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Gliotransmission: A Novel Target for the Development of Antiseizure Drugs

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Abstract

For more than a century, epilepsy has remained an incapacitating neurological disorder with a high incidence worldwide. Mesial temporal lobe epilepsy (TLE) is a common type of epilepsy without an effective pharmacological treatment. An increase in excitability and hypersynchrony of electrical neuronal activity during development are typically associated with an excitatory/inhibitory imbalance in the neuronal network. Astrocytes release gliotransmitters, which can regulate neuronal excitability and synaptic transmission; therefore, the classical neurocentric vision of the cellular basis of epileptogenesis has begun to change. Growing evidence suggests that the key contribution of astrocyte-to-neuron signaling in the mechanisms underlies the initiation, propagation, and recurrence of seizure activity. The aim of this review was to summarize current evidence obtained from experimental models that suggest how alterations in astroglial modulation of synaptic transmission and neuronal activity contribute to the development of this brain disease. In this article, we will summarize the main pharmacological, Ca^{2+}-imaging, and electrophysiological findings in the gliotransmitter-mediated modulation of neuronal activity and their possible regulation as a novel cellular target for the development of pharmacological strategies for treating refractory epilepsies.

Keywords

gliotransmission, drug-resistant epilepsy, purinergic receptors, glutamatergic receptors, pharmacological targets, chronic epilepsy models, ATP

Introduction

Epilepsy is a common neurological disorder affecting 50 to 70 million people worldwide, and it accounts for 0.5% of the global burden of disease and represents one of the most common neurological disorders (World Health Organization 2019). Epilepsy is characterized by a predisposition to experience seizures, which results in various social, psychological, and cognitive consequences. Despite great advances in the development of new treatments, approximately 20% to 25% of patients fail to achieve adequate seizure control (Janmohamed and others 2019), a condition called drug-resistant epilepsy (DRE) or refractory epilepsy. Among these, 10% to 50% of patients (representing 1.0-7.5 million people globally) are candidates for surgery (Schiltz and Fernandez-Baca Vaca 2018). Mesial temporal lobe epilepsy (TLE) is characterized by recurrent focal seizures originating from a network located discretely along the mesial aspect of the temporal lobe; it is usually accompanied by temporal or hippocampal sclerosis (HS) (Baulac 2015; Mathern and others 1998). This histopathological sign includes astrogliosis (i.e., reactive astrogliosis) and neuronal loss, which represent the most conspicuous sign in the TLE brain. Hippocampal sclerosis occurs in DRE and is a hallmark of focal epilepsy. It is the main marker for the prognosis of the surgical resection of focal DRE (Blumcke and others 2017). In patients with TLE, pharmacoresistance appears, on average, 9 years after starting pharmacological treatment (Janmohamed and others 2019; Shukla and Prasad 2012). Not all patients with TLE have DRE; however, patients with HS identified with

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magnetic resonance imaging are more prone to develop drug-resistance than are patients with other lesions or signs (Baulac 2015). The goal of antiseizure drug (ASD) treatment is to achieve full remission of seizures and involves chronic pharmacological therapy. Thus, the medical criteria for choosing ASDs must take into consideration the seizure type, use of other drugs, associated comorbidities, and personal preference regarding life quality. Two main neurobiological hypotheses used to explain DRE are (1) the "target hypothesis" and (2) the "transporter hypothesis" (Tang and others 2017). The target hypothesis proposes that changes in the molecular targets of the drug may reduce the targets' sensitivity to a drug's pharmacological effect, which includes intrinsic modifications (i.e., polymorphisms) or acquired modifications (i.e., by disease process, seizures, and mutagenesis). To date, the main pharmacological targets of ASDs are voltage-gated ion channels, transporters, receptors, and modulators of the neurotransmitter release machinery (Bialer and others 2018; Janmohamed and others 2019). Alternatively, the transporter hypothesis proposes that the failure of a drug's bioavailability at the target site results from the transporter-mediated extrusion of the ASDs from the brain (Feldmann and Koepp 2016). Both theories have been used to explain drug-resistance in DRE with the goal of mitigating alterations in neuronal excitability (e.g., lamotrigine) or synaptic neurotransmission (e.g., levetiracetam); however, the theories do not take into consideration other cellular components of the central nervous tissue such as glial cells, which have an active role in modulating synaptic plasticity and electrical properties in the normal brain (Amiri and others 2012; Bonansco and others 2011; Fellin and others 2004; Lalo and others 2014; Panatier and others 2011; Perea and Araque 2007; Perea and others 2016; Steinhaüser and others 2016). This neurocentric view of epilepsy is changing and a growing body of evidence reveals that reactive astrocytes contribute actively in epileptogenesis and in seizure activity (Alves and others 2017; Fedele and others 2005; Franke and Illes 2014; Gomez-Gonzalo and others 2010; Wellmann and others 2018). Astrocytes undergo morphological, molecular, and functional modifications that allow astrogliosis to develop, along with changes in the expression pattern of various proteins such as transporters, hemichannels, receptors, and enzymes, some of which are an integral part of signaling between astrocyte–neuron and astrocyte–astrocyte communication (Jabs and others 2008; Steinhaüser and others 2016). An active interplay between neurons and astrocytes in epilepsy has been proposed, and several groups have suggested astrocytes as a novel therapeutic target to control epileptic events (Carmignoto and Haydon 2012; Ding and others 2007; Jabs and others 2008; Steinhaüser and others 2016); however, only a few programs are working on developing ASDs directed at regulating glia-to-neuron interactions. In the following sections, we will review the main evidence showing how astrocytes regulate synaptic transmission in physiological conditions and how alterations in this form of paracrine signaling could act as a mechanism resulting in excitation and inhibition in the epileptic brain.

Neuronal Bases of Ictogenesis and Epilepsy

At the cortical level, several types of epileptiform activity (i.e., interictal and ictal activity) occur, all of which exhibit characteristic electrical activity patterns, which are identifiable through an electroencephalogram, associated with specific cellular correlates. Interictal activity is epileptiform activity occurring between ictal bursts (i.e., seizures) and include a broad frequency spectrum and longer duration depolarization waves (from $\geq$300 ms to $<$4 seconds) (Dash and others 2018). At the cellular level, rhythmic depolarizations of the membrane potential occur, and originate in the apical dendritic tree and spread toward the soma, thereby overlapping with bursts of action potentials of a paroxysmal depolarization shift (Dash and others 2018). The ictal activity subsequently reflects the somatic and synchronous discharge of action potentials from a restricted population of neurons, which then become superimposed with a sustained depolarization, followed by a phase of hyperpolarization of the membrane potential (i.e., postictal depression).

Neuronal hyperexcitability and hypersynchronization have been determined for several factors such as an imbalance in excitatory and inhibitory transmission and alterations in the intrinsic electrical properties of neurons and interneurons (Box 1; Bonansco and Fuenzalida 2016; Naylor 2010; Sessolo and others 2015). Thus, some evidence suggests that an imbalance between glutamatergic excitation and GABAergic inhibition helps to reduce the seizure threshold, and thereby trigger and maintain ictal discharge (Drexel and others 2017). Extracellular levels of glutamate increase up to 30 times in the human hippocampus during seizures (Soukupova and others 2015), whereas the number of GABAergic neurons and synapses, and the expression and function of γ-aminobutyric acid receptor (GABAR) are reduced (Goodkin and others 2008; Naylor 2010). In addition, the immunoreactivity pattern for α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPARs; GluR1, GluR2, and GluR3 subunits) and *N*-methyl-D-aspartate (NMDA) receptors (NMDARs; GluNR1 and GluNR2 subunits) show alterations in all hippocampal subfields in brain biopsies

from patients with TLE: GluNR1 immunoreactivity is strongly increased in the CA3-CA1 stratum radiatum (Mathern and others 1998). Prolonged seizures similarly decrease the number of postsynaptic GABA type A receptors ($GABA_A$Rs), which may be a resistance mechanism to the anticonvulsive effect of benzodiazepine in status epilepticus models (Goodkin and others 2008; Naylor 2010). Epileptiform activity alters intracellular Ca^{2+} concentrations and the activity of calcineurin, a Ca^{2+}- and calmodulin-dependent serine/threonine protein phosphatase, which are both associated with the reduced localization of the $GABA_A$R in the cell membrane and may increase seizure duration (Eckel and others 2015). The loss of GABAergic interneurons and a reduction in GABAergic synapses can decrease GABA release, diminish extracellular GABA availability, and reduce tonic inhibition, and thereby promote an imbalance toward the gain of excitatory activity. However, most current antiseizure treatments aim to decrease excitatory activity or increase inhibitory activity, but no treatment has achieved the expected efficacy. As presented in Table 1, the pharmacological mechanism of most drugs with clinical efficacy is directed at controlling neuronal activity, and diminishing neuronal excitability by using sodium channel inhibitors (e.g., eslicarbazepine, lamotrigine) or potassium channel openers (e.g., gabapentin), antagonists of AMPARs (e.g., perampanel), and $GABA_A$R agonists (e.g., ganaxolone and stiripentol). To date, experimental and clinical evidence has shown that ASDs are partially effective in achieving transient remission of seizures (Bialer and others 2018).

