of the brain for nearly four decades (Dunwiddie, 1980). Since then, considerable research has shed light on the concept that adenosine acts as a powerful endogenous neuroprotectant and anticonvulsant neuromodulator (Boison, 2016). Under normal physiological conditions, a main source of synaptic adenosine is the astrocytic release of ATP that is degraded to adenosine by a cascade of extracellular ectonucleotidase (EN) (Boison, 2008). However, adenosine is primarily synthesized in activated neurons and secreted to suppress excitatory neurotransmission in an autonomic feedback manner during prolonged neuronal activities (Lovatt et al., 2012). Excessive adenosine then is taken up by equilibrative nucleoside transporters (ENTs) to astrocytes where it undergoes metabolic clearance by adenosine kinase (ADK) to AMP (Boison et al., 2010) (Fig. 2). Intrahippocampal administration of adenosine to the site of stimulation was anticonvulsant in rats kindled by hippocampal electrical stimulation (Szybala et al., 2009); intraventricular release of adenosine after kainate-induced status epilepticus in rats prevented mossy fiber sprouting and resulted in a substantial long-lasting reduction in spontaneous recurrent seizures in terms of both seizure frequency and intensity (Williams-Karnesky et al., 2013). Conditional ablation of ADK in the forebrain attenuated seizures that were induced by intraamygdaloid injection of kainate in mice, whereas overexpression of ADK within the hippocampal CA3 region increased the spontaneous seizures in this brain region (Li et al., 2007; Li et al., 2008). In line, transient use of a small-molecule ADK inhibitor 5-iodotubercidin, administered twice daily during the latent phase of epileptogenesis from day 3-8 after the intrahippocampal injection of kainate, markedly decreased the percent time of epileptic mice in seizures (Sandau et al., 2019). These results are significant in that astrocyte-based ADK is highly upregulated in the epileptic hippocampus to decrease the adenosine levels at the synaptic sites, thereby contributing to epileptogenesis (Boison, 2008, 2012; Gouder et al., 2004).

To date, four adenosine receptor subtypes have been identified in humans and experimental rodents: A1, A2A, A2B and A3, which are a class of purinergic GPCRs and encoded by distinct genes. Adenosine A1 and A3 receptors are associated with $G_{\alpha i/o}$ to inhibit cAMP

biosynthesis; while A2A and A2B receptors are coupled to $G_{\alpha s}$ to promote cAMP-mediated signaling. Adenosine has been reported to modulate the neuronal excitability in the brain engaging all these four GPCRs, as it is conceivable that any shift in the ratio of stimulatory adenosine receptors to inhibitory adenosine receptors directly influences excitability of the brain and thus the seizure threshold (Boison, 2012, 2016). Though inhibition of ADK or ENTs can be used to increase adenosine levels at synaptic sites and thus represents an emerging strategy to suppress epileptic seizures, preclinical efforts have been made to explore the potential of these GPCRs – particularly A1 and A2A receptors – as antiepileptic and antiepileptogenic targets (Table 2) (Weltha et al., 2018).

3.3.1. Adenosine A1 receptor—Accumulating evidence from clinical and preclinical studies suggest that the G_i-coupled A1 adenosine receptor might exert an endogenous anticonvulsant effect via downregulating the postsynaptic cAMP signaling (Fig. 2). The A1 receptor expression was found lower in temporal cortex tissues surgically resected from patients with complex partial seizures than those in the normal control post-mortem tissues. This result suggests that the decreased expression of A1 receptor might reduce the G_i-mediated inhibitory function and thereby lower the threshold of epileptic seizures (Glass et al., 1996). Moreover, a study on 206 patients with severe traumatic brain injury (TBI) identified certain gene variants of adenosine A1 receptor associated with post-traumatic seizures, indicating that malfunctioning adenosine A1 receptor might contribute to the post-traumatic epileptogenesis (Wagner et al., 2010). The density of adenosine A1 receptor has also been shown to be decreased in the hippocampal nerve terminal membranes of rats following hippocampal kindling, leading to the deficiency in adenosine neuromodulation and failure of endogenous anticonvulsant mechanisms (Rebola et al., 2003). Adenosine A1 receptor knockout mice have been found to develop spontaneous seizures with homozygous knockout mice showing higher frequency and longer duration of electrographic seizures than their heterozygous cohorts (Li et al., 2007). The proconvulsive effect of adenosine A1 receptor ablation was aggravated by experimental traumatic brain injury, as well as the intrahippocampal injection of kainate, leading to lethal status epilepticus (Fedele et al., 2006; Kochanek et al., 2006). These studies on human patients and experimental animals together suggest that dysregulation of adenosine signaling via the inhibitory A1 receptor is involved in the pathophysiology of epilepsy, and thus the A1 receptor activation is required to prevent seizure intensification and dissemination within the brain (Fig. 2).

3.3.2. Adenosine A2A receptor—The stimulatory A2A adenosine receptor is coupled to $G_{\alpha s}$, and its activation by neuron-derived adenosine leads to elevated levels of cytosol cAMP and thereby neuronal hyperexcitability (Fig. 2). The expression of the A2A receptor was increased in the cerebral areas of Wistar Albino Glaxo/Rijswijk rats experiencing absence seizures, but not in their pre-symptomatic cohorts. Moreover, treatment with A2A receptor agonist CGS-21680 increased, whereas A2A antagonist SCH-58261 decreased, the number and duration of the unprovoked spike-wave discharges in these epileptic rats in a dose-dependent manner (D'Alimonte et al., 2009). Compared to the wildtype control cohorts, mice lacking the adenosine A2A receptor showed significant reduced intensity and frequency of seizures induced by the administration of PTZ or pilocarpine (El Yacoubi et al., 2009), suggesting that the A2A knockout mice exhibited partial resistance to limbic seizures.

In line, intracerebroventricular administration of another selective A2A antagonist ZM-241385 partially decreased seizure duration in rats after amygdala kindling (Li et al., 2012).

Early-life hyperthermic seizures in rats initially caused a short-term decrease of the adenosine A2A receptor density and 5'-nucleotidase activity in cerebral cortex within two days after seizure induction (Leon-Navarro et al., 2015), but later led to a long-lasting increase in A2A receptor expression, 5'-nucleotidase activity and A2A-mediated adenylate cyclase activity in the cortex when the animals reached to adulthood (Crespo et al., 2018). The activation of the A2A receptor by a selective agonist CPCA decreased the mortality and lowered the seizure threshold in a hyperthermia-induced seizure model in young rats (Fukuda et al., 2011). Thus, the adenosine A2A receptor signaling might aggravate febrile seizures and contribute to the pathogenesis of sudden unexpected death in epilepsy (SUDEP) in children. Taken together, it appears that neuronal hyperexcitability following precipitating insults likely causes the enhanced synaptic adenosine A2A receptor activation, which could exacerbate the aberration of normal circuitry leading to the chronic progression of epileptic seizures.

3.3.3. Adenosine A2B and A3 receptors—Compared to adenosine A1 and A2A receptors that are well studied in neuronal excitability, not much has been uncovered about the role of $G_{\alpha s}$-coupled A2B and $G_{\alpha i}$-coupled A3 receptors in the seizure generation or development of chronic epilepsy. Using the reverse microdialysis to deliver 5'-N-ethylcarboxamidoadenosine (NECA) – an adenosine analog as nonselective adenosine receptor agonist – into the striatum of freely moving mice, it was shown that the concentration of cytokine IL-6 in the perfusate was rapidly and robustly increased, whereas the NECA-induced IL-6 was selectively blocked by A2B receptor antagonist MRS-1706 (Vazquez et al., 2008). Given that IL-6, as a prototypical inflammatory cytokine, has been widely implicated in hyperexcitability of epileptic brains (Patel et al., 2017; Vezzani and Viviani, 2015), these findings suggest that adenosine stimulates IL-6 release likely by activating the A2B receptor and thereby facilitates epileptogenesis. Systemic administration of a selective A3 adenosine receptor agonist IB-MECA prior to NMDA or PTZ-induced seizures in mice decreased the percentage of mice with convulsions, delayed seizure onset, reduced neurological impairment, and increased animal survival (Von Lubitz et al., 1995). Though A2B and A3 receptors regulate cAMP signaling towards opposite directions (i.e., stimulatory vs. inhibitory), it has been shown that both A2B antagonist MRS-1706 and A3 antagonist MRS-1334 decreased the rundown of $GABA_A$ currents (Roseti et al., 2008), suggesting the A2B and A3 receptors might modulate the stability of GABA receptors and therefore fine-tune hyperexcitability of the brain. Nonetheless, it would be important to follow up on these pharmacological findings employing genetic strategies of gene deletion or overexpression in order to better understand the roles of adenosine A2B and A3 receptors in the pathophysiological processes related to epileptic seizures.

