

Targeting Neuroinflammation via Purinergic P2 Receptors for Disease Modification in Drug-Refractory Epilepsy

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Abstract: Treatment of epilepsy remains a clinical challenge, with >30% of patients not responding to current antiseizure drugs (ASDs). Moreover, currently available ASDs are merely symptomatic without altering significantly the progression of the disease. Inflammation is increasingly recognized as playing an important role during the generation of hyperexcitable networks in the brain. Accordingly, the suppression of chronic inflammation has been suggested as a promising therapeutic strategy to prevent epileptogenesis and to treat drug-refractory epilepsy. As a consequence, a strong focus of ongoing research is identification of the mechanisms that contribute to sustained inflammation in the brain during epilepsy and whether these can be targeted. ATP is released in response to several pathological stimuli, including increased neuronal activity within the central nervous system, where it functions as a neuro- and gliotransmitter. Once released, ATP activates purinergic P2 receptors, which are divided into metabotropic P2Y and ionotropic P2X receptors, driving inflammatory processes. Evidence from experimental models and patients demonstrates widespread expression changes of both P2Y and P2X receptors during epilepsy, and critically, drugs targeting both receptor subtypes, in particular the P2Y₁ and P2X₇ subtypes, have been shown to possess both anticonvulsive and antiepileptic potential. This review provides a detailed summary of the current evidence suggesting ATP-gated receptors as novel drug targets for epilepsy and discusses how P2 receptor-driven inflammation may contribute to the generation of seizures and the development of epilepsy.

Keywords: epilepsy, inflammation, purinergic signaling, ATP, P2 receptors, P2X₇ receptor, P2Y₁ receptor, disease modification, drug refractoriness

Introduction

Epilepsy encompasses a heterogeneous group of brain disorders which all share a predisposition to generate spontaneous, unprovoked seizures. With an incidence of ~1% and affecting up to 70 million persons worldwide, epilepsy is one of the most common chronic neurological diseases,¹ and consequently is associated with a substantial economic burden.² Adding significantly to the disease burden, epilepsy has a fourfold-increased risk of additional comorbidities (eg, mood, anxiety, and psychotic disorders).^{3,4} Epilepsy can result from genetic abnormalities (eg, de novo mutations, polymorphisms, copy-number variations) or insults to the brain, such as traumatic brain injury (TBI), stroke, prolonged seizures (eg, status epilepticus), or infection.^{5,6} Epileptogenesis, triggered following a precipitating insult to the brain, is the process of turning a normal healthy brain into a brain expressing epileptic

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seizures.⁷ Pathological changes occurring during epileptogenesis include ongoing neurodegeneration, structural and synaptic plasticity, increased permeability of the blood–brain barrier (BBB), aberrant neurogenesis, epigenetic changes, and inflammation.^{7,8} Temporal lobe epilepsy (TLE), which can be acquired following an insult to the brain, is the most common form of epilepsy in adults, is particularly prone to drug refractoriness, and involves structures within the limbic system, including the amygdala and hippocampus.¹ Hippocampal sclerosis, characterized by a pattern of selective neuronal loss and reactive gliosis, is the most common pathological finding in brains of TLE patients.⁹ Of note, whereas some recent studies have investigated a potential role of ATP-gated P2 receptors in epilepsies with a genetic cause (eg, use of WAG/Rij rats, which is a model of genetic absence epilepsy¹⁰), most effort has been invested in studying these receptors in TLE,¹¹ which is thus the main focus of the present review. While there are several options for the treatment of epilepsy, which include brain surgery, use of the ketogenic diet, or vagus nerve/deep-brain stimulation, antiseizure medication remains the first-choice treatment of epilepsy, with over 25 antiseizure drugs (ASDs) available.^{1,12} Current ASDs are, however, ineffective in >30% of patients, can cause severe side effects (eg, fatigue, irritability, and dizziness) and are thought to be mainly symptomatic.¹³ With the majority of ASDs targeting synaptic transmission (glutamate, γ -aminobutyric acid [GABA] and ion [$\text{Na}^+/\text{Ca}^{2+}$] channels) the focus is now on the identification of novel disease-modifying treatment strategies targeting underlying causes that provide long-lasting seizure control, either preventing the development of epilepsy or slowing down/reversing disease progression once epilepsy is established.^{14,15}

It is now well established that besides glutamatergic- and GABAergic-dependent neurotransmission, other signaling systems contribute to increased brain hyperexcitability, possibly representing alternative treatment targets for epilepsy. In this regard, exciting evidence gathered over the last few decades has demonstrated a role for purinergic signaling in both the generation of seizures and the development of epilepsy. Purinergic signaling encompasses a complex system comprising purine-release mechanisms, specific purinergic receptors (adenosine-sensitive P1 and adenine and uracil nucleotide-sensitive P2 receptors), and enzymes metabolizing and thereby removing extracellularly released purines, such as ATP or adenosine.^{16,17} While the anticonvulsant function of the

nucleoside adenosine via P1 receptors is well established,¹⁸ data generated over the last decade have also suggested an important role for P2 receptors during the generation of seizures and epilepsy.¹¹ This includes evidence showing ATP release following seizures and during epilepsy, widespread expression changes of P2 receptors during epilepsy within the brain, and importantly drugs targeting P2-receptor subtypes have been shown to suppress seizures and even impact on the development of epilepsy. In the present review, we provide a detailed description of what is known regarding the expression and function of P2 receptors observed in both animal models of epilepsy and patients, and how targeting of P2-receptor subtypes impacts on seizures, epilepsy, and the underlying inflammatory processes.

Epilepsy and Inflammation

Because seizures are thought to be the result of an imbalance between excitatory and inhibitory activity of neural circuits in the brain, epilepsy has been considered primarily to be a disease of neurons, with the targeting of neuronal ion channels, both GABA and glutamate receptors, the main approach to stop/suppress seizures.^{13,19} However, this perception has changed considerably over recent decades, with mounting evidence from both experimental models and patients demonstrating a contribution of other cell types to increased brain hyperexcitability, including astrocytes and microglia.^{20–22}

The role of astrocytes in epilepsy is well established. Astrocytes are key regulators of homeostasis in the central nervous system (CNS) during physiology and pathology, regulating crucial processes involved in gliotransmission, extracellular water and ion balance, maintenance of the BBB, regulation of arteriolar blood flow, energy supply, metabolism, oxidative stress, and neuroinflammation.²³ Suggesting a proepileptogenic function, reactive astrocytes, which are characterized by hypertrophy of primary processes and a dramatic increase in the expression of GFAP, display several characteristics believed to contribute to increased brain hyperexcitability and seizures. This includes the release of proinflammatory molecules (eg, IL1 β , IL6, or TNF α) and excitatory gliotransmitters (eg, ATP, glutamate), altered expression of ion channels (eg, inwardly rectifying potassium channel $\text{K}_{\text{ir}}4.1$), leading to perturbation in spatial K^+ buffering, changes in expression of Ca^{2+} and Cl^- transporters, uncoupling of gap junctions, and promotion of Ca^{2+} waves, which in turn are thought to modulate the release of a number of gliotransmitters that

may influence synaptic function.^{23–26} Moreover, astrocytes express the enzyme adenosine kinase (ADK), which catalyzes the conversion of adenosine into adenosine monophosphate, removing anticonvulsant adenosine from the extracellular space and thereby lowering seizure threshold.²⁷ Further support for a proepileptic function of activated astrocytes stems from a study showing that the activation of astrocytes in the absence of other pathologies is sufficient to cause epileptic seizures.²⁸