Box 1. Development of Antiseizure Drugs in Experimental Models of Epilepsy.

Under normal conditions, epileptogenesis requires a variable period of time called latency in which several molecular, cellular, and network level alterations promote the development of epilepsy (Janmohamed and others 2019). However, most in vivo and in vitro experimental models study epilepsy by solely replicating epileptiform activity associated with acute seizures (Ding and others 2007; Fellin and others 2004; Gomez-Gonzalo and others 2010; Taing and others 2017). These acute models are highly useful for studying alterations that may occur during and after a seizure; however, they do not replicate proconvulsive changes by which a healthy brain transforms into an epileptic brain. Most refractory epilepsies are characterized by the progressive emergence of spontaneous recurrent seizures; therefore, a sole acute seizure chemically or electrically induced in a naive nonepileptic animal does not generate the cellular and molecular changes characteristic of an epileptic brain (Löscher 2011, 2017; Mathern and others 1998). In several in vivo acute models of seizures induced by drugs such as noncompetitive channel blockers of the $GABA_A$R (e.g., noncompetitive antagonists such as pentylenetetrazole [PTZ], bicuculline, and picrotoxin), a single convulsant dose induces a state of continuous seizures (i.e., status epilepticus [SE]). Pilocarpine, a parasympathomimetic alkaloid cholinergic drug, similarly exerts its clinical effects via activating muscarinic acetylcholine M_3 receptors. It reproduces the latent period but not the progressive increase in frequency and severity of seizure that characterizes refractory epilepsy (Curia and others 2008). Single suprathreshold electrical stimulation or maximal electroshock seizure (MES) evokes an SE but without generating the latency phase. However, in vitro acute models using $GABA_A$R blockers (e.g., picrotoxin or bicuculline) and unspecific blockers of voltage-gated potassium channels (e.g., fampridine or 4-aminopyridine) applied in a magnesium (Mg^{2+})-free extracellular medium, kainic acid, local NMDA applications or high potassium (K^+) solution are frequently used to trigger epileptiform activity in brain slices; however, these models are not used to examine latency or seizure progression (Gomez-Gonzalo and others 2010; Taing and others 2017). These acute models have been used as the gold standard test for several old ASDs such as phenytoin, phenobarbital, and carbamazepine (Bialer and others 2018).Some in vitro and in vivo experimental models of chronic epilepsy exhibit several properties of TLE and reproduce most symptoms of intractable epilepsy (Löscher 2011). Kindling is an epileptogenic model, which is based on repeated exposure to subthreshold electrical or chemical stimulus that is initially harmless. Pioneering studies conducted in vivo in freely moving rodents and in vitro show that repeated electrical stimulation at subthreshold intensities produces an electroencephalographic pattern with after-discharges (ADs) or bursts of population spikes (Stasheff and others 1989). In vivo models of epileptiform activity are accompanied by motor and behavioral manifestations that increase gradually while the protocol progresses (Morales and others 2014). In the electrical kindling model, subthreshold stimulation of the amygdaloid complex produces a gradual and progressive increase in ADs and spike wave activity, which culminates in tonic-clonic generalized seizures similar to those exhibited by patients with TLE (Musto and others 2009). Some critical features of TLE strongly depend on the intensity, frequency, and duration of stimuli applied in pharmacological and electrical kindling models.At the histological level, chronic experimental models of epilepsy and patients with TLE exhibit astrogliosis in which glial fibrillary acidic protein–positive hypertrophic astrocytes change their gene expression pattern and overexpress a wide range of proteins such as the connexins (i.e., connexin hemichannel [HC-Cx]) and pannexins (i.e.,

pannexin hemichannel [HC-Panx]), glutamatergic and purinergic receptors, and ectonucleotidases and adenosine kinases (Jabs and others 2008). Moreover, a key hallmark of TLE, hippocampal sclerosis, occurs in the brain of kindled animals (Mathern and others 1998). Spontaneous recurrent seizures in TLE appear after a latent period, which similarly occurs in animals exposed to an extended protocol of kindling. No single model has completely reproduced refractory epilepsy; however, several governmental projects have focused on assessing novel targets that contribute to treating epileptogenesis and avoid pharmacoresistance in relevant epilepsy models. In this context, the National Institute of Neurological Disorders and Stroke (NINDS; Bethesda, MD, USA) of the National Institutes of Health (NIH; Rockville, MD, USA) has developed two major initiatives to identify novel antiseizure drugs and animal models to evaluate pharmacoresistance.In 1975, the NINDS-sponsored Anticonvulsant Screening Project aimed to increase the involvement between academia and industry to discover and develop new ASDs (Kehne and others 2017; Löscher 2017). In 2015, the NIH funded the Epilepsy Therapy Screening Program (ETSP) to identify differentiated agents for unmet medical needs of patients with epilepsy and drugs with efficacy against pharmacoresistant seizures by using a battery of acute and chronic animal models (e.g., MES, corneal kindling, intrahippocampal kainate model, and lamotrigine-resistant amygdala kindling) (Kehne and others 2017; Löscher 2017).

Neuronal Activity Modulated by Gliotransmitters

Astrocytes and neurons form a functional unit, called "tripartite synapsis" (Perea and Araque 2007). Astroglial processes envelope the synapses, and directly regulate neuronal excitability and neurotransmission through Ca^{2+}-dependent gliotransmitter release. Under physiological conditions, acetylcholine, glutamate, GABA, and adenosine triphosphate (ATP) released from neurons and glial cells can trigger intracellular Ca^{2+} elevations in neighboring astrocytes by activating a wide variety of metabotropic receptors such as the muscarinic acetylcholine receptor, mGluR1/5, GABA type B receptor ($GABA_BR$), and purine 2Y receptor/purine 2X receptor (P2YR/P2XR) (Navarrete and others 2012; Pascual and others 2012; Perea and Araque 2007). The intracellular Ca^{2+} elevations induce the exocytotic release of gliotransmitters such as D-serine, ATP, and glutamate from astrocytic processes enwrapping synaptic contacts (Mariotti and others 2016; Perea and Araque 2007). Glutamate, a major gliotransmitter, can have presynaptic or postsynaptic effects mediated by the activation of metabotropic glutamate receptors (mGluRs) and ionotropic glutamate receptors in excitatory and inhibitory neuronal networks (Fig. 1) (Fellin and others 2004; Perea and others 2016). At the glutamatergic terminal, astrocytic glutamate increases synaptic efficacy by regulating the release probability via the activation of group I metabotropic glutamate receptors (mGluR1/5), thereby setting the threshold for the induction of long-term plasticity at nearby glutamatergic synapses (Bonansco and others 2011; Panatier and others 2011; Perea and Araque 2007). At the postsynaptic level, glutamate released spontaneously from astrocytes produces slow inward currents (SICs) by extrasynaptic GluN2B-containing NMDA receptors on neighboring neurons, whereas glutamate released from synaptic terminals activate GluN2A-containing NMDA receptors within the synapse, thereby producing excitatory postsynaptic currents (EPSCs). The SICs are a source for dendritic plateau potentials, which have been associated with the simultaneous discharge in a neuronal population and promote synchronic activity at the neural network (Amiri and others 2012; Ashhad and Narayanan 2016). However, whether SICs contribute to hypersynchronous discharge in the epileptic brain remains unknown.