3.4. Metabotropic glutamate receptors

The metabotropic glutamate receptors (mGluRs) are members of the group C family of GPCRs that are bound and activated by glutamate, the amino acid that functions as the

primary excitatory neurotransmitter of the mammalian CNS. There are eight currently known types of mGluRs, namely from mGluR1 to mGluR8, which are classified into three subgroups I, II, and III based on their amino acid sequence homology profiles and pharmacological properties (Nicoletti et al., 2011). The mGluRs, via modulating other receptors, are involved in a variety of functional and pathological processes in the CNS, such as synaptic plasticity, learning, memory, anxiety, depression, neuropathic pain (Collingridge et al., 2017). The mGluRs are typically found in pre- and postsynaptic neurons of synapses in the cerebral cortex, hippocampus, striatum, cerebellum, spinal cord, and many other areas of the CNS, where their activation upon glutamate binding initiates biochemical signaling cascades, leading to the modulation of other associated proteins, e.g., ion channels (Notartomaso et al., 2017; Olivero et al., 2017; Sidorov et al., 2015). This can further result in alterations in the synaptic excitability through modulating the presynaptic release of neurotransmitters, postsynaptic reactions, or astrocytic functions (Johnson et al., 2017; Sheng et al., 2017; Umpierre et al., 2019). With the use of a variety of pharmacological and genetic approaches, the roles of these receptors in many physiological and pathological processes of the brain are unfolding. The majority of results from a large number of preclinical studies attest that activation of group I mGluRs is proconvulsant through lowering the seizure threshold and increasing excitability of the brain; whereas activation of groups II and III mGluRs pre-dominantly leads to anticonvulsant effects (Table 3) (Wong et al., 2005).

3.4.1. Group I mGluRs (mGluR1/5)—As the two currently known members in the group I mGluRs, mGluR1 and mGluR5 are coupled to $G_{q/11}$ proteins and, upon activation by glutamate binding, trigger the polyphosphoinositide hydrolysis of PIP2 by phospholipase C to produce IP3 and DAG, leading to Ca^{2+}-sensitive signaling pathways and PKC activation, respectively. These two receptors have been found on the postsynaptic dendrites of neurons in thalamus as well as on GABAergic interneurons in the cerebral cortex (Wong et al., 2005). Astrocytes also show significant expression of mGluR5 receptor (Parri et al., 2010; Umpierre et al., 2019).

Interestingly, the expression of mGluR1, but not mGluR5, was found to increase in the dentate gyrus of hippocampus in rats after electrical kindling or intraperitoneal injection of kainate as well as in patients with TLE (Blumcke et al., 2000). The overexpression of mGluR1 in mice did not alter the acute stages of seizures after pilocarpine application compared to the wildtype control cohorts; however, these mGluR1 transgenic mice showed a consistent increase in seizure frequency during the chronic epileptic phase beginning a few weeks after status epilepticus (Pitsch et al., 2007), suggesting a role for mGluR1 in increasing the susceptibility to spontaneous recurrent seizures during the process of epileptogenesis. In line, intracerebroventricular administration of AIDA, a selective antagonist for mGluR1 showed marked inhibition on PTZ-induced kindled seizures in mice in a dose-dependent manner; whereas this antiseizure effect was largely prevented by co-treatment with mGluR1-selective agonist (RS)-3,5-DHPG (Watanabe et al., 2011).

Inhibition of mGluR5 by a selective negative allosteric modulator CTEP corrected hyperactivity, decreased epileptic seizures, and enhanced *de novo* synthesis of synaptic proteins in a mouse model of tuberous sclerosis complex (TSC); while mGluR5 activation

by allosteric potentiator RO6807794 led to the exacerbation of these epileptic phenotypes (Kelly et al., 2018). However, treatment with another mGuR5-selective positive allosteric modulator VU0360172 attenuated acute seizures and decreased the pro-inflammatory cytokine-producing macrophages and microglia within the brain in the Theiler's murine encephalomyelitis virus (TMEV)-induced mouse model of TLE (Hanak et al., 2019). The mechanism underlying these seemingly conflicting results remains unclear, but the selectivity of compounds and the different animal models used in these two studies might be contributory factors, as mGluR5 inhibition by MTEP, another highly selective brain-permeable negative allosteric modulator which is an analog of CTEP, did not alter the seizure outcomes in the same virus-induced epilepsy model (Hanak et al., 2019).

The mechanisms whereby mGluR5-mediated G_q signaling regulates acute seizures and the development of epilepsy remain largely elusive, but could involve its functions in astrocytes, as mGluR5 has been found in astrocytes of resected tissues from epilepsy patients as well as in animals with experimental epilepsy. The mGluR5 receptor was thought to play an essential role in the regulation of structural and functional interactions between neurons and astrocytes at tripartite synapses (Panatier and Robitaille, 2016). It was revealed that mGluR5 expression was markedly increased in the mouse kainate model of TLE, and the mGluR5 function particularly persisted in astrocytes to regulate glutamate uptake throughout the entire course of epileptogenesis following status epilepticus induced by kainate. Meanwhile, animals with only transient mGluR5 expression in astrocytes after kainate-induced status epilepticus did not develop epilepsy (Umpierre et al., 2019), suggesting that astrocytic mGluR5 is an essential component of excitatory signaling regulation in the hippocampus during the process of acquired epileptogenesis.

3.4.2. Group II mGluRs (mGluR2/3)—There are two members in the group II mGluRs: mGluR2 and mGluR3, which are coupled to $G_{i/o}$ to suppress the production of cAMP through inhibiting the activity of adenylyl cyclase. Groups II mGluRs have been found predominantly on the presynaptic terminal, where they could inhibit the presynaptic glutamate release, as well as in astrocytes of cortex and thalamus, where they might regulate the expression of the excitatory amino acid transporter 1/2 (EAAT1/2) (Aronica et al., 2003; Ngomba and van Luijtelaar, 2018). It was widely reported that mGluR2/3 expression was substantially decreased in hippocampal CA1 and CA3 regions of mice and rats after pilocarpine-induced status epilepticus (Pacheco Otalora et al., 2006; Tang et al., 2004), and in patients with mesial TLE (Tang et al., 2004). A pharmacological study described that both central and systemic administration of two mGluR2/3-selective agonists LY379268 and LY389795 suppressed the sound-triggered or mGluR1/5 agonist DHPG-induced clonic seizures in DBA/2 mice in a dose and time dependent manner (Moldrich et al., 2001). Furthermore, these two mGluR2/3 agonists substantially reduced the spike and wave discharge (SWD) duration in lethargic (lh/lh) mouse model of absence seizures, as well as the electrically induced seizure score and after-discharge duration (ADD) in amygdala-kindled rats (Moldrich et al., 2001). Similarly, mGluR2/3-selective antagonist EGLU has been described to aggravate seizures in PTZ-induced kindled mice and antagonize the antiseizure effect from inhibiting the group I mGluRs (Watanabe et al., 2011). On the contrary, activation of mGluR2/3 by agonist LY379268 increased the numbers of SWD in

symptomatic WAG/Rij rats also in a dose-dependent manner, while treatment with mGluR2/3-selective antagonist LY341495 decreased the occurrence of SWD in this rat model of absence epilepsy (Ngomba et al., 2005). It is unknown whether these contradicting results were caused by the experimental differences in selectivity of ligands, pharmacological doses, animal species and models that were used in these studies. Nonetheless, future efforts in developing new ligands that would show preference to mGluR2 or mGluR3 together with strategies of gene deletion or overexpression will help to uncover the specific roles for each of these two G_i-coupled mGluR subtypes in epileptic seizures.

3.4.3. Group III mGluRs (mGluR4/6/7/8)—There are four currently known members in the group III mGluRs: mGlu4, mGlu6, mGlu7 and mGlu8, which like group II mGluRs, are also coupled to $G_{i/o}$ proteins to downregulate cAMP signaling when activated by glutamate. The mGluR6 is specifically expressed in the retina, where it regulates the responses of bipolar cells to light (Tian and Kammermeier, 2006), while all other group III mGluRs are mainly found in the cortico-basal ganglia-thalamo-cortical loop. The mGluR4 is predominantly expressed on glutamatergic terminals in the thalamic reticular nucleus (TRN) as well as the ventrobasal complex (VB), whereas mGluR7 and mGluR8 are also found on TRN neurons (Alexander and Godwin, 2006). The functions of group III mGluRs have been widely studied in the generation of absence seizures, a type of generalized nonconvulsive seizure that often involves the thalamus (Ngomba and van Luijtelaar, 2018).

Mice lacking mGluR4 showed marked resistance to absence seizures induced by low doses of $GABA_A$ receptor antagonists, such as PTZ, picrotoxin or bicuculline. Furthermore, the $GABA_A$ receptor antagonist-induced absence seizures were completely blocked by mGluR4 antagonist CPPG while exacerbated by the group I mGluRs agonist L-AP4, both of which were administered bilaterally into the TRN (Snead et al., 2000). In line, mGluR4 activation by systemic injection of an allosteric potentiator PHCCC enhanced absence-like seizures in Wistar Albino Glaxo/Rijswijk rats as well as the PTZ-treated mice (Ngomba et al., 2008), suggesting that mGluR4 activation facilitates the seizure generation in absence epilepsy. However, the ablation of mGluR4 in mice did not inhibit tonic-clonic seizures triggered by high doses of $GABA_A$ receptor antagonists, strychnine, or electroshock (Snead et al., 2000). On the contrary, mGluR4 knockout mice showed a significant increase of severity in acute seizures induced by pilocarpine injection and later had enhanced neuronal loss in the hippocampus during the chronic epileptic phase, though they did not display any increase in frequency of spontaneous recurrent seizures (Pitsch et al., 2007). Interestingly, a downregulation of mGluR4 expression and function also has been described in hippocampal CA3 region in rats after pilocarpine-induced status epilepticus (Dammann et al., 2018). These studies together suggest that the activation of mGluR4 might differentially regulate nonconvulsive and convulsive seizures, i.e., facilitation on absence seizures, but inhibition on tonic-clonic seizures.