Microglia are the brain's resident macrophages, and are crucial for physiological brain function. In their resting state, microglia have a ramified morphology with long protruding processes, and play a surveillance role by removing cellular debris and dead cells from the brain, synaptic pruning, and controlling the release of anti- and proinflammatory mediators. Following injury to the brain (eg, seizures), microglia are one of the first cell types to respond, and transition to an activated state.²⁹ In contrast to astrocytes, microglia's role during epilepsy is less clear, with both pro- and antiepileptic effects reported.³⁰ However, these pro- or antiepileptic effects are largely dependent on the stage following the initial insult. While previously believed to have a mainly proconvulsant function via the secretion of proinflammatory cytokines (eg, IL1 β),³¹ more recent research now has also suggested a protective function of microglia. In line with this, the depletion of microglia leads to an amplification and synchronization of neuronal activity, culminating in the generation of epileptic seizures. Interestingly, this effect is highly dependent on microglia's ability to sense and catabolize extracellular ATP that is released by neurons and astrocytes following neuronal activation.³² This detrimental effect was further confirmed by two recent studies wherein microglia were depleted in a similar fashion.^{33,34} These conflicting reports can be attributed to the heterogeneity of activated microglia that is largely present following an insult. Resting microglia can either transition to the proinflammatory and neurotoxic M₁ phenotype or the anti-inflammatory M₂ phenotype.³⁵ M₁ microglia secrete proinflammatory cytokines (such as TNF α , IL1 β , and IL6), produce reactive oxygen species to potentiate neuroinflammation and initiate phagocytosis essential for neuronal repair.³⁶ However, prolonged M₁ activation is neurotoxic, creating a positive-feedback loop of perpetuating inflammation and subsequent neurodegeneration.³⁷ M₂ microglia have an opposing function, secreting anti-inflammatory cytokines (such as IL4 and IL13) to promote neuronal protection, and are essential for repair mechanisms.³⁸

Microglia are polarized into an M₁ phenotype via classical proinflammatory cytokines, such as TNF α , IFN γ , and activation of TLR4-mediated NF κ B signaling, as well as induced by necrotic neurons, whereas the M₂ phenotype is induced via anti-inflammatory cytokines, such as IL4 and IL13.³⁹ Adding to the complexity, astrocyte microglia-dependent signaling occurs, with both cell types maintaining a tight relationship during seizures and epilepsy, impacting thereby on each other's activation status and function.⁴⁰ Microglia inflammatory action may precede astrocytic action. Microglia are known to stimulate the transition of astrocytes to both a neuroprotective⁴¹ and a neurotoxic phenotype,⁴² this stimulation being largely dependent on the proinflammatory cytokines released from presumably M₁ microglia. IL6, TNF α , and IL1 β induce a neuroprotective astrocyte phenotype,⁴¹ while IL1 α stimulates the neurotoxic phenotype.⁴² Interestingly, the classical anti-inflammatory cytokines TGF β or FGF released from M₂ microglia can inhibit the formation of neurotoxic astrocytes.⁴² Furthermore, in cocultures of astrocytes and microglia, the proliferative capability of astrocytes is drastically reduced, suggesting that microglia tightly regulate astrocytic proliferation.⁴³ Of note, neurogenesis is also regulated by microglia, with evidence suggesting that microglia may both promote and suppress aberrant neurogenesis seen in epilepsy models.^{21,44} While it is clear that microglia have a significant role in epilepsy and seizures, each phenotype's role has not yet been fully characterized in disease progression. In rodent models, a mixed-microglia phenotype is expressed following status epilepticus and also in established epilepsy.⁴⁵ However, less is known regarding the polarization state of microglia during epileptogenesis. Benson et al observed an elevated M₁ response during epileptogenesis; however, this was much less pronounced than in the acute and chronic stages of epilepsy. An emerging theoretical strategy to reduce the establishment of epilepsy is to elevate the M₂-activation profile following an insult whilst simultaneously reducing the effect of M₁ microglia.³⁶

In addition to astrocytes and microglia, other cells of innate immunoresponse (eg, monocytes and macrophages) have been ascribed a proepileptogenic role.⁴⁶ In animal models of seizures, microglia and neurons release chemoattractants to exacerbate BBB disruption and recruit monocytes, more specifically CCR2⁺ monocytes, across the BBB. Once infiltrated, they can release proconvulsant cytokines, such as IL1 β , via STAT3 signaling. However, while monocyte infiltration may exacerbate inflammation

and neuronal damage,^{47–49} no effect on seizure severity has been observed in a rodent model of status epilepticus.⁵⁰

Suggesting proinflammatory mediators directly interfere with neuronal function and excitability, cytokines have been reported to modulate the function of glutamate and GABA receptors, eg, IL1 β inhibits GABA-mediated inhibitory transmission and potentiates excitatory *N*-methyl-D-aspartate (NMDA)–receptor-dependent synaptic transmission^{51–53}, alter voltage-gated ion-channel function and expression eg, IL6 increases activity of Na_v1.7, thereby amplifying excitatory inputs on neurons,^{54–57} and modulate presynaptic exocytosis of both glutamate and GABA neurotransmitters.^{58,59} Cytokines increase the amplitude and network synchronicity of neuronal Ca²⁺ currents in neuronal culture via reducing GABAergic input.⁶⁰ Interestingly, IL1 β is able to reduce the anticonvulsant action of midazolam (a benzodiazepine that enhances endogenous GABA activity) in these cultures, suggesting IL1 β may cause the drug resistance seen in patients.⁶⁰ IL1 β 's ability to reduce GABA-neurotransmitter release in hyperexcitable networks in the mouse hippocampus may underlie this pharmacoresistance.⁶¹ Reinforcing this notion, in tissue resected from drug-refractory epileptic patients, cytokines (IL1 β) reduced GABA_A-mediated currents.⁵³ Furthermore, the IL1 type I receptor is closely associated with the NMDA-receptor subtype 2B (NR2B) subunit of the excitatory NMDA receptor,⁶² where IL1 β induces phosphorylation of NR2B. Phosphorylated NR2B induces excitotoxicity, enhances NMDA inward currents by ~45%, and inhibits NMDA outward currents, all promoting the generation of seizure.^{52,63,64} TNF α is also known to reduce inhibitory drive via inducing internalization of GABA_A receptors while concurrently promoting the expression of excitatory α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors.⁶⁵

Demonstrating the therapeutic potential of targeting inflammation in epilepsy, drugs targeting specific inflammatory pathways (eg, TLR4, HMGB1, IL1 β) have provided neuroprotection and disease modification, delaying the onset of epilepsy and suppressing epileptic seizures.^{66–68} Importantly, anti-inflammatory drugs have reached the stage of clinical trials,⁶⁹ which should accelerate their future use in the clinic.

There is now compelling evidence of inflammation impacting on both seizure generation and the development of epilepsy. Consequently, drugs targeting inflammatory

pathways represent a promising new therapeutic strategy to provide disease modification in epilepsy and treat patients not responding to current ASDs.