In inhibitory synapses contacting CA1 pyramidal neurons, astrocytic glutamate can potentiate or depress GABA release, depending on the receptors involved. Activating kainate receptors (KARs) in GABAergic terminals increases GABAergic transmission on inhibitory interneurons (Liu and others 2004), whereas the same receptor can depress monosynaptic inhibition on pyramidal neurons (Min and others 1999). Activation of the KARs at the axonal level increase neuronal excitability in inhibitory neurons and in pyramidal neurons (Lerma and others 2001). Astroglial glutamate can also activate presynaptic II/III mGluRs and decrease the frequency of miniature inhibitory postsynaptic currents (mIPSCs) and the amplitude of evoked inhibitory postsynaptic currents (eIPSCs) (Liu and others 2004). However, astroglial ATP and its main metabolite, adenosine, can exert dual effects, depending on the type of receptor activated, because receptors for both types of gliotransmitters are expressed at the pre- and postsynaptic level. In hippocampal neurons, the activation of adenosine type 1 receptors (A1Rs), which have antiseizure action, inhibits glutamate release (Etherington and Frenguelli 2004). In contrast, the activation of the purine receptor P2X and P2Y subtypes can facilitate or inhibit glutamate release, depending on the type of receptor involved: P2XRs facilitates glutamatergic transmission and P2YRs inhibits it (Mayhew and others 2018). Moreover, astroglial ATP can down- or up-regulate GABAergic transmission and increase the frequency and amplitude of spontaneous inhibitory postsynaptic currents (sIPSCs) via P2YRs activation or reducing IPSC amplitude by negatively

Table 1. Efficacy of Antiseizure Drugs in Kindling Models and in Patients with TLE.[a]

Main System Involved	Drug	Main Action Mechanism	Pharmacological Effects	Effect in the Kindling Models	Efficacy in Refractory Patients (%)	Reference(s)
GABAergic system	Ganaxolone It is not approved by the FDA, but it is used as an orphan drug to treat cyclin-dependent kinase-like 5 (CDKL5) gene-related early-onset infantile epileptic encephalopathy. Patients are being recruited for clinical trials NCT03350035 (recruiting), NCT02358538 (active, not recruiting), NCT03865732 (recruiting) and NCT02519439 (terminated)	Positive allosteric modulator of the $GABA_AR$	Increases inhibitory neurotransmission	Blocks seizure propagation and elevates the threshold seizure	13%-23% No meta-analysis	Greco and others 2018
	Vigabatrin It was approved by the FDA in 2009 and by the EMA in 2018	Irreversible inhibitor of 4-aminobutyrate aminotransferase (also known as GABA transaminase) (pKi = 3.1)	Increases the concentration of GABA in the brain	Reduces seizures in electrical and audiogenic kindling models	33%-62% Meta-analysis (risk ratio [RR], 2.58; 95% confidence interval [CI], 1.87-3.57)	Hemming and others 2013
	Gabapentin It was approved by the FDA in 1993	Gabapentin is a voltage-gated calcium channel modulator which acts via a cascade involving GABA receptors	Reduces the release of monoamine neurotransmitters such as norepinephrine, substance P, and glutamate	Reduces seizures in electrical and audiogenic kindling models	15%-22% Meta-analysis (RR, 1.89; 95% CI 1.40-2.55)	Panebianco and others 2018a
		Gabapentin is also a potent activator of voltage-gated potassium channels in the brain (Kv7.5 pEC_{50} = 8.7; Kv7.3 pEC_{50} = 8.3)	Reduces neuronal excitability by activating the voltage-gated potassium channels			
	Pregabalin It was approved by FDA and EMA in 2004	Pregabalin is a gabapentinoid. It appears to function as an inhibitor of voltage-gated calcium channels (Kd = 19 nM), rather than as a GABA receptor agonist	Reduces neuronal excitability by inhibiting voltage-gated calcium channels	Reduces seizures in electrical and pharmacological kindling models	31%–50% Meta-analysis (RR, 2.28; 95% CI 1.52-3.42)	Panebianco and others 2019
	Tiagabine It was approved by FDA in 1997	Tiagabine is classified as a GABA reuptake inhibitor that blocks GABA transporter 1 (GAT-1; pIC_{50} = 7.2)	Increases the concentrations of GABA in the brain	Reduces seizures in electrical kindling models	14%-28% Meta-analysis (RR, 3.16; 95% CI 1.97 to 5.07)	Bresnahan and others 2019b
	Stiripentol It was approved by the FDA in 2007 and the EMA in 2018	Positive allosteric modulator of $GABA_AR$	Increases inhibitory neurotransmission	Reduces seizures in kainate and pentylenetetrazol kindling models	49% (in children) Meta-Analysis (RR, 1.51; 95% CI 0.81-2.82)	Brigo and others 2018a
	Cenobamate (also called YKP3089) It is not approved by the FDA. Patients with primary generalized epilepsy are being recruited for phase 3 clinical trial NCT 03678753	The mechanism of action remains under investigation	Some evidence indicates it increases inhibitory neurotransmission and reduces neuronal excitability produced by voltage-gated sodium channels	Information is not available	40%-64% No meta-analysis	Krauss and others 2020
Glutamatergic system	Perampanel It was approved by the FDA and by the EMA in 2012	Noncompetitive AMPAR antagonists (pIC_{50} = 6.2)	Decreases excitatory neurotransmission	Increases the after-discharge threshold and retards the progression	33%–35% No meta-analysis	Frampton 2015
	Brivaracetam It was approved by the FDA and by the EMA in 2012	Inhibitor of synaptic vesicle glycoprotein 2A (SV2A) (pIC_{50} = 7.0)	Decreases GABA and glutamate exocytotic neurotransmitter release	Increases the threshold, shortens after-discharges, and increases the number of stimuli required to achieve racine 5	21%-55% Meta-analysis (RR, 1.79; 95% CI 1.51-2.12)	Lattanzi and others 2016
	Levetiracetam It was approved by the FDA in 1999 and by the EMA in 2000. It is the biologically active enantiomer of racemic formulation of etiracetam	Inhibitor of synaptic vesicle glycoprotein 2A (SV2A; pIC_{50} = 6.1; pKi = 5.8)	Decreases GABA and glutamate exocytotic neurotransmitter release	Reduces seizures in electrical, audiogenic and pharmacological kindling models	32%-72% Meta-analysis (RR, 2.17: 95% CI 1.93-2.43)	Chen and others 2019

(continued)

Table 1. (continued)

Main System Involved	Drug	Main Action Mechanism	Pharmacological Effects	Effect in the Kindling Models	Efficacy in Refractory Patients (%)	Reference(s)
	Topiramate It was approved by FDA in 1996	Inhibitor of carbonic anhydrases (pKi = 6.6-9.1) Positive modulator of the $GABA_AR$ Antagonist of ionotropic glutamate receptors (AMPA and kainate)	Increases inhibitory neurotransmission and decreases excitatory neurotransmission	Reduces seizures in electrical and pharmacological kindling models	37%-51% Meta-analysis (RR 2.71; 95% CI 2.05-3.59)	Bresnahan and others 2019a
	Zonisamide It was approved by the FDA in 2000 and the EMA in 2005	Inhibitor of carbonic anhydrases (pKi = 5.0-7.3) Positive modulator of the $GABA_AR$ NMDA receptor blocker	Increases inhibitory neurotransmission and decreases excitatory neurotransmission	Reduces seizures in electrical and pharmacological kindling models	26%-75% Meta-analysis (RR, 1.90; 95% CI 1.63-2.22)	Brigo and others 2018b
	Felbamate It was approved by FDA in 1993	Positive modulator of $GABA_AR$ Blocker of NMDA receptors	Increases inhibitory neurotransmission and decreases excitatory neurotransmission	Reduces seizures in electrical and pharmacological kindling models	38% No meta-analysis	Shi and others 2019
	Padsevonil (also called UCB 0942) It is not approved by the FDA. Patients with drug-resistant epilepsy are being recruited for Phase 3 clinical trial NCT03739840	Inhibits synaptic vesicle glycoprotein 2A (SV2A; pKi = 8.5), SV2B (pKi = 7.9) and SV2C (pKi = 8.5) Positive allosteric modulator of $GABA_A$ receptors (pKi = 6.4)	Acts selectively at pre- and postsynaptic levels.	Reduces seizure in electrical, audiogenic and pharmacological kindling models	30.8% No meta-analysis	Bialer and others 2018
Intrinsic properties and excitability	Eslicarbazepine It was approved by the FDA in 2013	Selective inhibitor of persistent sodium channels	Reduces repetitive firing by decreasing neuronal excitability	Increases the seizure threshold and reduces seizure severity by inhibiting kindling development	28%-54% Meta-analysis (RR, 2.9; 95% CI 1.49-5.68)	Chang and others 2017
	Oxcarbazepine It was approved by the FDA in 2000	Selective Inhibitor of persistent sodium channels	Reduces repetitive firing by decreasing neuronal excitability	Reduces seizures in electrical and pharmacological kindling models	26%-50% Meta-Analysis (RR, 2.96; 95% CI 2.20-4.0)	Castillo and others 2000
	Lamotrigine It was approved by the FDA in 1994. It is considered a WHO Essential Medicine	Voltage-gated sodium channel blocker (Nav1.2, pKi = 4.5)	Reduces repetitive firing by decreasing neuronal excitability	Reduces seizures in electrical, audiogenic, and pharmacological kindling models	9%-41% Meta-analysis (RR, 1.80; 95% CI 1.45-2.23)	Ramaratnam and others 2016
	Rufinamide It was approved by the FDA in 2007 and the EMA in 2008	Voltage-gated sodium channel blocker (Nav1.1)	Reduces repetitive firing by decreasing neuronal excitability	Reduces seizures in pharmacological kindling models	11%-32% Meta-analysis (RR, 1.79; 95% CI 1.44-2.22)	Panebianco and others 2018b
	Lacosamide It approved by FDA and EMA in 2008	Voltage-gated sodium channel blocker (pKi = 3.4-4.8)	Reduces repetitive firing by decreasing neuronal excitability	Reduces seizures in electrical and pharmacological kindling models	32%-41% Meta-analysis (RR, 1.70; 95% CI 1.38-2.10)	Weston and others 2015