Mice lacking mGluR7 showed hyperexcitability and increased susceptibility to tonic-clonic seizures induced by PTZ or bicuculline (Sansig et al., 2001), demonstrating an important role for mGluR7 in the regulation of neuronal excitability induced by inhibiting GABA functions. Disrupting the interaction between mGluR7 and its PDZ-interacting protein,

protein interacting with C kinase 1 (PICK1), by targeted mutation of the receptor or a cell-permeant dominant-negative peptide, led to behavioral symptoms and EEG discharges in both mice and rats, which are characteristic of absence epilepsy (Bertaso et al., 2008). Therefore, impaired interaction between mGluR7 and PICK1 might contribute to some form of epileptogenesis. Inhibiting mGluR7 with a selective brain-penetrant negative allosteric modulator ADX71743 enhanced thalamic synaptic transmission and induced absence epileptic seizures and lethargy, which were exacerbated by disrupting the interaction between the receptor and the PDZ protein PICK1 (Tassin et al., 2016).

The function of mGluR8 was found downregulated at the presynaptic terminals of the lateral perforant pathway but increased at the at Schaffer collateral-CA1 synapses in rats with spontaneous recurrent seizures after pilocarpine-induced status epilepticus (Dammann et al., 2018; Kral et al., 2003). These findings suggest that the development of epileptic seizures might be associated with the spatiotemporal alterations of mGluR8 expression that leads to the declined autoregulation of glutamate release. L-AP4, a selective agonist for mGluR4/8 has been demonstrated to inhibit seizure activities in PTZ-induced kindled mice. The antiseizure effect of mGluR4/8 activation was further enhanced by co-treatment with either group I mGluRs antagonist AIDA or group II mGluRs agonist APDC, but was completely blocked by MPPG, a group III mGluRs-selective antagonist (Watanabe et al., 2011). These interesting findings together suggest that $G_{q/11}$-coupled group I mGluRs are overall proconvulsant, while the activation of $G_{i/o}$-coupled groups II and III mGluRs causes anticonvulsant effects (Table 3).

3.5. Other GPCRs

In addition to the four major families of membrane receptors elaborated above, there are also a few other GPCRs that have been well investigated for their potential roles in regulating neuronal excitability and setting seizure threshold. They draw less attention compared to the well-recognized prostanoid, endocannabinoid, adenosine, and metabotropic glutamate receptors; however, their potential as novel antiepileptic and/or antiepileptogenic targets should not be neglected. We are only able to select the following three groups of GPCRs to discuss further due to the limited space and scope of this review (Table 4).

3.5.1. Histamine receptors—Histamine is a biogenic amine that is derived from the decarboxylation of amino acid histidine and exerts its physiological and pathological functions via four currently known GPCRs: H1, H2, H3 and H4 receptors (Seifert et al., 2013). Owing to the extensive activities of histamine as a local mediator and neurotransmitter, the histaminergic system is thought to be involved in the pathogenesis of diverse human diseases including epilepsy, as early evidence suggests that elevated histamine might suppress convulsive seizures and confer a neuroprotective role (Bhowmik et al., 2012). Among the four histamine receptors, $G_{q/11}$-coupled H1 receptor is widely expressed in the CNS as well as many peripheral tissues and contributes to nuclear factor κB (NF-κB)-mediated inflammatory processes. Antihistamines are used as anti-allergy and anti-inflammatory drugs by mainly acting on the H1 receptor (Canonica and Blaiss, 2011). Genetic deletion of H1 receptor or treatment with selective antagonist triprolidine in immature mice increased the susceptibility of animals to kainate-induced seizures,

demonstrated by augmented seizure severity and neuronal damage (Kukko-Lukjanov et al., 2010).

The $G_{i/o}$-coupled H3 receptor is primarily expressed in the CNS and functions as presynaptic autoreceptor to control the release of histamine and several other neurotransmitters, such as acetylcholine, dopamine, GABA, norepinephrine and serotonin, and thus plays important roles in homeostatic and cognitive processes. The H3 receptor also modulates several intracellular signaling pathways that are associated with epileptic seizures and neurotoxicity, thereby representing an attractive molecular target in the effort of searching for new antiepileptic and/or antiepileptogenic strategies. Though controversial, the pharmacological potential of H3 receptor selective antagonists and inverse agonists continues to receive considerable attention because of their prospective anticonvulsive properties, as an increasing body of evidence from animal studies demonstrates their effectiveness in seizure treatment (Table 4). Pitolisant – a highly selective antagonist for H3 receptor – showed extensive anticonvulsant effects in several preclinical seizure models, such as the mouse kainate model of temporal lobe seizures, the rat absence seizure model, and the mouse maximal electroshock model (Kasteleijn-Nolst Trenite et al., 2013). In a small phase II clinical trial, pitolisant showed dose-dependent beneficial effects in patients with photosensitive epilepsy, a rare type of epilepsy in which seizures are triggered by photic stimuli and resistant to current first-line AEDs (Kasteleijn-Nolst Trenite et al., 2013). Inspired by this promising outcome, a series of novel multiple-target ligands were designed by incorporating various antiepileptic structural motifs to the pharmacophore of pitolisant. These novel compounds displayed high affinities to H3 receptor, and one compound with hydantoin moiety that is present in phenytoin showed moderate anticonvulsant activities in both electroshock-induced and PTZ-kindled convulsions in Wistar rats (Sadek et al., 2014). However, whether the antiepileptic effect of this compound should be attributed to its actions on H3 receptors by the pharmacophore of pitolisant or voltage gated sodium channels by its hydantoin moiety remains elusive (Khanfar et al., 2016).

3.5.2. $GABA_B$ receptor—$GABA_B$ receptor is a metabotropic transmembrane receptor that is activated by inhibitory neurotransmitter GABA and linked via $G_{i/o}$ protein to potassium channels. There are two subunits of the $GABA_B$ receptor, $GABA_{B1}$ and $GABA_{B2}$, which appear to assemble to form a heteromeric dimer in a 1:1 stoichiometry through their intracellular C-terminal domains, and both subtypes are essential for the heterodimer receptor to be fully functional (Pin et al., 2004). As an inhibitory type of receptor, $GABA_B$ activation can stimulate the opening of potassium channels to reduce the frequency of action potentials, as well as the release of GABA and other neurotransmitters including glutamate. It also modulates the activity of calcium channels and inhibits adenylyl cyclase via $G_{i/o}$ protein. The $GABA_B$ receptor is highly expressed throughout the mammalian CNS and has been implicated in multiple neurological and psychiatric conditions, including both convulsive and nonconvulsive seizures (Han et al., 2012; Joshi et al., 2016). Baclofen, a lipophilic analog of GABA, is a highly selective agonist for $GABA_B$ receptor and binds to its orthosteric site. Baclofen has been used clinically to treat muscle spasms for decades and remains the only marketed drug specifically acting on the $GABA_B$ receptor. Its anticonvulsant effect in human epilepsy was first reported nearly four decades ago (Terrence

et al., 1983); however, its untoward muscle relaxant and sedative effects, as well as its rapid development of tolerance and short duration of action due to its relatively short *in vivo* half-life, potentially limit its clinical application. To overcome these pharmacodynamic and pharmacokinetic shortcomings, a number of positive allosteric modulators have been developed by several groups (Pin et al., 2004) and tested in diverse animal seizure models (Table 4). In a recent study, several positive allosteric modulators for $GABA_B$ receptor including GS39783, rac-BHFF, CMPPE, A-1295120, and A-1474713 showed robust anticonvulsant effects in the DBA/2J mouse audiogenic seizure model in a dose-dependent manner. However, the antiepileptic effects of these compounds were completely prevented by pre-treatment of the mice with $GABA_B$ antagonist SCH50911, suggestive of a $GABA_B$ receptor-mediated mechanism. Compared to baclofen, these novel allosteric potentiators showed decreased motor side-effects during the acclimation period of the test (Brown et al., 2016). However, more intense and robust studies are required to validate their antiepileptic and potentially antiepileptogenic effects in classical chemoconvulsant models of epilepsy.