Animal Models of Epilepsy

The heterologous nature of epilepsy syndromes is reflected in the plethora of experimental models available to mimic both acute seizures (eg, models of status epilepticus) and epilepsy. This includes both in vitro and in vivo approaches, with each having their inherent advantages and limitations. Whereas in vitro models mainly allow the study of molecular mechanisms of neuronal excitability, they cannot completely replicate the complexity of epilepsy and its genesis in a living being, which includes inflammatory processes, changes in the BBB, and complex neuron–glia interactions, though advantages of in vitro models include a higher number of possible replicates within a shorter time frame, with less variability between samples. While a detailed description of animal models of epilepsy is outside of the scope of the present review, we give a short summary of the main in vivo models used to investigate purinergic signaling in epilepsy. For a more comprehensive list of in vitro and in vivo models of seizures and epilepsy please refer to previous reviews published on this subject.^{15,70,71}

In vivo approaches to model seizures and epilepsy include the use of seizure-inducing chemoconvulsants (eg, KA, pilocarpine, or pentylenetetrazol [Ptz]), electrical stimulation (eg, perforant pathway, hippocampus, amygdala), genetic mutations, and injury models (eg, TBI, hyperthermia) with species spanning from flies to primates.^{71,72} To date, however, investigation of the impact of purinergic signaling on seizures and epilepsy has largely been restricted to the use of chemoconvulsants and electrical stimulation in rodent models using models of acute seizures and drug-refractory epilepsy. This primarily includes the glutamate agonist KA, the cholinergic agonist pilocarpine, and the GABA_A-receptor inhibitor Ptz, which will be described in more detail.

In vivo approaches modeling acute seizures include the Ptz model, widely used in the screening of anticonvulsant drugs. Here, intraperitoneal injections of Ptz lead to a single, brief, nondamaging seizure.⁷¹ Other models used to determine the impact of interfering with purinergic signaling during acute seizures include the maximal electroshock model and the 6 Hz psychomotor-seizure test, which has also been described as a model of pharmacoresistant partial seizures. Serial

administrations of subconvulsive doses of Ptz over a number of days (Ptz kindling model) are used to mimic the process of epileptogenesis and determine antiepileptic effects.⁷¹ Another widely used model of epileptogenesis is the amygdala kindling model, which consists in the repeated application of electrical stimuli via a depth electrode in the basolateral amygdala of rodents and induces permanently enhanced seizure susceptibility and other enduring brain alterations that are similar to those occurring in human TLE.⁷¹

KA- and pilocarpine-treated mice are usually the models of choice to study effects of drugs targeting the purinergic system on status epilepticus and TLE pathology. Systemically injected pilocarpine mimics certain aspects of TLE pathology, including a period of status epilepticus with subsequent development of spontaneous recurrent seizures and a pattern of hippocampal damage and sclerosis, particularly evident in the CA1 and hilar regions of the hippocampus.⁷³ This model is, however, associated with high mortality, peripheral immunoresponses prior to the onset of status epilepticus, including white blood-cell activation with consequent increase in IL1 β serum levels and BBB damage,⁷⁴ and high interanimal variability in pathology and neuronal injury, which most likely reflects a mixture of an ischemic and excitotoxic insult.^{71,73} Similarly to systemic pilocarpine, systemic injections of KA also cause prolonged seizures and the development of epilepsy with high variability in hippocampal pathology in mice and variable epilepsy development.⁷⁵ In contrast, intracerebral injections of KA (eg, intrahippocampal and intraamygdala [IA]), seem to be associated with less mortality, more consistent and reliable focal and unilateral hippocampal lesions, and spontaneous seizure onset and refractoriness to ASDs.^{75,76} Of note, a recent study demonstrated that intracerebral (ie, IA and intrahippocampal) KA causes similar changes in hippocampal gene expression to human TLE.⁷⁷ However, each of these models replicates slightly different aspects of seizure-induced pathologies, which is important to keep in mind when interpreting data and potential conflicting results among studies.^{15,71}

Purinergic Signaling via P2 Receptor — Overview

ATP Release

ATP acting as an intercellular signaling molecule was first described by Geoffrey Burnstock in 1972.⁷⁸ It is well

recognized that ATP functions as either a sole transmitter or co-transmitter in both the peripheral nervous system and the CNS.⁷⁹ Here, ATP can act as a fast, excitatory neurotransmitter or as a neuromodulator, and is involved in a plethora of short- and long-term physiological and pathological processes, including inflammation, cellular survival, proliferation, cellular differentiation, and synaptic plasticity.^{17,80,81} Under physiological conditions, ATP is usually present at micromolar concentrations in the extracellular space; however, under pathological conditions (eg, inflammation, hyperexcitability, and cell death) extracellular ATP levels can reach the millimolar range.^{79,81,82} As such, ATP acts as an endogenous danger signal, known as a damage-associated molecular pattern (DAMP).⁸³ ATP can enter the extracellular space passively by crossing the compromised membranes of damaged and dying cells⁷⁹ or be actively released from different cell types, including neurons, astrocytes, microglia, and endothelial cells.^{79,84} ATP-release mechanisms include exocytosis of secretory granules, vesicular transport involving the vesicular nucleotide transporter (VNUT), and membrane channels such as ABC transporters, pannexins (eg, pannexin 1), connexins, and via purinergic receptors themselves (eg, P2X₇ receptor).^{79,84} Once released into the extracellular space, ATP is rapidly metabolized by ectonucleotidases, including the ectonucleoside triphosphate diphosphohydrolase family, the ectonucleotide pyrophosphatase/phosphodiesterase family, and alkaline phosphatases, into such breakdown products as ADP and adenosine, which are also important neurotransmitters and neuromodulators in their own right.^{17,18,85}

Purinergic P2 Receptors

Activated by adenine and uridine nucleotides, purinergic P2 receptors are further subdivided into two families according to their sequence homology, pharmacology, and mechanism of action. This includes the ionotropic P2X receptors and metabotropic P2Y receptors, both expressed throughout the CNS and functional on both neurons and glial cells.⁸⁶

To date, seven mammalian subunits of the P2X-receptor family have been cloned (P2X₁₋₇), ranging in length from 379 (P2X₆) to 595 (P2X₇) amino acids.⁸⁷ Each subunit comprises two transmembrane domains: a large extracellular loop and an intracellular N- and C-terminus.⁸⁸ P2X receptors can form either functional homo- or heterotrimers, which upon binding to their endogenous agonist ATP allow the passage of small cations, such as Na⁺, Ca²⁺,

and K^+ , depolarizing the cell membrane. All P2X receptors are sensitive to ATP; however, with differing levels of affinity ($P2X_1 > P2X_3 > P2X_2 > P2X_4, P2X_5 > P2X_6 > P2X_7$).⁸⁹ P2X receptors are activated within milliseconds, and with the exception of P2X₇, display fast desensitization ($P2X_3 > P2X_1 > P2X_2 > P2X_4 > P2X_7$).^{88,90} While the contribution of P2X receptors to fast synaptic transmission is well-established,⁹¹ they have also been implicated in several other cellular processes, such as proliferation, differentiation, maturation, survival, cell adhesion, migration, and inflammation.⁹² Among the P2X receptors, due to its unique structural and functional characteristics, P2X₇ has attracted particular attention as a target for brain diseases, including epilepsy.⁹³ P2X₇ has a relatively low affinity for ATP ($EC_{50} \geq 100 \mu\text{M}$, activation threshold 0.3–0.5 mM, which has been reported decreased during inflammation [0.05–0.1 mM])⁹⁴, suggesting that P2X₇ activation occurs mainly under pathological conditions of high ATP release (which may occur during a seizure). It also has slow desensitization dynamics, the ability to permeabilize the cell membrane to molecules up to 900 Da in size, and is a key driver of inflammation via activation of the NLRP3 inflammasome.⁹⁴ Moreover, suggesting a direct impact on neurotransmission, P2X₇ has been shown to modulate both uptake and release of GABA and glutamate during epilepsy.^{95,96} P2X₇ is highly expressed on cells of the immune system, including microglia. P2X₇ expression has also been reported on oligodendrocytes and endothelial cells.^{97,98} Its expression and function with regard to astrocytes and neurons remains, however, controversial.^{99,100}