AMPAR = α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor; ASD = antiseizure drug; EMA = European Medicines Agency; EPSP = excitatory postsynaptic potential; FDA = Food and Drug Administration; GABA = γ-aminobutyric acid; $GABA_AR$ = γ-aminobutyric acid type A receptor; $GABA_BR$ = γ-aminobutyric acid type B receptor; IPSP = inhibitory postsynaptic potential; SIC = slow inward current; TLE = temporal lobe epilepsy; WHO = World Health Organization.

[a]The abbreviation Kv7 is slow potassium voltage-gated channels (also called M-current); RR is the response rate of treatment group minus the response rate of the placebo group (expressed as a percentage); SV2A is a protein integral transmembrane glycoprotein in synaptic vesicles, which presumably acts as a modulator of Ca^{2+}-dependent neurotransmitter release; pKi is the negative logarithm of inhibition constant for a ligand; and pIC_{50} is the negative logarithm of the molar concentration of a ligand (agonist or antagonist) that reduces a response by 50% of the maximal inhibition.

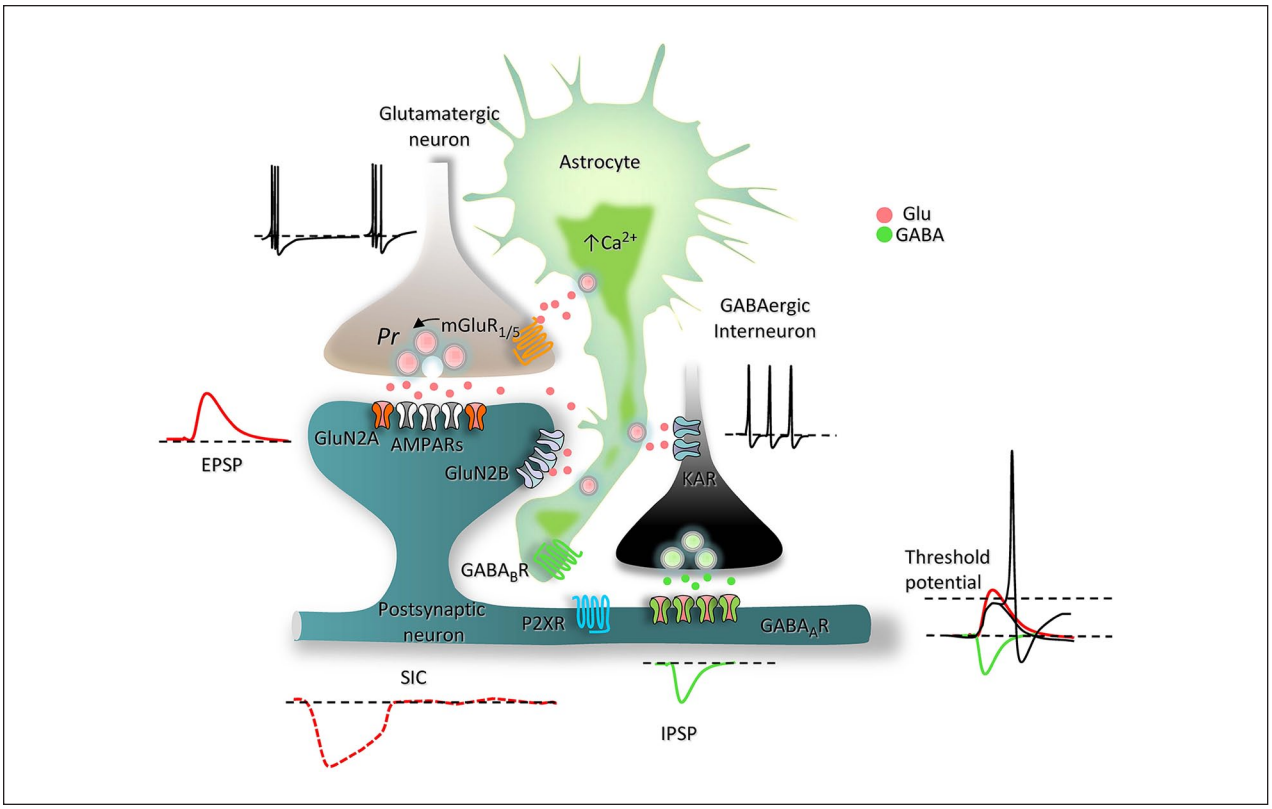

Figure 1. Schematic of the signaling pathways of gliotransmitter release and neuronal responses.
Under physiological conditions, astrocytes have a low rate of calcium (Ca^{2+})-dependent glutamate release, which modulates the probability of neurotransmitter release of glutamatergic terminal by metabotropic glutamate receptor 1 and 5 (mGluR1/5) activation. In addition, spontaneous astrocytic glutamate release can produce a low rate of *N*-methyl-D-aspartate receptor (NMDAR)-mediated slow inward currents in glutamatergic postsynaptic neurons, whereas the activation of KAR and P2XR can increase or decrease GABAergic transmission, respectively. This astrocytic glutamatergic "tonus" could set the basal neurotransmission and the electrical activity of inhibitory and excitatory neurons that form a neural network.
AMPAR = α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor; EPSP = excitatory postsynaptic potential; GABA = γ-aminobutyric acid; $GABA_AR$ = γ-aminobutyric acid type A receptor; $GABA_BR$ = γ-aminobutyric acid type B receptor; Glu = glutamate; GluN2A = glutamate N2A subunit; GluN2B = glutamate N2B subunit; IPSP = inhibitory postsynaptic potential; KAR = kainate receptor; NMDAR = *N*-methyl-D-aspartate receptor; P2XR = purine 2X receptor; Pr = probability of neurotransmitter release; SIC = slow inward current.

modulating the GABAAR through P2XRs activation (Lalo and others 2014).

In short, astrocytes can facilitate or depress excitatory and inhibitory signals by activating different types of gliotransmitter receptors that are expressed at the presynaptic and the postsynaptic level. The astrocytes can modulate the excitatory/inhibitory balance in a given neural network, and its disruption represents a potential mechanism by which neural networks become epileptic.