3.5.3. Galanin receptors—Galanin is a neuropeptide that is widely expressed in the mammalian CNS and involved in the modulation and inhibition of action potentials in neurons by acting on three GPCR subtypes: GalR1, GalR2, and GalR3. Several lines of evidence from animal studies using both genetic and pharmacological approaches reveal that galanin, via these three GPCRs, is a potent and effective modulator of neuronal excitability in the hippocampus. Galanin receptors, particularly GalR1 and GalR2, have been explored as potential targets for new antiepileptic therapeutics in multiple animal seizure models (Table 4). Genetic ablation of $G_{i/o}$-coupled GalR1 increased the vulnerability of mice to seizures induced by systemic treatment of Li-pilocarpine or electrical stimulation of the perforant path of the hippocampus (Mazarati et al., 2004). However, the deletion of GalR1 did not affect the severity and duration of seizures induced by kainate injection in mice (Mazarati et al., 2004; Schauwecker, 2010). These seemingly conflicting results from different seizure models might be explained by the well-recognized resistance of the C57BL/6 mice used in these studies to kainate-induced seizures and neuronal injury (McKhann et al., 2003). In another recent study, the deletion of $G_{q/11}$-coupled GalR2, but not the $G_{i/o}$-coupled GalR3, increased the frequency and duration of seizures after intrahippocampal administration of kainate in mice, although the genetic ablation of the galanin receptors had no effect on PTZ-induced seizures (Drexel et al., 2018). Systemic administration of NAX 5055, a bioavailable brain-permeable analog of galanin with binding preference to GalR1, showed profound anticonvulsant activities in several mouse models, including corneal kindling model, audiogenic seizure model, and the 6 Hz model of pharmacoresistant epilepsy (White et al., 2009). However, NAX 5055 was not active in the mouse traditional maximal electroshock model and only showed minimal anticonvulsant activity in the mouse subcutaneous PTZ seizure model even at the highest tested dose (White et al., 2009). In addition, NAX 5055 did not show any benefit in the rat multiple-hit model of symptomatic infantile spasms (Jequier Gygax et al., 2014). NAX 810-2, a GalR2-preferring galanin analog, also showed dose-dependent antiepileptic effects in both mouse corneal kindling and 6 Hz seizure models (Metcalf et al., 2017). These studies together suggest that GalR1 and GalR2 should be explored further for their potential as novel antiepileptic and/or antiepileptogenic targets.

4. Concluding remarks and outlook

4.1 Challenges

GPCRs have gained new momentum in CNS indications, as demonstrated by the scientific impetus in GPCR neurobiology and neuropharmacology, leading to many emerging new drug targets for various conditions associated with the brain. As such, an increasing body of evidence from a large number of preclinical and clinical studies points to indispensable roles for GPCRs in the pathophysiological processes related to acute seizures and potentially the development of acquired forms of epilepsy. Yet, there is a lack of drug that interrupts epileptic seizures via directly acting on GPCRs to date. Moreover, there is no currently known clinical trial to evaluate the therapeutic potential of targeting GPCRs for acquired epilepsy (ClinicalTrials.gov). As the most recently introduced antiseizure medication, cannabidiol (Epidiolex®) was approved by the US FDA as a treatment for two severe forms of epilepsy, i.e., Dravet syndrome and Lennox-Gastaut syndrome, in young patients who are two years of age or older (FDA, 2018). Cannabidiol as an adjuvant treatment can dramatically decrease seizure frequency in childhood-onset drug-resistant epilepsy, but like any other AEDs, it does not lead to becoming seizure-free and treatment-related adversity still remains a considerable concern (O'Connell et al., 2017; Stockings et al., 2018). The exact mechanism of action whereby cannabidiol executes anticonvulsant effects remains unknown and does not seem to be through interaction with CB receptors, and a multimodal action engaging more than 10 potential molecular targets identified has been proposed (Chen et al., 2019). Cannabidivarin (GWP42006), another non-psychoactive analog of cannabidiol that is believed to target the orphan GPCR 55 (GPR55) – a likely cannabinoid receptor (Ryberg et al., 2007) – has recently failed to outperform placebo in a phase 2 clinical trial for focal seizures (https://clinicaltrials.gov/show/NCT02365610; https://clinicaltrials.gov/show/NCT02369471).

The reasons why there is still a lack of GPCR-targeted drug for epilepsy treatment, despite tremendous preclinical and clinical efforts, remain unknown. However, there might be multiple contributory factors and the nature of GPCRs per se certainly could help to explain this disappointing fact. GPCRs in the brain are often expressed by both neuronal and nonneuronal cells, in which they might mediate different, in some cases even opposite, functions due to the distinct cellular contents. For instance, prostaglandin receptor EP2 in neurons mediates a number of important normal physiological functions and also can confer early neuroprotective effects in some injury settings (Andreasson, 2010; Jiang and Dingledine, 2013; Serrano et al., 2011), but the activation microglial EP2 contributes to chronic inflammation in the brain, leading to detrimental effects on neuronal cells (Johansson et al., 2013; Johansson et al., 2015). Conventional agonists or antagonists of the receptor might provide some beneficial effects through acting on EP2 in one cell type, but also can cause undesirable effects via its counterpart in another cell type. Because neurons and microglia may respond to precipitating injuries in different time-courses, it is not uncommon that GPCR-targeted agents, when administered with different treatment regimens, may lead to quite different outcomes even in the same animal model (Du et al., 2016; Jiang et al., 2015). In addition, unlike most ion channels that, when activated, allow certain ions to pass through, resulting in more specific cellular responses, activation of a

GPCR usually initiates several downstream signaling pathways, thereby leading to multiple consequences, which can be desirable or undesirable. Developing GPCR-targeted therapeutic must ultimately hinge on a well understanding of the GPCR downstream signaling pathways (Eichel and von Zastrow, 2018), as the downstream molecular effectors could provide alternative targets with more specificity than the receptors themselves.

4.2 Opportunities

Our current understanding of the contribution of GPCRs to epileptic seizures was profoundly based on pharmacological studies in animal seizure models, which largely rely on the selectivity and pharmacokinetic properties of drugs used in those studies. Genetic approaches of gene deletion or overexpression with cell type-specificity have been increasingly used recently to validate the roles for GPCRs in neurons and glial cells of epileptic brains (Stempel et al., 2016; Stoppel et al., 2017; Sugaya et al., 2016; Umpierre et al., 2019), as their functions may depend on the cellular contents that determine the downstream signaling pathways and responding components. A combination of genetic and pharmacological strategies is encouraged to fully understand their complex roles and assess the feasibility of pharmacologically targeting these receptors for epilepsy. As tools and techniques for studying GPCRs grow, so too will our understanding of their physiological and pathological functions in the brain, which can guide translational efforts in developing next-generation antiepileptic and/or antiepileptogenic therapeutics.

To date, the majority of preclinical studies indicate that the activation of $G_{q/11}$-associated EP1, FP, and mGluR1/5 receptors and G_s-coupled EP2, A2A and A2B receptors is overall proconvulsive in animal models of epilepsy. Conversely, $G_{i/o}$-linked CB1, CB2, A1, A3, mGluR2/3, mGluR4/7/8, $GABA_B$, and GlaR1 act as endogenous anticonvulsants. More in-depth studies are needed to clarify the roles of other GPCRs such as DP1/2, EP3/4, IP, TP, mGluR6, H1, H3, and GalR2 in epileptic seizures due to conflicting results or lack of definitive evidence from previous studies (Tables 1–4). Nonetheless, it appears that GPCRs might regulate neuronal excitability through both G_s/G_i-controlled cAMP and $G_{q/11}$-dependent Ca^{2+} pathways that control the expression and function of various voltage-gated or ligand-gated ion channels including sodium channels, ionotropic glutamate receptors, potassium channels, etc., which work together to control the excitability of the brain and set the seizure threshold (Aizpurua-Olaizola et al., 2017; Collingridge et al., 2017; Weltha et al., 2018). However, the molecular mechanisms whereby GPCRs regulate these ion channels remain largely unknown. Elucidating the downstream effectors of G proteins might provide alternative strategies to targeting the receptors or G proteins themselves for acquired epilepsy with more specificity.

4.3 Downstream effectors and biased ligands

As the second messenger for stimulatory GPCRs, cAMP regulates various important biological processes under both normal and disease conditions. The effects of cAMP within the brain were historically believed to be mediated by either PKA or cyclic nucleotide-regulated ion channels. During the past two decades, PKA-independent cAMP action that is mediated by EPAC, a family of guanine nucleotide exchange factors (GEFs) called cAMP-GEFs, became widely recognized for their roles in a variety of physiological and

pathological processes (de Rooij et al., 1998; Kawasaki et al., 1998). In the CNS, the cAMP/EPAC signaling has been reported to regulate glutamate transmitter release in the central neurons, as well as hippocampal long-term potentiation (LTP) (Yang et al., 2012). Interestingly, upon cAMP binding, EPAC can block ATP-sensitive potassium channels (K_{ATP}), leading to Ca^{2+}-dependent glutamate release. Genetic ablation of EPAC proteins increases the open probability of K_{ATP} channels in the dentate granule neurons and decreases glutamate release, thereby reducing the vulnerability of epileptic seizures in mice following kainate treatment (Zhao et al., 2013). Therefore, the G_s downstream EPAC might represent an alternative molecular target for acute seizures and potentially acquired epileptogenesis.