The metabotropic P2Y-receptor family consists of eight G protein-coupled receptors — $P2Y_{1,2,4,6,11-14}$.^{17,101} P2Y receptors can be activated by adenosine or uridine nucleotides: ATP ($P2Y_2, P2Y_{11}$), ADP ($P2Y_1, P2Y_{12}, P2Y_{13}$), uridine-5'-triphosphate (UTP) ($P2Y_2, P2Y_4$), UDP ($P2Y_6, P2Y_{14}$), and UDP-glucose ($P2Y_{14}$). P2Y receptors have an extracellular amino terminus, intracellular carboxyl terminus, and seven transmembrane-spanning motifs.¹⁰² P2Y receptors can also be grouped according to their coupling to specific G proteins: $P2Y_1, P2Y_2, P2Y_4, P2Y_6$, and $P2Y_{11}$ receptors are coupled with G_q/G_{11} proteins, stimulating phospholipase C and leading to the release of calcium from intracellular stores and the activation of PKC. $P2Y_{11}$ can also couple with G_s , stimulating adenylate cyclase and increasing the production of cyclic adenosine monophosphate (cAMP).¹⁰¹ $P2Y_{12}, P2Y_{13}$, and $P2Y_{14}$ receptors are coupled to G_i proteins, inhibiting adenylate

cyclase and thereby decreasing cAMP production.¹⁰¹ Similarly to P2X receptors, P2Y receptors have been described to be expressed and functional on all brain cells and implicated in several cellular functions and pathological processes relevant to epileptogenesis and epilepsy, including synaptic reorganization, changes in neurotransmitter release, neurodegeneration, and neuroinflammation.^{103,104}

Purinergic Receptors and Neuroinflammation

P2X Receptors and Neuroinflammation

With many inflammatory pathologies resulting in altered purinergic signaling and with ATP being one of the primary DAMPs to be released at sites of inflammation, the close association of P2 receptors with inflammatory signaling comes as no surprise.¹⁰⁵ This positive-feedback loop created between inflammation and ATP release creates continuous neuroinflammation in the brain.⁸¹

All P2X-receptor subtypes have some degree of expression on immune-system cells of the body; however, within the brain, only *P2rx1, P2rx4, P2rx6*, and *P2rx7* mRNA is predominantly expressed in mouse microglia.^{106,107} Microglia *P2rx3* and *P2rx5* expression is observed at much lower levels, with *P2rx5* expression restricted to neonatal animals and no evidence of microglia *P2rx2* expression at any age analyzed.¹⁰⁶ All P2X receptors aside from P2X₆ are expressed in astrocytes.¹⁰⁸ Of all P2X receptors, P2X₄ and P2X₇ possess the largest body of evidence of a well-defined functional role in inflammation. P2X₁ and P2X₂ inflammatory function within the brain is currently unknown, and their association with inflammation stems from their expression changing at sites of inflammation observed in ischemia, axotomy, stab-wound injuries, and cerebellar lesions.^{109–112} Despite their expression in glial cells, an immunomodulatory function of P2X₃, P2X₅, and P2X₆ within the brain is currently unknown.

Of P2X₇'s various roles, its proinflammatory function has been widely explored, with many touting it as the gatekeeper of inflammation.¹¹³ P2X₇ is known to be the most potent contributor to the release of proinflammatory cytokines,⁹⁴ including but not limited to IL18,¹¹⁴ IL6,¹¹⁵ IL1 β , and IL1 α .¹¹⁶ With varying affinity of ATP required to activate P2X₄ or P2X₇, they work in tandem to respond to stimuli over a wide range of ATP concentrations.¹¹⁷ In many disease pathologies, including seizures, the

proinflammatory cytokines IL1 β , IL18, and TNF α are released. It is well known that pharmacological inhibition or genetic knockout of P2X₄ or P2X₇ can hinder the release of proinflammatory cytokines, such as IL1 β .^{118–122} Proinflammatory and proconvulsive IL1 β release is a two-signal process: priming (an inflammatory signal acts upon TLR4 to synthesise proIL1 β) and activation (activation of the NLRP3 inflammasome and caspase1 is required to cleave pro-IL1 β and for subsequent secretion of IL1 β). P2X₇ is important for NLRP3 activation, with P2X₇'s pore-forming ability described as necessary to create a drop of local intracellular K⁺ concentration needed for activation of the inflammasome without compromising global cellular K⁺ homeostasis.^{123,124} P2X₄ also possesses the ability to form a large conductance pore that can facilitate inflammasome activation, with P2X₄-knockout mice showing impaired inflammasome activation and subsequent IL1 β release following a spinal cord injury.¹¹⁸ However, P2X₄ may act as the initial trigger, due to a higher affinity for ATP, and then via working with pannexin 1 to release ATP, with P2X₇ amplifying the signal and elevating inflammation within the brain.⁹² P2X₇ stimulation can also promote the activation of microglia and elevate their proliferation.¹²⁵ Furthermore, P2X₇ may contribute to further release of DAMPs, which act to potentiate inflammation via facilitating pyroptosis.¹²⁶ Pyroptosis, a form of inflammatory programmed cell death, is downstream of the P2X₇-NLRP3 axis, where the permeability of the cell membrane increases, inducing swelling and subsequent cell death. P2X₇ activation to induce pore formation can also contribute to cell death and subsequent DAMP release via rearrangements of the cell cytoskeleton.^{127,128} Interestingly, P2X₄ pore formation does not lead to cell death.¹²⁹ The release of DAMPs is a fundamental component of inflammation, and can further act upon P2X₇ to potentiate inflammation. Furthermore, P2X₇ activation can stimulate production of cytokine precursors via upregulation of both the NF κ B¹³⁰ and NFAT¹³¹ proinflammatory transcription factors.

Aside from P2X₇'s defined role in microglia, its astrocytic expression has also been reported. Functional astrocytic P2X₇ has been observed in cortical brain slices¹³² and astrocytic cultures, where it induces cell death and the release of radical oxygen species and proinflammatory IL6.¹³³ Furthermore, human primary astrocytes secrete IL1 β mediated via the P2X₇-NLRP2 signaling cascade in tandem with pannexin 1 in a manner similar to what is seen in microglia.¹³⁴

With P2X₄ higher ATP affinity than P2X₇, P2X₄ may have a more physiological role in inflammation. Upregulation of P2X₄ is observed in many disease models that are associated with inflammation.^{135–137} P2X₄ immunoreactivity has been observed in astrocytes,¹³⁸ however, conflicting reports from electrophysiological experiments suggest no functional role of astrocytic P2X₄.¹³⁹ P2X₄ is widely considered to drive microglia motility, process extension, activation, and recruitment to the site of damage, with ATP acting as a chemotactic factor for P2X₄⁺ microglia.^{140,141} In fact, P2X₄ activation drives microglia recruitment and motility via the PI3K–Akt pathway.^{140,142} P2X₄ expression is mainly restricted to activated microglia, with P2X₄ localized in intracellular lysosomal compartments of microglia and trafficked to the cell surface under inflammatory conditions.¹⁴³ Microglia are shifted to an activated state in response to noxious stimuli and governed via the transcription factors IRF8 and IRF5 directly inducing expression of the *P2rx4* gene.¹⁴⁴ Furthermore, P2X₄ also plays an anti-inflammatory role, which may occur following its acute proinflammatory function. P2X₄ is associated with mediating microglia cell death as a physiological response, with suggestions that the repopulating microglia are regenerative.¹⁴⁵ Following a spinal cord injury, microglia also release brain-derived neurotrophic factor¹⁴⁶ mediated via P2X₄ to promote regeneration.¹⁴⁷ In multiple sclerosis models, activation of microglia P2X₄ promotes an anti-inflammatory phenotype and facilitates remyelination.¹⁴⁸