Disruption of the Astrocyte–Neuron Interplay in the Epileptic Brain

A main feature of epileptic tissue is astrogliosis in which reactive astrocytes undergo a shift in their gene expression pattern, which generates profound changes at the structural and morphological levels (Jabs and others 2008). These expression changes include a wide variety of proteins that mediate astrocyte-to-neuron and astrocyte-to-astrocyte interactions, and include receptors for several neuronal and glial transmitters, hemichannels and transporters (Steinhaüser and others 2016). Astrocytes are primarily responsible for glutamate clearance from the synaptic cleft through the high-affinity glial glutamate transporters excitatory amino acid transporter 1 (EAAT1) and excitatory amino acid transporter 2 (EAAT2) (Lehre and Danbolt 1998). Relevant evidence indicates that alterations in the expression or the functioning of these transporters represent a key role in epilepsy (Pajarillo and others 2019; Sarac and others 2009). Selective deletion of astroglial glutamate transporter 1 (GLT1) in mice generates spontaneous seizures, which is associated with the hyperexcitability of the epileptic focus (Sugimoto and others 2018). The expression of

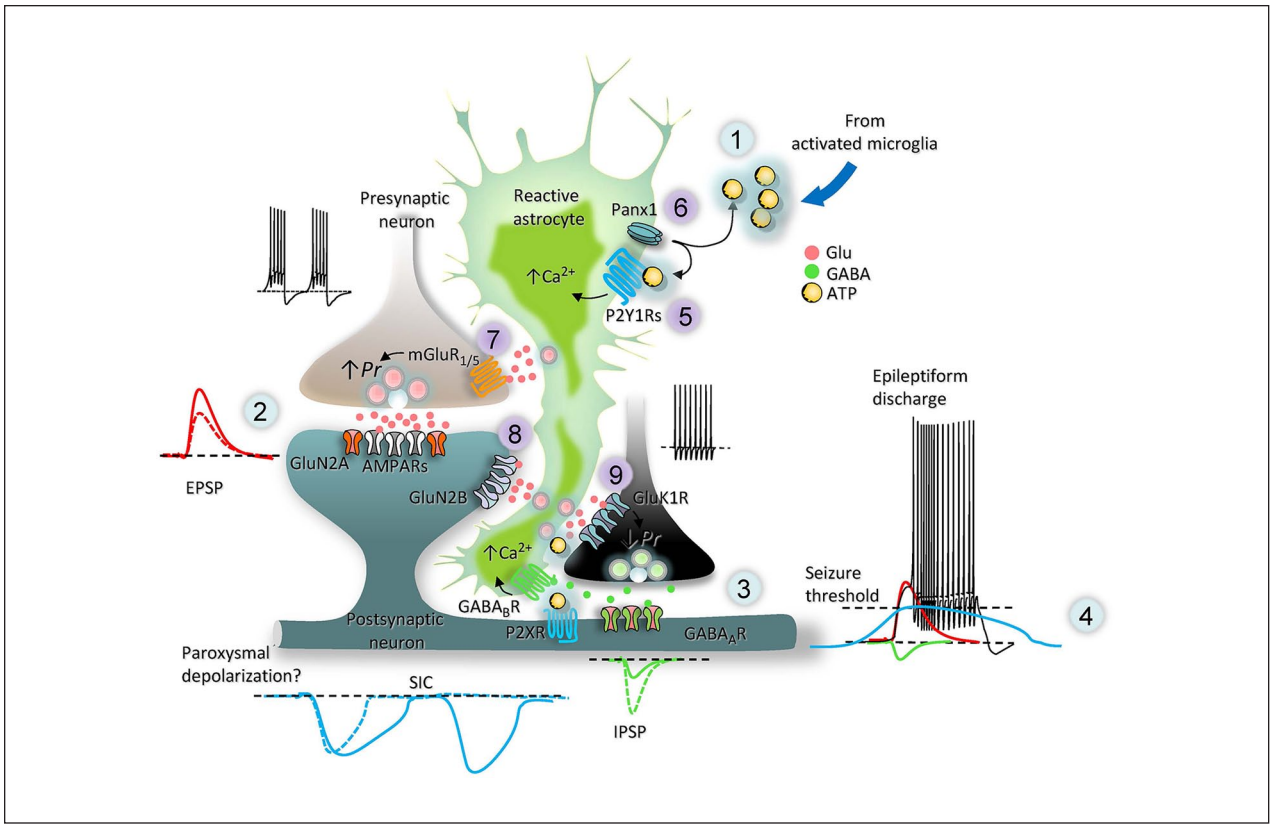

Figure 2. Scheme of the proposed mechanisms for gliotransmission increasing promote the epileptiform activity and the potential targets to control the astroglial signaling.

The figure shows (1) the increase in astrocyte-to-astrocyte signaling mediated by adenosine triphosphate (ATP)—probably released by activated microglia—increase slow calcium (Ca^{2+}) transients in astrocytes and subsequently increase glutamate release from astrocyte; (2) activation of mGluR1/5 increase glutamatergic transmission (EPSP in red; dot = before and line = after); (3) transmission of GABA is decreased by the activation of KARs rich in the GluR5 subunit or P2XR (IPSP in green dot = before and line = after); (4) hyperexcitability and hypersynchronicity induced by the increased production of a high rate of NMDAR-mediated SICs in glutamatergic postsynaptic neurons (SIC in blue; dot = before and line = after), presumably associated with paroxysmal depolarization; (5) MRS2179, an antagonist of P2Y1Rs, inhibits the long-lasting astroglial Ca^{2+} waves in reactive astrocytes; (6) blockers of the mimetic peptides ^{10}Panx1 and A-438079, an antagonist of P2X7R, inhibit the release of ATP from astrocytes; (7) LY367385 and MPEP, an antagonist of the mGluR1/5 subtypes, diminish the up-regulation of glutamate release; (8) ifenprodil, an antagonist of NMDARs rich in GluN2B subunits, reduce SIC frequency and amplitude; (9) NS3763 and LY382884, antagonists of GluK1 decrease GABAergic depression and hyperactivity of excitatory neurons.

AMPAR = α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor; EPSP = excitatory postsynaptic potential; GABA = γ-aminobutyric acid; $GABA_AR$ = γ-aminobutyric acid type A receptor; $GABA_BR$ = γ-aminobutyric acid type B receptor; Glu = glutamate; GluN2A = glutamate N2A subunit; GluN2B = glutamate N2B subunit; IPSP = inhibitory postsynaptic potential; KAR = kainate receptor; MPEP = 2-methyl-6-(phenylethynyl)pyridine; NMDAR = *N*-methyl-D-aspartate receptor; P2X7R = purine 2X7 receptor; P2Y1R = purine 2Y1 receptor; Pr = probability of neurotransmitter release; SIC, slow inward current.

astrocytic glutamate transporter EAAT-1 and EAAT-2 are diminished in the CA1 region of the hippocampus and in the temporal lobe of patients with TLE (Sarac and others 2009). A further revision regarding the role of astrocytic glutamate transporters in neurologic disorders has been discussed in a recent review (Pajarillo and others 2019).

The immunoreactivity patterns for GluR1, GluR2/3, NR1, and NR2 subunits are altered in all hippocampal subfields obtained from patients with TLE (Mathern and others 1998). Immunoreactivity in the NR1 subunit is preferentially increased in the CA3-CA1 stratum radiatum, whereas GluR2/3 are strongly expressed in the soma and proximal dendrites on pyramidal neurons and dentate granule cells. Group I/II mGluRs are also overexpressed in astrocytes and neurons from hippocampal tissue resected from patients with TLE, and in experimental models (i.e., SE) (Aronica and others 2000). In addition, in the hippocampal pyramidal neurons of kindled rats, the accumulation of stable ternary SNAP receptor (SNARE) complex (i.e., VAMP-2/SNAP-25/synaxin-1) with synaptic vesicle protein 2 (SV2), which is correlated with enhanced glutamate release, has been demonstrated, and suggests that excitatory neurotransmission is up-regulated (Matveeva and others 2012). In kindled rats, the