GPCRs can also mediate G protein-independent signaling through coupling to β-arrestin proteins, which were originally discovered for their roles in desensitization and internalization of the GPCRs (Ferguson et al., 1996; Goodman et al., 1996). Moreover, the recruiting β-arrestins to GPCRs appears to trigger conformational changes that initiate interaction with the endocytic machinery, thereby linking the trafficking and signaling events. β-arrestin-mediated signaling has been found in nearly all GPCR families including those are discussed in this review (Blume et al., 2017; Eng et al., 2016; Jiang and Dingledine, 2013; Mundell and Kelly, 2011). For instance, β-arrestin-1/2 is involved in EP2 receptor-mediated cytokine production in neuroinflammatory conditions (Chu et al., 2015). The interaction between β-arrestin-1 and CB1 receptor during their endocytic trafficking was also reported recently (Delgado-Peraza et al., 2016). β-arrestin-2 couples mGLuR5 to mediate neuronal protein synthesis in the hippocampus, which is independent of $G_{q/11}$-mediated signaling pathways (Stoppel et al., 2017). Moreover, β-arrestin-2, but not β-arrestin-1, regulates synaptic plasticity that is mediated by mGluR1 in CA3 pyramidal neurons and mGluR5 in CA1 via Src kinase and MAPK/ERK pathways (Eng et al., 2016). However, whether β-arrestin signaling contributes to GPCRs-mediated pathophysiological processes related to epilepsy remains to be determined. It is expected that pharmacological manipulation of GPCRs with emerging biased ligands targeting β-arrestin-dependent signal transduction could potentially help answer this question (Reiter et al., 2012).

4.4 Allosteric modulators

Marked progresses in developing allosteric modulators for GPCRs during the past decade have led to appealing therapeutic strategies for various CNS conditions including certain forms of epilepsy (Conn et al., 2014; Nickols and Conn, 2014). Though conventional orthosteric agonists and antagonists have traditionally been pursued to target GPCRs, allosteric modulators provide multiple mechanistic advantages owing to their potential capability of providing temporal and spatial context-dependence and distinguishing among closely associated receptor subtypes that are activated by common natural ligands, such as biolipids, neurotransmitters, and nucleosides (Conn et al., 2014; Foster and Conn, 2017; Lane et al., 2013; Nickols and Conn, 2014). Therefore, allosteric modulators could potentially avoid the adverse effects associated with orthosteric agonism or antagonism of the targeted GPCRs. Interestingly, cannabidiol has been identified as a non-competitive negative allosteric modulator (NAM) for CB1 receptor (Laprairie et al., 2015), which might help to explain why it does not appear to have any psychotropic effects such as those caused

by Δ9-tetrahydrocannabinol (THC) in marijuana, although the exact mechanism of action for its antiepileptic effects has not been determined. We recently reported positive allosteric modulators (PAMs) for the EP2 receptor that can augment cAMP signaling only in the presence of its natural agonist – PGE_2 and do not affect other PGE_2 receptor subtypes or GPCRs in other families. These small-molecule PAMs have proven to be valuable tools to elucidate the roles of EP2 receptor following neuronal insults (Jiang et al., 2010; Jiang et al., 2018). Remarkably, allosteric modulators have been developed for all three mGluR groups owing to their essential roles in multiple psychiatric and neurological disorders (Conn et al., 2009). Currently using these valuable chemical tools in the epilepsy research field is mostly limited to absence epilepsy, as mGluRs are known for their regulation of SWD, the electrographic hallmark of absence seizures (Duveau et al. ,2019; Ngomba and van Luijtelaar, 2018). In the future it is important to test them in convulsive seizure models to fully explore their potential to be developed as antiepileptic and/or antiepileptogenic therapies.

4.5 Where do we go from here?

One of the major concerns with GPCR-based therapies is the receptor desensitization, referring to acute and prolonged decreases in response to continuous or repeated stimulation. Desensitization is considered a strategy of mammalian cells to avoid unrestricted growth or toxicity caused by overstimulation. During the short-term desensitization, GPCR remains at the cell membrane, but becomes refractory to repeated stimuli, as β-arrestins uncouple the receptor and G protein. Long-term desensitization occurs when the GPCR is phosphorylated by GPCR kinases (GPCRKs), followed by the degradation in lysosomes, and later the receptor would be restored by protein synthesis and processing, i.e., resensitization (Kelly et al., 2008; Rajagopal and Shenoy, 2018). Desensitization disables the receptor from functioning despite the continuous presence of stimuli, and thus compromises the efficacy of therapeutic agents. As such, desensitization might represent a common molecular mechanism whereby some GPCR-targeted therapies engaging traditional ligands (e.g., baclofen for $GABA_B$ receptor) fail to achieve desired antiepileptic or antiepileptogenic effects, which often require extended treatment. In this regard, allosteric modulators and biased ligands might have less propensity for developing tolerance caused by receptor desensitization than conventional agonists or antagonists (Gjoni and Urwyler, 2008; Lin et al., 2018; Slivicki et al., 2018). These nontraditional ligands do not occupy the orthosteric binding site; rather, they stabilize receptor conformation preferentially modulating one specific pathway, and thereby allow more targeted intervention of cellular function and treatment of disease (Gjoni and Urwyler, 2008; Hitchinson et al., 2018; Michel and Charlton, 2018). Owing to these benefits and other advantages discussed earlier, there has been an increasing number of biased ligands along with allosteric modulators targeting GPCRs in clinical trials over the past decade, particularly in the CNS disease indications (Hauser et al., 2017). Future efforts should be encouraged to focus on these nontraditional agonists or antagonists for the next-generation antiepileptic and potential antiepileptogenic strategies targeting GPCRs. Remarkably, we have already witnessed the emerging attention to their potential uses in controlling certain types of epileptic seizures in a number of recent preclinical studies (Brown et al., 2016; Hanak et al., 2019; Kelly et al., 2018; Tassin et al., 2016).

Acknowledgements

This work was supported by the National Institutes of Health (NIH)/National Institute of Neurological Disorders and Stroke (NINDS) grants R00NS082379 (J.J.), R01NS100947 (J.J.), and R21NS109687 (J.J.). We sincerely apologize for not being able to discuss and cite all papers that are relevant to topics in this review due to the space limitation.

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Highlights

- GPCRs are involved in regulating neuronal excitability and setting seizure threshold
- Targeting various groups of GPCRs has not yet been translated to clinical use to date
- GPCR downstream effectors may provide antiepileptic and/or antiepileptogenic targets
- Allosteric or biased agents of GPCRs have potential as novel antiseizure therapeutics

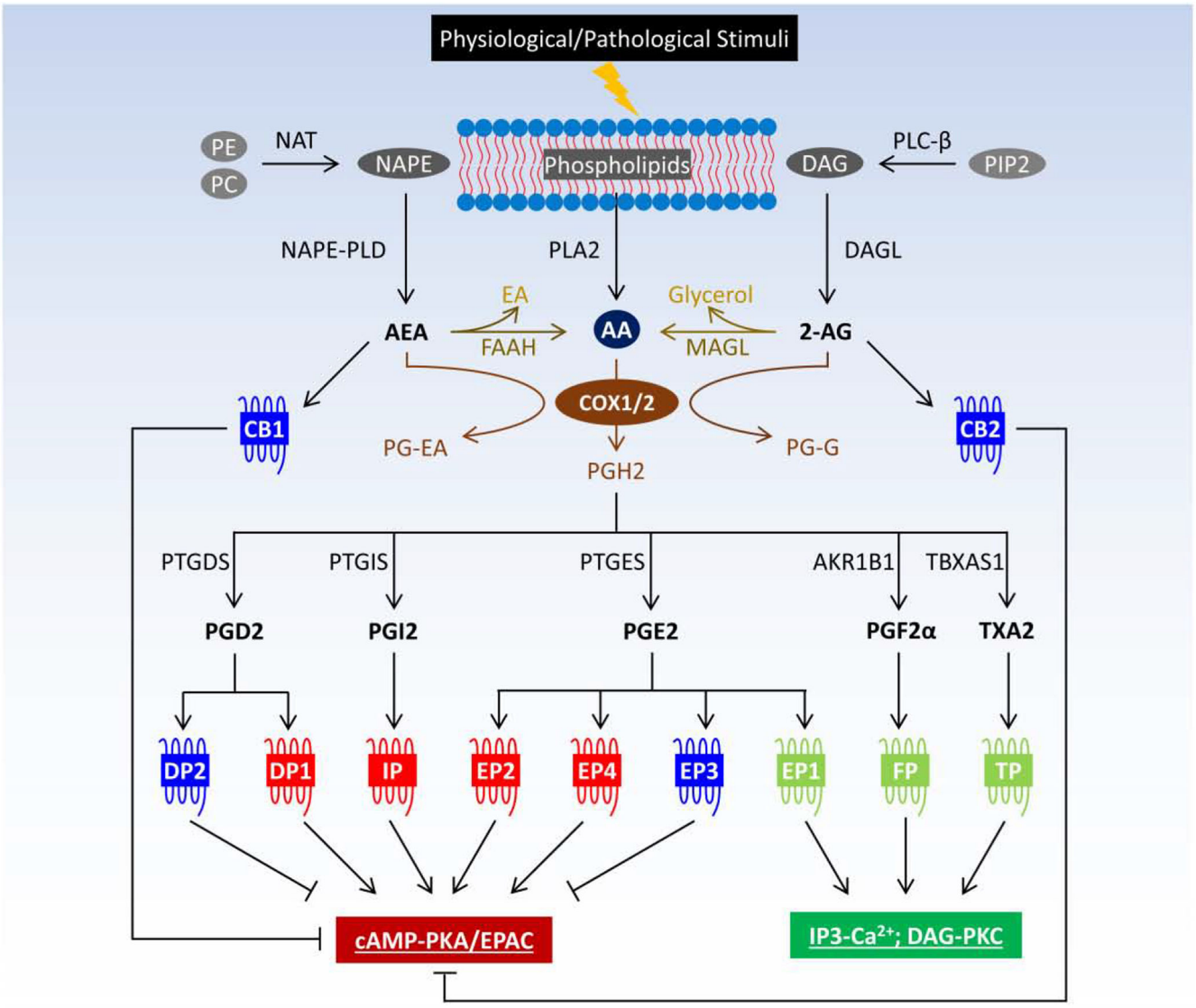

Figure 1.