P2Y Receptors and Neuroinflammation

P2Y receptors have been repeatedly implicated in neuroinflammation; however, their exact role is not fully understood and seems to be dependent on downstream-signaling pathways.¹⁴⁹ As with P2X receptors, many P2Y receptors are expressed on inflammatory cells. In isolated microglia, mRNA of all known rodent P2Y receptors can be detected.¹⁰⁶ Among P2Y receptors, P2Y₁ is one of the predominant targets of ATP in mediating danger signals in the brain during, eg, ischemia¹⁵⁰ or trauma.¹⁵¹ As such, there is a plethora of evidence of P2Y₁ inflammatory action. Glial P2Y₁ expression is known to regulate astroglial proliferation and astrogliosis.¹¹² In a model of cerebral ischemia, antagonism of P2Y₁ reduced the astrocytic transcription of cytokines (eg, TNF α) and chemokines.¹⁵² Overexpression of P2Y₁ in astrocytes can lead to the propagation of intracellular Ca²⁺ waves, which is responsible for the release of ATP and cytokines.^{152,153} Oxidative

stress potentiates the activation of P2Y₁ and the release of IL6.¹⁵⁴ Interestingly, blockade of P2Y₁ increases astrocytic response and is neuroprotective in TBI.⁴¹ Reinforcing tight cross-interaction of glial cells, microglia-derived cytokines have been demonstrated to downregulate P2Y₁ expression and function on astrocytes, promoting formation of glial scars and increasing astrocyte process extension.⁴¹ Interestingly P2Y₁ and P2Y₁₂ activation can induce astrocyte proliferation in vitro.¹⁵⁵ In the same study, activation of P2Y₁₄ had no influence on proliferation. Though less explored, an anti-inflammatory role of P2Y₁ through the release of IL6 has also been suggested.¹⁵⁶

P2Y₁₂ is heavily implicated in mediating inflammatory responses. In fact, P2Y₁₂ immunoreactivity is widely accepted as a marker of resting microglia,¹⁵⁷ ie, microglia in nonpathological conditions. Similarly to P2X₄, the primary role of P2Y₁₂ activation is to induce reactivity of microglia, elicit microglia-process outgrowth, and stimulate their migratory movement via PI3k/PLC signaling to sites of inflammation.^{140,158–160} As such, P2Y₁₂ may promote neuronal damage in brain pathologies by guiding the inflammatory response to the site of injury.^{161,162} Interestingly, P2Y₁₂ expression is downregulated during the phenotypic M₁/M₂ shift,¹⁶³ reinforcing its role in resting microglia. Additionally, microglial P2Y₁₂ and P2Y₁₃ are involved in preventing astroglial proliferation mediated by ADPβS in cocultures of astrocytes and microglia.¹⁶⁴ P2Y₁₂ and P2Y₁₃ are integrally linked, with P2Y₁₃ acting to potentiate the chemotaxis response of P2Y₁₂,¹⁶⁵ and have been widely reported to be expressed in microglia and astrocytes.^{106,166–168} As reported for the other P2Y receptors, P2Y₁₃ activation also increases intracellular Ca²⁺ in glial cells,^{167–169} contributing to the release of several proinflammatory cytokines (IL1β, IL6, and TNFα) from microglia cells.¹⁷⁰ Furthermore, a recent study demonstrated that joint activation of P2Y₁ and P2Y₁₃ induce the retraction of microglial processes in epileptic and peritumoral human tissue.¹⁷¹

Much less is known about the remaining P2Y receptors in inflammation. P2Y₆ has been implicated in microglia activation, leading to activation of inflammation signaling pathways.¹⁷² P2Y₆ stimulation is also involved in inducing microglia phagocytosis of cell debris, which is beneficial to neuroregeneration.¹⁷² Interestingly, P2Y₆ activation can inhibit P2X₄ functioning in microglia, which results in reduced motility.¹⁷³ P2Y₂ plays a neuroprotective role via upregulation of antiapoptotic genes in astrocytes¹⁷⁴

and promoting migration of astrocytes.¹⁷⁵ Furthermore, P2Y₂ mediates amyloid β-protein phagocytosis by microglia cells, suggesting a neuroprotective role in Alzheimer's disease.¹⁷⁶ Unfortunately, current understanding of P2Y₄'s functional role in neuroinflammation is lacking, despite it being known to be expressed in microglia.¹⁰⁶

Purinergic Signaling During Seizures and Epilepsy

ATP Release During Seizures and Epilepsy

Initial evidence suggesting a link between increased extracellular ATP levels and hyperexcitability was provided by two studies, one showing increased extracellular ATP concentrations in hippocampal brain slices of a seizure-prone strain of mice (inbred DBA/2 [D2] mice),¹⁷⁷ and the other evidencing motor seizures caused by microinjections of ATP into the prepiriform cortex of rats.¹⁷⁸ In vitro evidence suggesting that ATP can be released during increased neuronal activity stems from a study using depolarizing high K⁺ concentrations in slices of rat hippocampus.¹⁷⁹ Further in vitro evidence was provided by Lopartar et al, showing increased extracellular ATP mediated via pannexin 1 in rat hippocampal slices treated with the glutamate agonist (*S*)-3,5-dihydroxyphenylglycine.¹⁸⁰ In contrast to these findings, other methods, such as stimulation of the Schaffer collateral, did not increase extracellular ATP concentrations.¹⁸¹ In agreement with pannexin 1 activation contributing to extracellular ATP increases during seizures, a recent study using resected tissue from patients with epilepsy showed that extracellular ATP increased 80% during high K⁺-induced ictal discharge, which was blocked by pharmacological inhibition of pannexin 1.¹⁸² Of note, blocking pannexin 1 channels provides potent anticonvulsive effects in a mouse model of KA-induced seizures, suggesting a proconvulsant function of extracellular released ATP.¹⁸² In vivo evidence showing increased extracellular ATP during seizures/epilepsy was provided by Dona et al using a rat model of epilepsy induced by pilocarpine administration (360 mg/kg, IP). The authors showed that status epilepticus led to an increase of the ATP metabolites ADP, AMP, and adenosine, but not ATP. On the other hand, the same ATP metabolites were found to be decreased during epilepsy, though increased significantly following an epileptic seizure. This also included an approximately fourfold increase in extracellular ATP levels.¹⁸³ Further suggesting a proconvulsant function of extracellular adenosine

nucleotides, injection of ATP, the ATP analogue 2,3-*O*-(4-benzoylbenzoyl) ATP (BzATP) or ADP, into the lateral ventricle of mice caused high-amplitude, high-frequency polyspiking on electroencephalography (ATP)¹⁸⁴ and increased seizure severity during KA (0.3 µg, IA)-induced status epilepticus (BzATP and ADP).^{119,185} In contrast to a proconvulsant function of extracellular ATP, injection of uridine-5'-triphosphate into the lateral ventricle of mice reduced seizure severity during IA KA-induced status epilepticus.¹⁸⁵

P2-Receptor Expression Following Seizures and During Epilepsy

P2 receptors, including both P2X and P2Y receptors, are widely distributed throughout the brain, where they are expressed and functional on both neurons and glial cells, contributing to a plethora of physiological and pathological processes ranging from neurotransmission and neurogenesis to glial cell communication.^{93,104} While we still lack a detailed description of potential expression changes during epilepsy for most of the P2 receptors, this has changed in recent years, in particular for the P2X₇ and P2Y₁ subtypes.