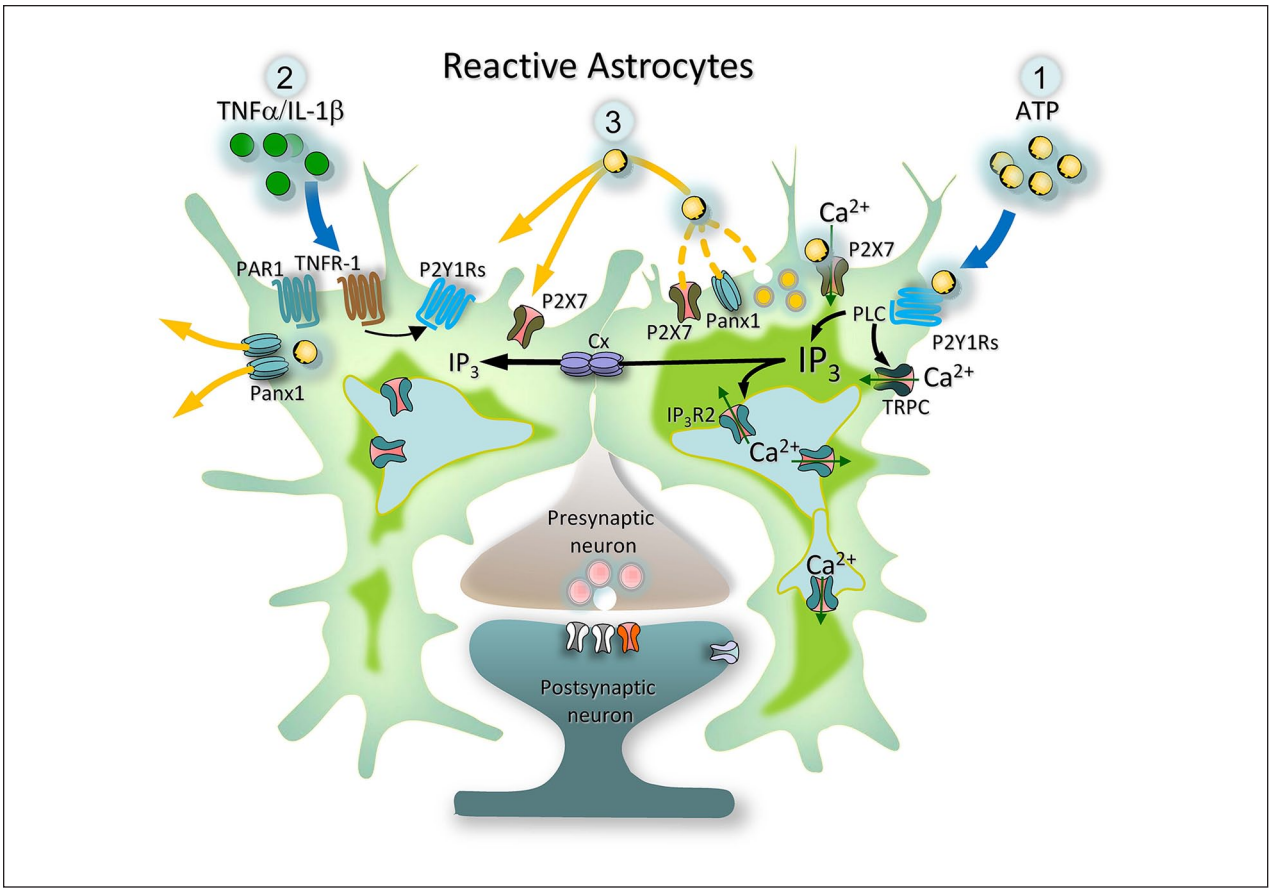

Figure 3. Scheme of the proposed mechanisms for the activation of astrocyte–astrocyte signaling in epilepsy.
(1) Adenosine triphosphate (ATP) and (2) cytokines such as tumor necrosis factor alpha (TNFα) and interleukin 1-beta (IL-1β) released from microglia can activate P2Y1Rs directly or indirectly through the astrocytic TNF receptor (TNFR1). Activation of P2YRs G-protein-coupled receptors, which are overexpressed in reactive astrocytes, elevates the levels of inositol-1,4,5-trisphosphate ($InsP_3$) and diacylglycerol (DAG) via phospholipase C (PLC), and subsequently causes slow calcium (Ca^{2+}) transients and triggers the release of gliotransmitters, including ATP and glutamate, from astrocytes. The diffusion of $InsP_3$ between astrocytes occurs through gap junctions formed by connexin hemichannels (HC-Cxs), which allows Ca^{2+}-waves to spread in the astrocytic network. The generation of InsP3 and DAG also open transient receptor potential channels (TRPCs), which, along with ATP-gated purinergic channels (P2X7), provide an additional entry point of extracellular Ca^{2+} and contributes to astrocytic hyperactivity of the epileptic tissue. (3) The permeation of ATP through pannexin channels (Panx1), HC-Cxs, and P2X7 hemichannels constitute the main conductive pathways that allow a direct ATP efflux into the extracellular space, which, along with vesicular ATP release, is a mechanism of amplifying autocrine purinergic signaling during seizure generation.

number of adenosine A1Rs in the glutamatergic terminal of the hippocampal formation, which is an endogenous anticonvulsant, are diminished (Etherington and Frenguelli 2004). Along with other molecular changes, these alterations associated with astrogliosis modify the astroglial Ca^{2+}-dependent signaling and gliotransmission, which could lead to dysfunction in astrocyte–neuron signaling (Fig. 2) and astrocyte–astrocyte signaling (Fig. 3), and affect the physiology of the neural circuits. Ortinski and coworkers (2010) demonstrated that the selective induction of reactive astrogliosis via adeno-associated virus reduces inhibitory but not excitatory synaptic transmission in hippocampal slices. This finding suggests that an insult that induces astrogliosis is sufficient to affect the excitatory/inhibitory synaptic balance in a manner similar to that in the epileptic brain (Ortinski and others 2010). In addition, evidence from acute epilepsy models show that microglial activation, which occurs in several types of epilepsy, triggers ATP release from astrocytes and exacerbates seizure activity (Jimenez-Pacheco and others 2016; Pascual and others 2012). Thus, impaired gliotransmitter-mediated astrocyte–neuron signaling by reactive astrocytes can contribute to the development of hyperexcitability is a new mechanism that promotes synaptic imbalance in epileptic circuits (Fig. 2) (Alvarez-Ferradas and others 2015; Gomez-Gonzalo and others 2010; Steinhaüser and others 2016). Considerable variations in frequency, amplitude, and duration of astroglial Ca^{2+}-dependent signals occur, depending on the affected central nervous system region, phase of the

disease, experimental model, disease severity, and so on (Ding and others 2007; Heuser and others 2018). Moreover, astrocytes in cell cultures and in brain slices from the hippocampal formation of patients with TLE exhibit an elevated frequency of astrocytic Ca^{2+} waves, which is correlated with increased glutamate release from astrocytes (Heuser and others 2018). Our group consistently showed that hippocampal astrocytes from full-kindled rats exhibit a higher incidence of long-lasting Ca^{2+} transients and consequently increased glutamate-mediated gliotransmission (Alvarez-Ferradas and others 2015; Wellmann and others 2018). The up-regulation of glutamatergic gliotransmission enhances synaptic efficacy via a presynaptic mechanism mediated by mGluRs1/5 activation, thereby increasing the probability of neurotransmitter release in neighboring glutamatergic synapses. Selective inhibition of Ca^{2+} transients by loading astrocytes with the Ca^{2+} chelator 1,2-bis(*o*-aminophenoxy) ethane-*N,N,N′,N′*-tetraacetic acid (BAPTA) strongly reduces the incidence of long-lasting astroglial calcium transients and diminished glutamate-mediated gliotransmission, which set CA3-CA1 glutamate neurotransmission near the control values (Alvarez-Ferradas and others 2015).

Abnormal astroglial Ca^{2+}-dependent signaling and glutamate-mediated gliotransmission can up-regulate the efficacy of glutamatergic transmission at CA3-CA1 synapses and is a mechanism for the development of hypersynchronous neuronal firing, which is another key feature of epileptic circuits (Amiri and others 2012). Astrocytes can depolarize neighboring neurons in a synchronic fashion through extrasynaptic NMDAR-dependent SICs (Amiri and others 2012), which can cause paroxysmal depolarization shifts. This shift is a key cellular manifestation of epilepsy.

Moreover, recent in vivo research employing a kainic acid model of TLE demonstrated that the first cells activated in the hippocampus are astrocytes; their activation precedes the hypersynchronous neuronal activity at the onset of a seizure (Dossi and others 2018). These findings support the notion that astrocytes can recruit and synchronize neurons to seizure activity, and regulate the ictal threshold, seizure propagation, and seizure duration, which may result from the development of excitatory feedback loops between astrocytes and neurons (Carmignoto and Haydon 2012; Gomez-Gonzalo and others 2010).

Selective optogenetic activation of inhibitory interneurons—particularly fast-spiking parvalbumin-GABAergic interneurons—promote the spread of focal epileptiform activity in hippocampal slices, although this process would seem paradoxical (Sessolo and others 2015). In addition, inhibition mediated by parvalbumin- and somatostatin-expressing interneurons remains invariable throughout the preictal and early ictal periods (Miri and others 2018). This finding suggests that epileptiform activity cannot be explained by the lack of GABAergic inhibition exerted by these cells. Some modulatory effects mediated by astroglial glutamate over inhibitory interneurons and the consequences on astroglial Ca^{2+}-mediated activity can partially explain these results; however, this modulatory effect has been studied to a lesser extent.