Crosstalk between prostanoids and endocannabinoids in GPCR signaling. Prostanoids and endocannabinoids are eicosanoids derived from cell membrane-bound phospholipids. Endocannabinoids, such as AEA and 2-AG, can be metabolized to AA, which is the precursor for prostanoid biosynthesis. In addition, COX also metabolizes endocannabinoids to prostaglandin analogs, i.e., PG-EA from AEA and PG-G from 2-AG. Responding to extracellular stimuli, prostanoids and endocannabinoids are rapidly synthesized to mediate wide-ranging physiological and pathological processes via directly acting on a myriad of GPCRs. Abbreviations: 2-AG, 2-arachidonoylglycerol; AA, arachidonic acid; AEA, *N*-arachidonoylethanolamine; AKR1B1, aldo-keto reductase family 1 member B1 or aldose reductase; cAMP, cyclic adenosine monophosphate; CB, cannabinoid receptor; COX, cyclooxygenase; DAG, diacyl glycerol; DAGL, diacylglycerol lipase; DP, PGD_2 receptor; EA, ethanolamine; EP, PGE_2 receptor; EPAC, exchange factor directly activated by cAMP; FAAH, fatty acid amide hydrolase; FP, $PGF_{2\alpha}$ receptor; IP, PGI_2 receptor; IP3, inositol

1,4,5-triphosphate; MAGL, monoacylglycerol lipase; NAPE, *N*-arachidonoyl-phosphatidylethanolamine; NAPE-PLD, *N*-acylphosphatidylethanolamine-specific phospholipase D; NAT, *N*-acyltransferase; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, prostaglandin; PG-EA, PG(D_2/E_2/$F_{2\alpha}$/I_2)-ethanolamide or prostamide; PG-G, PG(D_2/E_2/$F_{2\alpha}$/I_2)-glycerol ester; PIP2, phosphatidylinositol 4,5-bisphosphate; PKA, protein kinase A; PKC, protein kinase C; PLA2, phospholipases A2; PLC-β, phospholipase C-β; PTGDS, PGD_2 synthase; PTGES, PGE_2 synthase; PTGIS, PGI_2 synthase; TP, TXA_2 receptor; TBXAS1, TXA_2 synthase 1; TX, thromboxane.

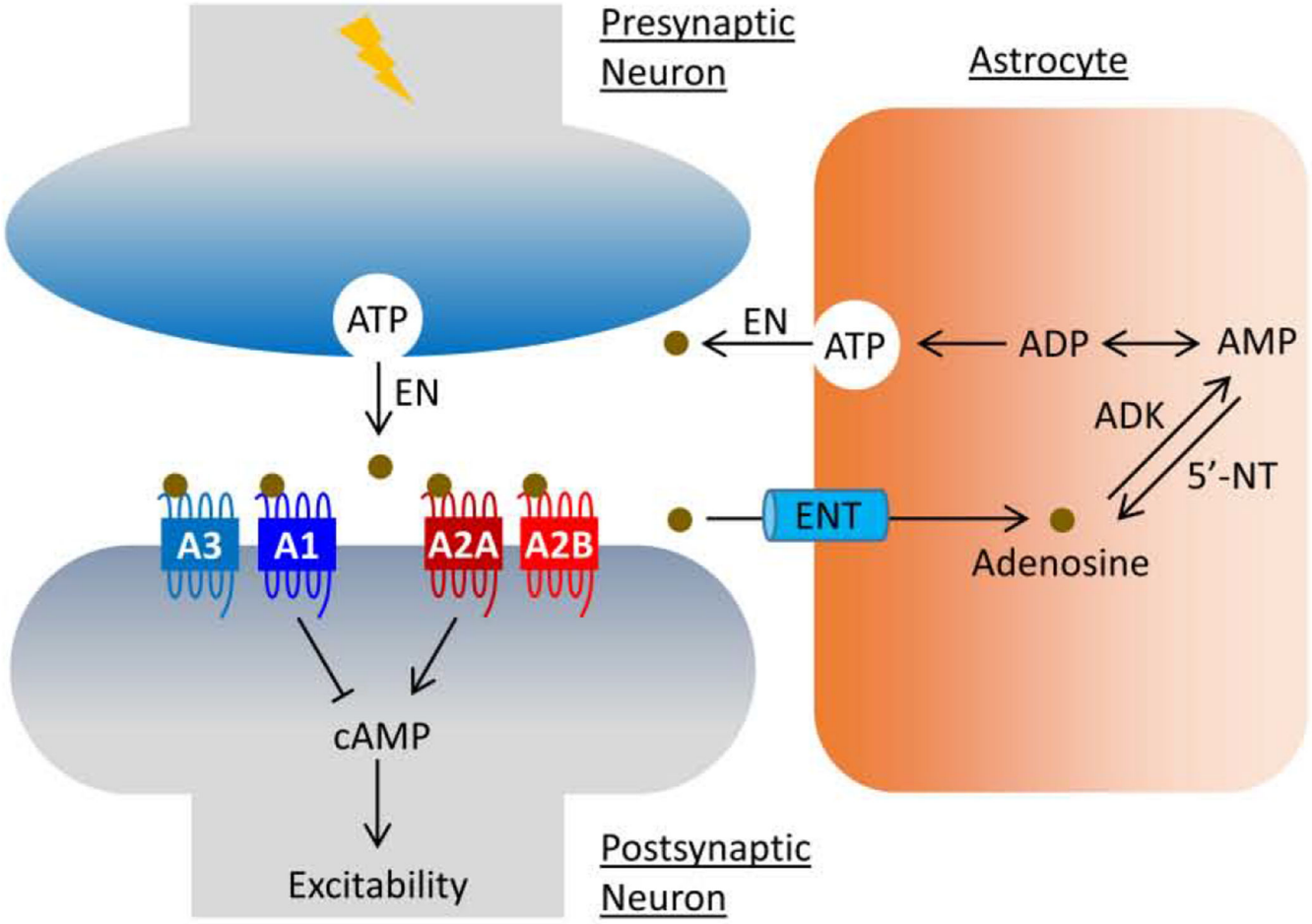

Figure 2.

Adenosine signaling at tripartite synapse. As the primary source of adenosine, ATP is released from astrocytes and neurons via vesicles under normal physiological and pathological conditions, respectively. Upon release, ATP undergoes quick digestion to adenosine by a cascade of EN. Excessive adenosine is then taken up by ENT to astrocytes where it undergoes metabolic clearance by ADK to AMP to complete the balance of the ATP/adenosine conversion cycle. Adenosine regulates neuronal excitability via GPCR-mediated cAMP signaling in the epileptic brain. Abbreviations: 5'-NT, 5′-nucleotidase; A1, adenosine receptor subtype A1; A2A, adenosine receptor subtype A2A; A2B, adenosine receptor subtype A2B; A3, adenosine receptor subtype A3; ADK, adenosine kinase; ADP, adenosine diphosphate; AMP, adenosine monophosphate; ATP, adenosine triphosphate; cAMP, cyclic adenosine monophosphate; EN, extracellular ectonucleotidase; ENT, equilibrative nucleoside transporter.

Table 1.

Prostanoid and cannabinoid receptors in seizure disorders.