Expression changes for P2X receptors following seizures (eg, status epilepticus) or during epilepsy have been reported for P2X₂, P2X₄, and P2X₇. No changes have been found so far in expression of the remaining P2X receptors (ie, P2X₁, P2X₃, P2X₅, and P2X₆).¹¹⁹ While P2X₂ has been shown to be downregulated following KA (3 µg, IA),¹¹⁹ P2X₄ expression increases in the hippocampus following systemic KA injections (8–22 mg/kg, IP),^{142,186} but not IA KA- or pilocarpine (360 mg/kg, IP)-induced status epilepticus.^{119,187} In contrast, P2X₄ expression is decreased in the hippocampi of pilocarpine (360 mg/kg, IP)-treated epileptic rats.¹⁸⁷ As mentioned previously, among the P2X-receptor family, most data are available for P2X₇. P2X₇ expression has been reported to be upregulated in mice following intraperitoneal KA (hippocampus),¹⁸⁶ IA KA-induced status epilepticus in mice (hippocampus, cortex, striatum, thalamus, and cerebellum),^{119,188,189} and pilocarpine-induced status epilepticus in rats (hippocampus).¹⁸⁷ Studies have suggested P2X₇ to be upregulated on neurons post-status epilepticus using immunohistochemical approaches, detecting P2X₇ at glutamatergic nerve terminals,¹⁸⁷ and a soluble EGFP BAC transgenic P2X₇ mouse model, where EGFP is under the transcriptional control of the *P2rx7*

promoter.^{119,188} Others, however, have failed to detect neuronal expression of P2X₇ using P2X₇ reporter mice where P2X₇ is fused to EGFP.^{189,190} Of note, a recent study comparing both transgenic P2X₇ reporter mouse lines observed aberrant P2X₇ expression and increased P2X₄ expression in the soluble EGFP BAC transgenic P2X₇ mouse model, questioning the validity of this reporter mouse.¹⁹¹ P2X₇ expression has also been shown on oligodendrocytes post-status epilepticus; however, its expression was not detected on astrocytes.^{119,189} During epilepsy, P2X₇ expression has been shown to be increased in the hippocampus and cortex in both experimental rodent models and patients with TLE.^{95,187,188,192} Regarding its cell type-specific expression using immunohistochemistry, P2X₇ expression has been shown to increase on microglia, mossy fibers, and glutamatergic nerve terminals of epileptic pilocarpine (360–380 mg/kg, IP)-treated rats.^{187,193} A mainly microglial and neuronal P2X₇ induction during epilepsy was confirmed by using the soluble EGFP BAC transgenic P2X₇ mouse model.^{188,192} Regarding neuronal expression, Barros-Barbosa found P2X₇ to be increased in neocortical nerve terminals of patients with epilepsy.⁹⁵ However, similarly to status epilepticus, neuronal expression of P2X₇ receptors during epilepsy could not be confirmed in a more recent study using the P2X₇ reporter mouse where P2X₇ is fused to EGFP.¹⁸⁹ P2X₇ expression has also been found on oligodendrocytes, while absent on astrocytes during epilepsy in mice.¹⁸⁹ Based on these results, whereas there is broad consensus on P2X₇ receptors being increased on microglia following status epilepticus and during epilepsy, whether P2X₇ receptors increase on neurons is still a matter of debate.

Changes in the transcription and expression of the P2Y receptors have been reported in different brain structures, including the hippocampus and cortex, using different mouse models, including KA (3 µg, IA and 18–22 mg/kg, IP)^{185, 186} and pilocarpine (340 mg/kg, IP),¹⁸⁵ pilocarpine (340 mg/kg, IP),¹⁸⁵ and KA (18–22 mg/kg, IP)-induced status epilepticus mouse model.¹⁸⁶ In the IP KA-induced status epilepticus mouse model, *P2ry6*, *P2ry12*, and *P2ry13* mRNA transcripts were increased in the hippocampus.¹⁸⁶ A more detailed study using the IA KA-induced status epilepticus mouse model, identified an interesting pattern between changes in P2Y-receptor transcript levels and the two main agonists of these receptors (adenosine and uridine nucleotides). While transcript levels of adenosine-sensitive receptors were decreased (*P2ry1*, *P2ry12*, and *P2ry13*), transcript levels of uridine-

sensitive receptors were increased (*P2ry2*, *P2ry4*, and *P2ry6*) in the hippocampus. At the protein level, hippocampal P2Y receptors coupled with G_q were increased (P2Y₁, P2Y₂, P2Y₄, and P2Y₆) and P2Y receptors coupled with G_i downregulated or unchanged (P2Y₁₂, P2Y₁₃, P2Y₁₄).¹⁸⁵ In contrast, P2Y-receptor expression was mainly upregulated in the cortex post–status epilepticus¹⁹⁴ and in the hippocampi of epileptic mice and patients with drug-refractory epilepsy.^{185,195} Regarding cell type-specific expression, while P2Y₁ has been shown to be expressed in microglia following IA KA-induced status epilepticus,¹⁹⁶ P2Y₂ and P2Y₄ have been shown to be mainly expressed in astrocytes in brain tissue from patients with intractable epilepsy.¹⁹⁵

Targeting of P2X Receptors During Seizures and Epilepsy

As stated before, among the P2X receptors, though not the only one investigated, P2X₇ has attracted most attention as a potential therapeutic target for seizure suppression and treatment of epilepsy.⁹³

Treatment with P2X₇ antagonists has been shown to provide anticonvulsive effects in several experimental models of status epilepticus and acute seizures. P2X₇ antagonists (A438079 and Brilliant Blue G [BBG]) reduce both seizure severity during status epilepticus and resulting neurodegeneration in the KA (3 µg, IA) mouse model (pre- and posttreatment regime).^{119,188} Using the same IA KA mouse model, seizure suppression is also evident in P2X₇ Knockout mice and via P2X₇-targeting antibodies delivered into the lateral ventricle.¹¹⁹ Interestingly, P2X₇ antagonists potentiated effects of the anticonvulsant lorazepam, suggesting their potential as adjunctive treatment for pharmacoresistant status epilepticus.¹¹⁹ Confirming an anticonvulsive effect of P2X₇ antagonism, a more recent study using three P2X₇ antagonists (BBG, A438079, and A740003) showed reduced seizure severity during coriaria lactone (40 mg/kg, IP)-induced status epilepticus in rats.¹⁹⁷ Suggesting anticonvulsive effects of P2X₇ antagonism not being dependent on developmental stage, P2X₇ antagonism also reduces seizures in a KA (2 µg, ICV) rat model of early-life seizures using the P2X₇ antagonist A438079¹⁹⁸ and in a model of hypoxia-induced seizures (5% O₂ for 15 minutes) in 7-day-old mouse pups using two P2X₇ antagonists (A438079 and JNJ47965567).¹²¹ The effects of P2X₇ antagonism on neonatal seizures are even more exciting, as commonly used ASDs, such as phenobarbital, show only