Astroglial glutamate is a source for KAR activation, particularly KARs rich in GluR5 subunits, which can modulate GABAergic-mediated inhibition in two distinct and opposite ways: (1) by reducing GABAergic-mediated inhibition via G-protein coupled receptors that stimulate the protein kinase C pathway and (2) by increasing the excitability of inhibitory interneurons through depolarization-mediated cationic influx mediated by the activation of axonal KAR receptors (Lerma and others 2001). Recent research demonstrates that GABA released from CA1 interneurons induces long-lasting Ca^{2+} oscillations in astrocytes through $GABA_BR$ activation (Mariotti and others 2016), and that bursting action potential discharge from CA1 interneurons evokes Ca^{2+}-dependent glutamate release from neighboring astrocytes (Perea and others 2016). The aforementioned findings support the hypothesis that GABAergic transmission could ease the development and spread of epileptiform activity by triggering astroglial Ca^{2+}-dependent activity that increase glutamate-mediated gliotransmission, which then (1) increases the probability of neurotransmitter release at glutamatergic synapses via presynaptic mGluR1/5 activation; (2) synchronizes glutamatergic neurons via extrasynaptic NMDAR activation; and (3) exacerbates GABA release through the activation of axonal KAR in inhibitory interneurons.

Given that astrocytes act as integrators and modulators of synaptic activity, astroglial glutamate could be a robust common trigger of epileptiform activity in hyperexcitable neuronal networks, and thereby regulate the ictal threshold and seizure spread, duration, and severity. However, how astrocyte dysfunction arises and where signals generating astroglial hyperactivity/hyperexcitability originate remain unclear.

Astrocytes release ATP, which has a modulatory effect over neurons and neighboring astrocytes. It is the main extracellular messenger for astrocyte–astrocyte communication (Fig. 3). Adenosine triphosphate enters the extracellular space via exocytotic and nonexocytotic mechanisms such as hemichannels formed by HC-Cx and HC-Panx (Cheung and others 2014). This gliotransmitter is the main signaling molecule for the propagation of astroglial Ca^{2+} waves via activating ionotropic and metabotropic purinergic receptors (i.e., P2X and P2Y, respectively) (Cieslak and others 2017).

Several reports suggest that the purinergic pathways involved in ATP-mediated astrocyte–astrocyte signaling

are increased in the epileptic brain (Alvarez-Ferradas and others 2015; Pascual and others 2012; Wellmann and others 2018) and extracellular concentrations of ATP can be augmented (Dossi and others 2018). Thus, autocrine ATP release can trigger astroglial Ca^{2+} signaling by activating tumor necrosis factor alpha (TNFα) and purinergic receptors (Dossi and others 2018; Nikolic and others 2018; Shigetomi and others 2018). In the epileptic brain, several connexin types (Deshpande and others 2017) and pannexin 1 (Alves and others 2017; Dossi and others 2018) are overexpressed, which can regulate the release of ATP and other paracrine signals, thereby deregulating astroglial-mediated modulation of synaptic transmission. In particular, functional in vitro and in vivo studies demonstrate that inhibiting hemichannels, which are primarily expressed in astrocytes rather than in neurons, have antiseizure effects (Deshpande and others 2017; Dossi and others 2018; Santiago and others 2011) and diminish seizure duration and severity. However, the expression of P2Y and P2X purinergic receptors is up-regulated in astrocytes in epileptic tissue. Alves and colleagues (2017) recently demonstrated that astroglial P2Y1R overexpression in the hippocampus is a ubiquitous change observed in several epilepsy animal models and in hippocampal tissue from patients with TLE. Furthermore, in the same work, administering the specific P2Y1R agonist adenosine diphosphate (ADP) exacerbated seizure severity and duration. We recently demonstrated that astroglial Ca^{2+}-mediated hyperexcitability in the epileptic hippocampus requires P2Y1R activation in astrocytes; P2Y1Rs are also inhibited by the specific antagonist MRS2179 (Wellmann and others 2018). We likewise showed that astroglial HC-Px1 from kindled hippocampus is hyperactive and that HC-Px1 blockade restores normal astroglial Ca^{2+} signaling, which also mimics the effect of MRS2179. Dossi and colleagues (2018) used human epileptic brain samples and kainic acid mouse model of TLE samples and consistently obtained very similar results, demonstrating that HC-Px1 blockade, P2R blockade, or genetic deletion of pannexin-1 channels have antiseizure properties—all reduced spontaneous seizures by 70%. These findings altogether show the key role of astroglial-mediated purinergic signaling in the epileptogenic process and its involvement in modulating seizure threshold, duration, severity, and propagation of epileptiform activity (Cieslak and others 2017; Pascual and others 2012). Moreover, ATP and P2R provide the main extracellular pathway for astrocyte–astrocyte signaling; therefore, alterations in interastrocyte communication should be considered a potential mechanism underlying the excitatory/inhibitory imbalance in epileptic circuits, being the modulation of astroglial Ca^{2+}-dependent activity weighted as a direct pharmacological target for the development of antiseizure drugs.

Pharmacological Tools as Modulators of Astroglial Ca^{2+}-Dependent Signaling and Gliotransmission

The evidence collected from animal models and human TLE specimens indicates that disruption in astrocyte Ca^{2+}-dependent excitability and its modulatory effect over synaptic circuits could have a pivotal role in the breakdown of the excitatory/inhibitory balance in DRE. In this article, we have presented a model (Fig. 2) that summarizes the changes that alter neuronal excitability and synaptic transmission arising from a disturbance in astroglial Ca^{2+}-dependent and gliotransmitter-mediated neuromodulation. This model outlines most astrocyte-to-astrocyte and an astrocyte-to-neuron interaction associated with epilepsy to date and allows us to propose novel pharmacological targets.

In experimental models, several pharmacological tools have been employed to control or modulate the interplay between astrocytes and neurons (Table 2). Reactive astrocytes exhibiting an increased Ca^{2+}-dependent activity (e.g., an increase in transient frequency or single event duration) can trigger (1) an up-regulation in astrocyte network activity (i.e., astrocyte-to-astrocyte signaling) mediated by ATP, thereby increasing the incidence of somatic slow Ca^{2+} transients; (2) an increase in glutamate-mediated gliotransmission and consequently an increase in glutamate-mediated neurotransmission by activating mGluR at the presynaptic level; (3) a decrease in GABAergic neurotransmission mediated by the activation of KAR; and (4) an increase in the excitability of neighboring neurons and synchronized activity of neurons within the epileptogenic foci, thereby decreasing the seizure threshold and promoting the propagation and recurrence of seizures (Fig. 2). These crucial features of epileptic activity and underlying mechanisms remain poorly understood and may be partially explained by astrocytic dysfunction. However, only a few programs exist for antiepileptic drug development with the aim of regulating astrocyte-to-neuron interactions.

An inflammatory process or a hypoxic or traumatic brain insult could initially activate microglia, which release ATP and stimulate neighboring astrocytes through activating purinergic receptors, including P2Y (i.e., P2Y1) and P2X (i.e., P2X7) (Franke and Illes 2014; Pascual and others 2012). Activated astrocytes consequently release more ATP through Ca^{2+}-dependent exocytotic and nonexocytotic processes, with the spread of Ca^{2+} waves within the astrocyte network as the amplifying mechanism (Pascual and others 2012). The ATP released from astrocytes can enter the extracellular space, likely via connexin and pannexin hemichannels, which can be inhibited by 10PanX and GAP26 mimetic peptides (Shigetomi and others 2018; Wellmann and others 2018),

Table 2. Potential Drugs for Controlling Astroglial Activity in Chronic Models of Epilepsy.[a]