Receptor	G protein	Recent studies and therapeutic outcomes	References
EP1	$G_{q/11}$	EP1 antagonist SC-19220 (10 nmol, i.c.v.) 15 min before PTZ injection (60 mg/kg, i.p.) reduced PTZ-provoked acute seizures in adult male Wistar rats.	Oliveira et al., 2008
		Treatment with EP1 antagonist SC-51089 (10 mg/kg, i.p., b.i.d.) starting 30 min before the first pilocarpine injection interrupted seizure-promoted expression of efflux transporter P-glycoprotein at the blood-brain barrier following lithium-pilocarpine-induced status epilepticus in adult female Wistar rats and restored the anticonvulsant effect of a low dose of phenobarbital (6 mg/kg, i.p.).	Pekcec et al., 2009
		Pre-Treatment with EP1 antagonist SC-51089 (3-30 mg/kg, i.p.) significantly decreased the seizure severity in adult male NMRI mice after amygdala kindling.	Fischborn et al., 2010
		Genetic ablation of EP1 receptor increased seizure threshold and showed anti-inflammatory and neuroprotective effects after systemic administration of kainate in male adult C57BL/6 mice.	Rojas et al., 2014
		Pre-treatment with EP1 antagonist ONO-8713 (10 mg/kg, s.c.) 30 min before PTZ injection (60 mg/kg, i.p.) attenuated, whereas EP1 agonist ONO-DI-004 (10 mg/kg, s.c.) exacerbated PTZ-induced seizures in adult male Swiss mice.	Reschke et al., 2018
EP2	G_s	Treatment with EP2 selective antagonist TG4-155 (5 mg/kg, i.p.) reduced neuronal injury in the hippocampus after pilocarpine (280 mg/kg, i.p.)-induced status epilepticus in male adult C57BL/6 mice.	Jiang et al., 2012
		Post-treatment with EP2 antagonist TG6-10-1 (5 mg/kg, i.p.) brought extensive benefits following status epileptics in pilocarpine (280 mg/kg, i.p.)-treated male adult C57BL/6 mice, i.e., reduction in delayed mortality, acceleration of recovery from weight loss and functional impairment, prevention of the blood-brain barrier breakdown, and decrease in neuronal inflammation and injury in the hippocampus.	Jiang et al., 2013; Jiang et al., 2015
		Post-treatment with TG6-10-1 treatment (5 mg/kg, i.p.) was neuroprotective and accelerated functional recovery after status epilepticus in male adult Sprague Dawley rats treated by DFP (9.5 mg/kg, i.p.).	Rojas et al., 2015
		Post-treatment with TG6-10-1 (5 mg/kg, i.p.) prevented status epilepticus-induced deficits in the novel object recognition task in male adult Sprague Dawley rats.	Rojas et al., 2016
		Post-treatment with TG6-10-1 (5 mg/kg, i.p., b.i.d.) reduced prolonged seizure-promoted functional deficits, cytokine induction, reactive gliosis, blood-brain barrier disruption, and hippocampal damage in adult male C57BL/6 mice treated by kainate (30 mg/kg, i.p.).	Jiang et al., 2019
EP3	$G_{i/o}$	EP3 antagonist L-826266 (1 nmol, i.c.v.) 15 min before PTZ injection (60 mg/kg, i.p.) increased the latency for clonic seizures induced by PTZ in adult male Wistar rats.	Oliveira et al., 2008
		Pre-treatment with EP3 antagonist ONO-AE3-240 (10 mg/kg, s.c.) 30 min before PTZ injection (60 mg/kg, i.p.) attenuated, whereas EP3 agonist ONO-AE-248 (10 mg/kg, s.c.) potentiated PTZ-induced seizures in adult male Swiss mice.	Reschke et al., 2018
EP4	G_s	EP4 antagonist L-161982 (750 pmol, i.c.v.) 15 min before PTZ injection (60 mg/kg, i.p.) increased the latency for PTZ-evoked acute seizures in adult male Wistar rats.	Oliveira et al., 2008
FP	$G_{q/11}$	Intracisternal administration of $PGF_{2\alpha}$ (700 ng/35 g) after pre-treatment with COX inhibitor indomethacin (10 mg/kg, i.p.) prevented seizures and decreased neuronal injury in the hippocampus after systemic injection of kainate for seizure induction in adult male ICR mice; $PGF_{2\alpha}$ alone had no effect on kainate-induced seizures in these animals.	Kim et al., 2008
		Intracisternal injection of FP antagonist AL-8810 (10 or 50 ng/35 g) 20 min before kainate injection aggravated seizure activity in adult male ICR mice in a dose-dependent manner.	Kim et al., 2008
		Intracisternal administration of $PGF_{2\alpha}$ (700 ng) 20 min before kainate injection reduced seizure activity and mortality in immature CD-1 mice.	Chung et al., 2013
DP1	G_s	PGD_2 (5 μg, i.c.v.) or an active analog ZK-118.182 (1-100 ng, i.c.v.) 5 min before PTZ injection (60 mg/kg, i.p.) delayed the onset of generalized convulsions and reduced mortality in adult male Wistar rats.	Akarsu et al., 1998
		Supression of PGD2 activity by AH-6809 (50 ng, i.c.v.) 20 min before PTZ injection (60 mg/kg, i.p.) or inhibition of PGD_2 synthesis by sodium selenite (0.2 μg, i.c.v.) 15 min before PTZ injection (60 mg/kg, i.p.) increased the seizure incidence and intensity in adult male Wistar rats.	Akarsu et al., 1998

Receptor	G protein	Recent studies and therapeutic outcomes	References
		Genetic ablation of DP1 receptor delayed seizure onset, reduced the duration of generalized tonic-clonic seizures, and decreased number of seizure spikes in adult male C57BL/6 mice treated by PTZ.	Kaushik et al., 2014
TP	$G_{q/11}$	TP activation by U-46619 (300 μg/kg, s.c.) 30 min before the injection of PTZ (60 mg/kg; i.p.) increased the latency for PTZ-induced myoclonic jerks and tonic-clonic seizures in adult female C57BL/6 mice.	Freitas et al., 2018
		TP antagonist, SQ-29548 (up to 300 μg/kg, i.p.) 30 min before the injection of PTZ (60 mg/kg; i.p.) neither modified PTZ-induced seizures nor blunted the anticonvulsant effect of the agonist U-46619 in adult female C57BL/6 mice.	Freitas et al., 2018
CB1	$G_{i/o}$	Adult male C57BL/6 mice lacking CB1 showed decreased seizure threshold in kainate model of temporal lobe epilepsy.	Marsicano et al., 2003
		Non-selective CB agonist WIN 55212-2 showed dose-dependent anticonvulsant effects in hippocampal neuronal culture models of status epilepticus and acquired epilepsy; whereas the anticonvulsant action of WIN 55212-2 in these models was specifically blocked by a CB1 antagonist SR141716.	Blair et al., 2006
		Genetic ablation of CB1 receptor or pre-treatment with the antagonist SR141716 (10 mg/kg) increased seizure severity without altering seizure-promoted cell proliferation and neuronal death after pilocarpine injection in male adult C57BL/6 mice.	Kow et al., 2014
		Pre-treatment with CB1 agonist CP55940 (0.3 mg/kg, i.p.) did not show any obvious effect on pilocarpine-induced seizures in male adult C57BL/6 mice.	Kow et al., 2014
		Treatment with CB1 selective inverse agonist AM251 (20 mg/kg, i.p.) 60 min before kainate injection (30 mg/kg, i.p.) shortened the latency to onset of generalized tonic-clonic seizures and increased mortality in adult male and female C57BL/6 mice.	Sugaya et al., 2016
CB2	$G_{i/o}$	CB2 inverse agonist AM630 (2 mg/kg, i.p.), when co-administered with non-selective CB receptor agonist WIN 55212-2 (21 mg/kg, i.p.), but not by itself alone, showed considerable antiepileptic effects in the maximal dentate activation model of partial epilepsy in adult male Wistar rats.	Rizzo et al., 2014
		Co-treatment with CB2 selective inverse agonist AM630 (2 mg/kg, i.p.) exacerbated the susceptibility of AM251(20 mg/kg, i.p.)-treated adult male and female C57BL/6 mice to kainate (30 mg/kg, i.p.)-induced seizures.	Sugaya et al., 2016
		Deletion of both CB1 and CB2, but not either CB1 or CB2 alone, showed spontaneous seizures or handling-induced seizures in adult male and female C57BL/6 mice.	Rowley et al., 2017

Abbreviations: DFP, diisopropyl fluorophosphate; PTZ, pentylenetetrazol.

Table 2.

Adenosine receptors in seizure disorders.

Receptor	G protein	Recent studies and therapeutic outcomes	References
A1	$G_{i/o}$	Deletion of A1 receptor exacerbated the neuronal loss, convulsions and subsequent mortality in adult male and female C57BL/6 mice after unilateral intrahippocampal injection of kainate (1 nmol).	Fedele et al., 2006
		The proconvulsive effect of A1 receptor ablation was aggravated by experimental traumatic brain injury in adult male and female C57BL/6 mice.	Kochanek et al., 2006
		Adenosine A1 receptor deficiency mice (adult male C57BL/6) developed spontaneous seizures with homozygous knockout mice showing higher frequency and longer duration of electrographic seizures than their heterozygous cohorts.	Li et al., 2007
A2A	G_s	Focal bilateral microinjection of A2A receptor agonist CGS-21680 (up to 3 μg/side) increased, whereas A2A antagonist sCh-58261 (up to 20 μg/side) decreased, the number and duration of the unprovoked spike-wave discharges in young and adult male WAG/Rij rats in a dose-dependent manner.	D'Alimonte et al., 2009
		Ablation of A2A receptor decreased intensity and frequency of seizures induced by PTZ or pilocarpine in adult CD1 mice.	El Yacoubi et al., 2009
		A2A selective agonist CPCA (2 mg/kg, i.p.) decreased the mortality and lowered the seizure threshold in a hyperthermia-induced seizure model in young male Lewis rats.	Fukuda et al., 2011
A2B	G_s	Delivery of nonselective adenosine receptor agonist NECA (0.1 mM) into the striatum of adult BALB/c mice via reverse microdialysis increased IL-6, and this cytokine elevation was blocked by A2B receptor antagonist MRS-1706 (0.01 mM).	Vazquez et al., 2008
A3	$G_{i/o}$	Treatment with selective A3 adenosine receptor agonist IB-MEC (100 μg/kg, i.p.) prior to NMDA or PTZ-induced seizures in adult male C57BL/6 mice decreased the percentage of animals with convulsions, delayed seizure onset, reduced neurological impairment, and increased animal survival.	Von Lubitz et al., 1995

Abbreviations: IL-6, interleukin 6; NECA, 5′-N-ethylcarboxamidoadenosine; NMDA, *N*-methyl-D-aspartic acid; PTZ, pentylenetetrazol.