limited effects in suppressing seizures in infants.^{199,200} Moreover, the use of commonly used ASDs is undesirable at a time when the brain is developing rapidly and particularly vulnerable to any toxic effects. Therefore, the targeting of P2X₇, thought to be mainly activated during pathological release of ATP,²⁰¹ may represent a promising novel treatment strategy for neonates.²⁰² Other studies, however, have shown only a moderate or no anticonvulsant effect of P2X₇ antagonism. Using the maximal electroshock seizure-threshold test and the Ptz (87 mg/kg, IP) seizure-threshold test in mice, Fischer et al showed no anticonvulsant effects provided by P2X₇ antagonism using four antagonists (JNJ47965567, AFC5128, BBG, and tanshinone IIA sulfonate). However, further strengthening the potential of P2X₇ antagonism as adjunctive treatment, P2X₇ antagonists potentiated anticonvulsant effects of carbamazepine in the maximal electroshock-seizure test.²⁰³ In line with this, a subsequent study by Nieczym et al²⁰⁴ found only a weak anticonvulsant effect of the P2X₇ antagonist BBG on Ptz (1% Ptz 2 mL/min, IV) seizure threshold, maximal electroshock-seizure threshold, and 6 Hz psychomotor seizure-threshold tests. Moreover, no effect of P2X₇ antagonism on seizures was observed in WAG/Rij rats, a model of genetic absence epilepsy.¹⁰ Suggesting a proconvulsive function of the P2X₇ receptor, Kim and Kang reported exacerbated seizure severity in P2X₇-KO mice using a pilocarpine (150, 175, 200, 225, or 250 mg/kg, IP) mouse model.²⁰⁵ In addition, research by the same group using the pilocarpine (380 mg/kg, IP) rat model showed that P2X₇ antagonism (oxidized ATP [OxATP] and BBG) protected against astroglial cell death,²⁰⁶ reduced the infiltration of neutrophils into the frontoparietal cortex,²⁰⁷ and increased status epilepticus-induced hippocampal neurodegeneration.²⁰⁸ Suggesting, however, a model-specific effect, research by the same group showed that P2X₇ deficiency did not alter seizure severity in the systemic KA (25 mg/kg, IP) mouse model or the picrotoxin (5 mg/kg, IP) mouse model.²⁰⁵ As such, while P2X₇ antagonism impacts on seizure severity in models of status epilepticus, these effects seem to be minor or absent during acute nondamaging seizures.

Suggesting antiepileptogenic potential of P2X₇ antagonism, Soni et al showed that the P2X₇ antagonist BBG decreased mean kindling score and restored cognitive deficits and motor coordination using the Ptz (30 mg/kg, IP) kindling model in rats.²⁰⁹ Similar results were obtained in a more recent study by Fischer et al using the P2X₇ antagonists JNJ47965567, AFC5128, and BBG.²⁰³ In the

same vein, Amorim et al showed that injection of a P2X₇-targeting siRNA post-status epilepticus delayed the emergence of the first epileptic seizure and reduced the frequency and severity of seizures in the pilocarpine (370 mg/kg, IP) rat model.²¹⁰ Interestingly, a recent study by Jamali-Raeufy et al showed that the P2X₇ antagonist BBG and linagliptin, which blocks the enzyme dipeptidyl peptidase-4 and is used for the treatment of diabetes, reduced seizure severity and neuronal cell death more efficiently when given in combination in a KA (4 µg, intrahippocampal) rat model. Epileptic rats treated with both inhibitors also showed improved spatial learning at a later stage, probably caused by fewer seizures during the initial insult.²¹¹ In agreement with P2X₇ antagonism impacting on epilepsy-related comorbidities, treatment of pilocarpine-induced epileptic rats with the P2X₇ antagonist BBG promoted antidepressive and anti-anxiety effects. BBG did not, however, affect the development of spontaneous recurrent seizures, but inhibited microglia activation following pilocarpine (single dose of 30 mg/kg and repeated doses of 10 mg/kg, IP)-induced status epilepticus in rats.²¹² However, similarly to status epilepticus, treatment with the P2X₇ antagonists AZ10606120 and BBG following pilocarpine (300 mg/kg, IP)-induced status epilepticus in mice resulted in the development of a more severe epileptic phenotype.²¹³

Suggesting P2X₇ antagonism as treatment for established epilepsy, Amhaoul et al showed that treatment with the P2X₇ antagonist JNJ47965567 during epilepsy reduced seizure severity, though without affecting the total number of seizures. In this study, epileptic rats were treated for 1 week 3 months after KA (22.2±2.02 mg/kg, IP)-induced status epilepticus.²¹⁴ The antiepileptic potential of P2X₇ antagonism was further demonstrated in a study carried out by Jimenez-Pacheco et al. In this study, mice subjected to IA KA (0.3 µg) were treated with the P2X₇ antagonist JNJ47965567 for 5 days, starting at 10 days post-status epilepticus. In contrast to the previous study, P2X₇ antagonism reduced the total number of seizures during treatment, and remarkably, during an additional recording period following treatment withdrawal, suggesting disease-modifying potential.¹⁹² Therefore, in contrast to acute seizures, P2X₇ seems to play a more prominent role during epilepsy once pathological processes have been initiated.

While compelling evidence has now demonstrated a role for P2X₇ mediating hyperexcitability in the brain, the mechanisms by which it modulates seizure severity

are still to be determined. While affecting numerous pathways in the CNS, the most likely explanation is that P2X₇ effects are mediated, at least in part, via their prominent role in driving inflammatory processes. P2X₇ is an important driver of microglial activation and proliferation,¹²⁵ and has been shown to be highly expressed under physiological conditions and during epilepsy.^{189,190} Suggesting a functional role of P2X₇ regulating inflammatory processes during seizures and epilepsy, blocking of P2X₇ during status epilepticus leads to decreased hippocampal IL1β levels.¹¹⁹ Moreover, targeting of P2X₇ receptors during epilepsy reduces both microgliosis and astrogliosis.¹⁹² Although not shown to be increased on astrocytes following seizures or during epilepsy, astrocytes can also be activated via P2X₇.²¹⁵ As outlined before, astrocytes can reduce seizure thresholds via various mechanisms (eg, dysregulation of extracellular ionic balance, impaired neurotransmitter reuptake, release of proinflammatory cytokines and purines, eg, ATP, and removal of extracellular adenosine via ADK).^{27,28,216,217} Interestingly, P2X₇ antagonism has been shown to protect against astrocyte death following pilocarpine (380 mg/kg, IP)-induced status epilepticus, possibly contributing to its proconvulsive function in this model.²⁰⁶ Further in line with P2X₇ impacting on astrocytes, P2X₇-deficient mice subjected to KA (25 mg/kg, IP) show decreased astroglial autophagy through the regulation of FAK- and PHLPP1/2-mediated Akt-S473 phosphorylation.²¹⁸ P2X₇ has also been shown to promote microglial activation and astroglial apoptosis via PDI-mediated redox/S-nitrosylation following pilocarpine (380 mg/kg, IP)-induced status epilepticus in rats.²¹⁹ It is, however, important to keep in mind that while suppressing inflammatory pathways is the most likely explanation, P2X₇ is involved in a myriad of different effects in pathological conditions, including BBB disruption, changes in neurotransmitter levels, synaptic reorganization, and neurogenesis, to name just a few.²²⁰ Of note, suggesting P2X₇ activation modulating extracellular levels of the neurotransmitters GABA and glutamate, Barros-Barbosa et al demonstrated that by using isolated nerve terminals of the neocortex from patients with epilepsy, P2X₇ antagonism prevented the uptake of GABA more efficiently in tissue from epileptic patients, thereby potentially increasing GABAergic rundown.⁹⁵ Further supporting a role of P2X₇ receptors in the control of neurotransmitter levels in epilepsy, a more recent study observed that P2X₇ modulated both GABA and glutamate release depending on the availability of extracellular Ca²⁺.⁹⁶

The only other P2X receptors for which a functional role has been investigated in the setting of seizures and epilepsy are P2X₃ and P2X₄. In the case of P2X₄, Ulmann et al showed that despite experiencing no changes in seizure severity during status epilepticus, P2X₄-deficient mice were partially protected from seizure-induced neurodegeneration following KA (8–22 mg/kg, IP)-induced status epilepticus, possibly via regulating the activation of microglia.¹⁴² As for P2X₃, Xia et al showed that treatment with the P2X₃ NF110 reduced mean kindling scores and improved other pathological parameters, such as memory deficits, motor activity, neuronal damage, and hippocampal inflammation, in the Ptz (30 mg/kg, IP)-induced kindling rat model.²²¹