System	Drug	Mechanism	Cellular Target	Effect in Chronic Models of Epilepsy	Reference(s)
Purinergic signaling	MRS2179	Competitive $P2Y_1$ antagonist (pKi = 7.0-7.1)	Reactive astrocytes	Inhibits Ca^{2+}-mediated gliotransmission from reactive astrocytes	Alvarez-Ferradas and others 2015; Pascual and others 2012; Wellmann and others 2018
	JNJ-47965567 A-438079	P2X7 antagonist (pKi = 7.9) P2X7 antagonist (pIC_{50} = 6.9)	Microglia-activated astrocytes	Reduces spontaneous seizures and gliosis through decreasing neuroinflammatory signals Inhibits astroglial Ca^{2+}-transients	Jimenez-Pacheco and others 2016; Jimenez-Pacheco and others 2013
Glutamatergic signaling	LY456236 LY367385	$mGlu_1$ allosteric modulator (pIC_{50} = 6.9) $mGlu_1$ antagonist (pIC_{50} = 5.1)	Neurons astrocytes	Decreases glutamate release up-regulated from presynaptic terminals and perisynaptic astrocytic processes	Alvarez-Ferradas and others 2015; Gomez-Gonzalo and others 2010
	MPEP	$mGlu_5$ allosteric modulator (pKi = 6.4)	Neurons astrocytes	Decreases glutamate release up-regulated from presynaptic terminals and perisynaptic astrocytic processes	
	Ifenprodil	Selective antagonist of GluN2B-rich subunits containing NMDARs (pIC_{50} = 7.1)	Projection neurons	Inhibits SIC-mediated paroxysmal depolarization and hypersynchronicity neuronal activity	Wang and others 2017; Zhu and others 2015
	NS3763 LY382884	GluK1 noncompetitive antagonist (pIC_{50} = 5.8) GluK1 antagonist (pIC_{50} = 4.0)	Interneurons Projection neurons	Inhibition of KAR-mediated depression of GABAergic transmission Decreases ictal and interictal spikes	Bonfardin and others 2010; Peret and others 2014

EMA = European Medicines Agency; FDA = Food and Drug Administration; GABA = γ-aminobutyric acid; $GABA_AR$ = γ-aminobutyric acid type A receptor; $GABA_BR$ = γ-aminobutyric acid type B receptor; GluK1, glutamate kainate 1; mGlu1, metabotropic glutamate receptor 1; NMDAR = *N*-methyl-D-aspartate receptor; KAR, kainate receptor; P2X7 = purine 2X7 receptor; SIC = slow inward current; TLE = temporal lobe epilepsy.

[a]The abbreviation pKi is the negative logarithm of inhibition constant for a ligand, and pIC_{50} is the negative logarithm of the molar concentration of a ligand (agonist or antagonist) that reduces a response by 50% of the maximal inhibition.

and by Ca^{2+}-dependent vesicular exocytosis. This pathway generates slow, long-lasting P2Y1-mediated astrocytic Ca^{2+} transients that propagate on a large scale (Shigetomi and others 2018; Wellmann and others 2018), thereby inducing astrogliosis in the astrocyte network (Franke and Illes 2014; Shinozaki and others 2017). Reactive astrocytes subsequently overexpress P2Y1 receptors, which have been implicated in the generation of long-lasting astrocytic Ca^{2+} transients in astrocytes (Alves and others 2017; Franke and Illes 2014; Pascual and others 2012; Shigetomi and others 2018). These slow P2Y1R-mediated Ca^{2+} oscillations up-regulate glutamate gliotransmission and enhance the glutamatergic tone in synapses wrapped by processes emitted by reactive astrocytes. In particular, P2Y1R expressed in astrocytes is blocked by MRS2179, which decreases the duration of astroglial Ca^{2+}oscillations by reducing the frequency of slow Ca^{2+} transients and restoring a normal pattern of astroglial activity (Alvarez-Ferradas and others 2015; Wellmann and others 2018). This up-regulation of astrocytic Ca^{2+}-waves increases glutamate release from astrocytes. This action then increases the probability of neurotransmitter release in excitatory synapses by activating presynaptic mGluR1 and mGluR5 receptors, which are inhibited by LY367385 and 2-methyl-6-(phenylethynyl)pyridine (MPEP), respectively (Alvarez-Ferradas and others 2015). At the postsynaptic component, enhanced glutamate-mediated gliotransmission can synchronize neurons within and outside the epileptic network by heightening SIC-mediated depolarization through extrasynaptic NMDAR activation, which can be selectively blocked by specific inhibitors such as ifenprodil (Wang and others 2017; Zhu and others 2015). The specific extent and contribution of SIC-mediated depolarization to the genesis of hyper-synchronic discharge of glutamatergic neurons within and outside the epileptic network remains an open question. However, the up-regulation in glutamate gliotransmission can induce KAR activation at the presynaptic terminal of GABAergic interneurons (Bonfardin and others 2010), and thereby reduce GABA release and contribute to the development of the characteristic excitatory/inhibitory imbalance. Specific antagonists of GluK1/5-containing KARs such as NS3763, LY377770, and LY382884 can rescue GABAergic depression and prevent the development of epileptiform activity in chronic epilepsy models.

Most inhibitors included in this article have mostly been tested in in vitro models and tested only in some in vivo models; however, their potential effect as an antiepileptic drug has not been tested in patients with refractory epilepsy. Moreover, despite the findings of several works (e.g., seizure mitigation), the interpretation of how these drugs exert their anticonvulsive effect is not regularly associated with the modulation of astrocyte–neuron interactions (Wang and others 2017). Based on this rationale, we invite researchers working in epilepsy physiopathology and pharmacology to consider and develop drugs that regulate astroglial Ca^{2+}-dependent activity and/or gliotransmission as potential treatment that could effectively control seizures and/or impede the development of epilepsy.

Remarks and Perspectives

At the clinical level, antiepileptogenic therapies aim to prevent and cure epilepsy, whereas ASD treatments are directed at the remission of symptoms and preventing the propagation of seizures. At the brain level, the aim of treatment requires raising the seizure threshold and, to some extent, restoring the excitatory/inhibitory balance. At the circuit level, astroglial Ca^{2+}-dependent activity and gliotransmission could synchronize and modify the excitability of neurons outside the network, and thereby simultaneously modulate the activity of several neural circuits. At the synaptic level, the quantity and type of gliotransmitters released seems tightly associated with astroglial Ca^{2+} dynamics in astrocytic microdomains, which are finely modulated and modulates back neuronal activity as in a loop. Thus, the spatiotemporal modulatory power exerted by astrocytes can encompass a few synapses up to large neuronal territories. The altered astroglial network dysregulates brain integration on a large scale, which is observed in patients with TLE. In this scenario, we proposed a model that has reactive astrocytes and astrocyte networks in its very core and that explains most functional alterations in the epileptic brain (i.e., hyperexcitability, hypersynchronization), based on the modulatory power gliotransmission exerts over the excitatory/inhibitory balance. The body of evidence reviewed in this article suggests that astroglial activity and gliotransmission can be pharmacologically modulated for treating epilepsy at three different levels: (1) by regulating astrocytic hyperactivity/hyperexcitability via reducing hemichannel-mediated ATP release from microglia and astrocytes (i.e., pannexin and connexin inhibitors) or by inhibiting astroglial P2Y and P2X purinergic receptors; (2) by modulating astrocyte-mediated regulation of excitatory glutamatergic neurons via inhibiting presynaptic group I mGluRs (i.e., mGluR subtype 1 and mGluR subtype 5 antagonists) and inhibiting postsynaptic NMDA-dependent depolarization; and (3) by modifying astrocyte-mediated regulation of inhibitory GABAergic interneurons via inhibiting kainate receptors that down-regulate GABA release. However, pharmacological agents have not been developed that specifically control astroglial Ca^{2+}-mediated excitability, gliotransmission, or the expression of specific molecules in astrocytes implicated in epileptogenesis.

Finally, our review attempts to make a critical view of the neurocentric hypothesis of epilepsy, summarizes recent advances in epilepsy physiopathology that focuses on the astrocytes as a target, and highlights the relevance of employing chronic epilepsy animal models to test classic and new ASDs, which could improve the outcome of the assays. The incorporation of astrocytes as active modulators of neurotransmission and neuronal excitability undoubtedly represents an exciting challenge for development of new treatment alternatives. In the future, the search for drugs that can achieve effective seizure control through different mechanisms against astroglial dysfunction would increase the available options for patients with pharmacoresistant epilepsy. These new ASDs could potentially reduce some adverse effects such as disturbances in mood, attention, cognition, and awareness that are frequently associated with drugs used to treat epilepsy.

In our view, the dysregulation of astroglial signaling and gliotransmission is a novel target for the development of specific drugs and constitutes a therapeutic alternative for some brain diseases resistant to treatment with conventional drugs. In that regard, the degree to which the specific role of astrocytes in the physiopathology of epilepsy is understood can be key in discovering new drugs to treat patients still awaiting an effective intervention.

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