Table 3.

Metabotropic glutamate receptors in seizure disorders.

Receptor	G protein	Recent studies and therapeutic outcomes	References
Group I mGluR1/5	$G_{q/11}$	Overexpressing mGluR1 increased seizure susceptibility in pilocarpine-treated adult male BL6SJ/F1 hybrid mice.	Pitsch et al., 2007
		Treatment with mGluR1 antagonist AIDA (up to 1 μmol/site, i.c.v.) inhibited, while treatment with group I mGluR agonist DHPG (up to 50 nmol/site, i.c.v.) exacerbated, seizures in PTZ-treated young male ICR mice in a dose-dependent manner.	Watanabe et al., 2011
		Selective mGluR5 negative allosteric modulator CTEP (2 mg/kg, p.o.) decreased, while mGluR5 positive allosteric modulator RO6807794 (0.3 mg/kg, i.p.) increased the hyperactivity and seizures in Tsc2-deficient male and female mice.	Kelly et al., 2018
		Positive modulation of mGluR5 by VU0360172 (50 mg/kg, i.p.) attenuated seizures induced by TMEV in young male C57BL/6 mice.	Hanak et al., 2019
		Conditional ablation of mGluR5 in astrocytes decreased the rate of glutamate clearance in the hippocampus during epileptogenesis of adult male and female mice.	Umpierre et al., 2019
Group II mGluR2/3	$G_{i/o}$	Activation of mGluR2/3 by selective agonists LY379268 and LY389795 (0.001-10 nmol, i.c.v. or 1-30 mg/kg, i.p.) suppressed the sound-triggered or mGluR1/5 agonist DHPG-induced clonic seizures in young male and female DBA/2 mice a dose-dependent manner.	Moldrich et al., 2001
		Activation of mGluR2/3 by LY379268 (0.33 or 1 mg/kg, i.p.) increased, while mGluR2/3 inhibition by LY341495 (0.33, 1 or 5 mg/kg, i.p.) decreased, the occurrence of SWDs in an adult male rat model of absence epilepsy in a dose-dependent manner.	Ngomba et al., 2005
		Treatment with mGluR2/3 agonist APDC (20 nmol/site, i.c.v.) inhibited, while treatment with group II mGluR antagonist EGLU (up to 200 nmol/site, i.c.v.) exacerbated, seizures in PTZ-treated young male ICR mice in a dose-dependent manner.	Watanabe et al., 2011
Group III mGluR4/6/7/8	$G_{i/o}$	Ablation of mGluR7 elevated excitability and increased susceptibility to tonic-clonic seizures induced by PTZ or bicuculline in mice.	Sansig et al., 2001
		Genetic deletion of mGluR4 increased the severity of pilocarpine-induced seizure in adult male CD-1 mice but did not alter the spontaneous recurrent seizures.	Pitsch et al., 2007
		Positive modulation of mGluR4 by allosteric ligand PHCCC (10 mg/kg, s.c.) enhanced absence-like seizures in Wistar Albino Glaxo/Rijswijk rats as well as the PTZ-treated adult male WAG/Rij and ACI mice.	Ngomba et al., 2008
		Treatment with mGluR4/8 agonist L-AP4 (up to 20 nmol/site, i.c.v.) inhibited, while treatment with group III mGluR antagonist MPPG (up to 50 nmol/site, i.c.v.) exacerbated, seizures in PTZ-treated young male ICR mice in a dose-dependent manner.	Watanabe et al., 2011
		Negative modulation of mGluR7 by allosteric inhibitor ADX71743 (100 mg/kg, i.p.) enhanced thalamic synaptic transmission and induced absence epileptic seizures and lethargy in male adult mice.	Tassin et al., 2016

Abbreviations: PTZ, pentylenetetrazol; SWD, spike and wave discharge; TMEV, Theiler's murine encephalomyelitis virus; TSC, tuberous sclerosis complex.

Table 4.

Histamine, $GABA_B$, and galanin receptors in seizure disorders.

Receptor	G protein	Recent studies and therapeutic outcomes	References
H1	$G_{q/11}$	Genetic ablation of H1 receptor or pre-treatment with H1-selective antagonist triprolidine (10 mg/kg, i.p.) increased the susceptibility of immature C57Bl/6J mice to kainate-induced seizures.	Kukko-Lukjanov et al., 2010
H3	$G_{i/o}$	H3 receptor selective antagonist pitolisant (20 mg/kg, i.p.) reduced both the frequency and duration of spike-and-wave discharges in genetic absence epilepsy rat from Strasbourg (GAERS).	Kasteleijn-Nolst Trenité et al., 2013
		Pitolisant (100 mg/kg, i.p.) completely suppressed EEG epileptiform activity and seizures in the maximal electroshock test in mice, a model for primary and secondarily generalized tonic-clonic seizures.	Kasteleijn-Nolst Trenité et al., 2013
		Pitolisant (10 or 20 mg/kg, i.p.) decreased the number and cumulated duration of hippocampal discharges in mice treated by kainate.	Kasteleijn-Nolst Trenité et al., 2013
		H3 receptor selective antagonist pitolisant (20, 40, and 60 mg) showed dose-dependent beneficial effects in patients with photosensitive epilepsy.	Kasteleijn-Nolst Trenité et al., 2013
		Pre-treatment with an H3 receptor-targeted compound with a hydantoin moiety (10 mg/kg, i.p.) was anticonvulsant in electroshock-induced and PTZ-kindled convulsions in adult male Wistar rats.	Sadek et al., 2014
$GABA_B$	$G_{i/o}$	$GABA_B$ activation by baclofen (< 60 mg/day) was anticonvulsant in a human study involving 12 patients.	Terrence et al., 1983
		Allosteric potentiation of $GABA_B$ by small-molecule modulators GS39783 (10-100 mg/kg, i.p.), rac-BHFF (30-300 mg/kg, i.p.), CMPPE (10-100 mg/kg, i.p.), A-1295120 (3-30 mg/kg, i.p.), and A-1474713 (10-100 mg/kg, i.p.) before seizure induction showed anticonvulsant effects in the DBA/2J mouse audiogenic seizure model in a dose-dependent manner. These beneficial effects were largely blocked by the $GABA_B$ antagonist SCH50911 (50 mg/kg, i.p.).	Brown et al., 2016
GalR1	$G_{i/o}$	Genetic ablation of GalR1 increased the vulnerability of male C57BL/6 mice to seizures induced by lithium-pilocarpine or electrical stimulation of the hippocampus.	Mazarati et al., 2004
		GalR1 receptor deletion did not affect either the severity or duration of seizures induced by kainate injection in adult male C57BL/6 mice.	Mazarati et al., 2004; Schauwecker, 2010
		GalR1 receptor activation by galanin analog NAX 5055 (4 mg/kg, i.p.) suppressed audiogenic seizures in male and female Frings mice in a time-dependent manner.	White et al., 2009
		NAX 5055 (i.p.) was highly potent against acute psychomotor seizures induced by 6 Hz corneal stimulation in adult male albino CF-1 mice with low ED_{50} values: 0.7 mg/kg for 22 mA, 0.8 mg/kg for 32 mA, and 2.9 mg/kg for 44 mA stimulations.	White et al., 2009
		NAX 5055 (6 mg/kg, i.p.) showed marked anticonvulsant effects against the fully expressed secondarily generalized seizures in corneal kindling model of partial seizures in adult male albino CF-1 mice.	White et al., 2009
		NAX 5055 even at a very high dose (20 mg/kg, i.p.) was not active in the mouse traditional maximal electroshock model and was minimally active in the subcutaneous PTZ seizure model in adult male albino CF-1 mice.	White et al., 2009
		NAX 5055 (0.5-4 mg/kg, i.p.) had no effect in a multiple-hit model of symptomatic infantile spasms of male Sprague-Dawley rats.	Jequier Gygax et al., 2014
GalR2	$G_{q/11}$	GalR2-preferring analog NAX 810-2 (0.2-3 mg/kg, i.v.) showed antiepileptic effects in both adult male albino CF-1 mouse corneal kindling and 6 Hz seizure models.	Metcalf et al., 2017
		Genetic deletion of GalR2 increased both the frequency and duration of seizures after intrahippocampal administration of kainate in adult male C57BL/6 mice but had no effect on PTZ-induced seizures.	Drexel et al., 2018
GalR3	$G_{i/o}$	Genetic ablation of GalR3 did not affect either the seizure frequency or duration in mouse intrahippocampal kainate model or systemic PTZ seizure model in adult male C57BL/6 mice.	Drexel et al., 2018

Abbreviations: GABA: γ-aminobutyric acid; PTZ, pentylenetetrazol.