Targeting of P2Y Receptors During Seizures and Epilepsy

While P2X receptors have attracted most attention as possible drug targets for epilepsy, increasing evidence also suggests a role of the metabotropic P2Y receptors,^{11,222} with P2Y₁ and P2Y₁₂ being the most studied.²²² Evidence suggesting a role for P2Y₁₂ during status epilepticus stems from a study using P2Y₁₂-knock-out mice. Using the KA (18–22 mg/kg, IP) mouse model of status epilepticus, Eyo et al found that P2Y₁₂-deficient mice showed a more severe seizure phenotype and reduced microglial process extension in the hippocampus.¹⁶⁰ They suggested that neuronal NMDA-receptor activation contributes to an influx of Ca²⁺, which increased ATP release from neurons to activate microglial P2Y₁₂, potentiating the extension of microglia processes.¹⁶⁰ In line with P2Y₁₂ regulating microglial processes, Avignone et al also showed an increase in microglia motility following treatment with the P2Y₁₂ agonist 2-Me-ADP in a mouse model of KA (15 and 5 mg/kg, IP)-induced status epilepticus.²²³ In a more recent study, using resected hippocampal tissue from TLE patients, Milior et al showed that while low doses of ADP induced microglial process extension via blocking of P2Y₁₂, high doses of ADP caused microglial process retraction and membrane ruffling, which was blocked by the coapplication of antagonists against P2Y₁ and P2Y₁₃.¹⁷¹ Moreover, microglial P2Y₁₂ has been suggested to contribute to status epilepticus-induced aberrant neurogenesis and increased immature neuronal projections.²²⁴

Initial evidence suggesting a role for P2Y₁ during seizures showed that P2Y₁ antagonism (MRS2179)

reduced neuronal cell death following KA (10 mg/kg, IP)-induced status epilepticus in rats, though without affecting seizure severity.²²⁵ In contrast, a more recent study published by our group showed that P2Y₁-deficient mice subjected to IA KA (3 µg) presented a lower seizure threshold, experiencing more severe seizures during status epilepticus and increased seizure-induced neurodegeneration in the hippocampus.¹⁹⁶ In line with genetic deletion of P2Y₁, treatment with the P2Y₁ antagonist MRS2500 prior to IA KA exacerbated seizure severity during status epilepticus and resulting neurodegeneration. Moreover, mice pretreated with the P2Y₁ agonist MRS2365 had less severe seizures during status epilepticus and less seizure-induced neurodegeneration.¹⁹⁶ However, suggesting a context-specific role for P2Y₁ during status epilepticus, treatment with the P2Y₁ agonist MRS2365 or P2Y₁ antagonist MRS2500 10 minutes following IA KA injection when mice had presented their first seizure burst showed the opposite results when compared to the pretreatment regime. Here, the P2Y₁ antagonist reduced seizure severity and neurodegeneration, while treatment with the P2Y₁ receptor agonist increased seizure severity. These results suggest that P2Y₁-based treatment is highly dependent on the timing of intervention. Why P2Y₁ behaves differently according to the timing of treatment initiation remains elusive. However, immunohistochemical analysis shows that while P2Y₁ receptors are mainly expressed in neurons during physiological conditions, they become rapidly upregulated in microglia following IA KA. Therefore, a likely explanation may be a cell type-specific contribution of P2Y₁, depending on where it is increased, with predominant neuronal P2Y₁ expression reducing hyperexcitability and predominant P2Y₁ microglial expression contributing to increased hyperexcitability via driving inflammatory processes.¹⁹⁶ To fully address a possible cell type-specific contribution of P2Y₁ to seizures requires, however, the use of a cell type-specific deletion of P2Y₁ (ie, in neurons and microglia). The role of P2Y₁ is, however, not restricted to microglia, and P2Y₁ has also been shown to be functional on astrocytes.¹¹² In a rapid amygdala kindling model, P2Y₁ has been proposed to contribute to seizure generation and epilepsy via the mediation of astrocytic Ca²⁺ oscillations²²⁶ and to restore normal excitatory synaptic activity in the hippocampus by blocking TNF α -induced Ca²⁺ release from astrocytes.²²⁷ Recently and in line with these results, Martorell et al, using a rapid kindling model in rats consisting of ten daily trains of biphasic rectangular current

pulses at subthreshold postdischarge intensity applied for 3 consecutive days, showed that P2Y₁ inhibition rescued both the abnormal pattern of astroglial Ca²⁺ activity and plastic properties of CA3–CA1 synapses in the epileptic hippocampus.²²⁸

In contrast to studies analyzing the impact of targeting P2Y receptors during status epilepticus, only one study has investigated a possible contribution of P2Y receptors to epileptogenesis and epilepsy.¹⁹⁶ Here, the authors showed that treatment with the P2Y₁ antagonist MRS2500 post-IA KA (3 μg)-induced status epilepticus, delayed the onset of epilepsy, and when applied during epilepsy, suppressed epileptic seizures, though this effect did not persist during treatment withdrawal. Changes in inflammation during treatment were not analyzed.¹⁹⁶

Targeting of P2Y receptors has shown promising results during acute seizures and also during epilepsy (Table 1). Again, as with P2X receptors, the exact mechanisms of how these receptors impact on seizures or epilepsy remains to be established; however, results so far suggest a prominent role of P2Y receptor–driven inflammation. While P2Y₁₂ seems to be more important for the activation of microglia,¹⁶⁰ effects mediated via P2Y₁ have been suggested for both microglia and astrocytes.¹⁹⁶ Of note, in the case of P2Y₁, neuronal expression seems to be associated with protective mechanisms,²²⁶ and thus targeting of P2Y₁ in specific cell types may represent a better and possibly more efficient pharmacological strategy to treat seizures and epilepsy. Whether a cell type–specific function is also the case for other P2Y receptors remains to be established.

Conclusion

Over the last decade, compelling evidence suggesting an important role of P2 receptors during pathological brain hyperexcitability has been steadily increasing, with encouraging data showing that targeting of these receptors not only suppresses neuroinflammatory processes but also provides seizure-suppressive and disease-modifying effects (Figure 1). However, to advance therapeutic strategies based on P2-receptor targeting further toward clinical application, several issues should be addressed.

1. While mounting data suggest anticonvulsive and antiepileptogenic effects via targeting of P2 receptors, the mechanisms of action of these receptors during seizures and epilepsy are still poorly understood. P2 receptors are described as one of the

gatekeepers of inflammation, with continuous P2-receptor activation believed to contribute to sustained pathological neuroinflammation in the brain, possibly contributing to seizures and the development of epilepsy. However, how exactly P2 receptors contribute to inflammation during seizures and epilepsy is yet not fully understood.

2. P2 receptors have been shown to change their expression in different brain regions following seizures and during epilepsy; however, we still lack reliable data regarding their cell type–specific expression or their subcellular localization, which is probably due to a lack of reliable detection tools for most of the purinergic receptors. For example, while it is well accepted that P2X₇ is expressed in microglia during epilepsy, its expression on neurons is still a matter of debate.⁹⁹ As such, better detection tools (eg, nanobodies, P2-receptor reporter mice¹⁹⁰) are needed to establish the exact expression profile of these receptors during epilepsy.
3. New data suggest a cell type–specific function of P2 receptors during seizures. For example, neuronal P2Y₁ expression seems to be anticonvulsive, whereas P2Y₁ expressed on microglia may be proconvulsive.¹⁹⁶ Therefore, further investigation is needed to clarify the exact cell-specific contribution of these receptors to disease progression, requiring the use of cell type–specific knockout mice (eg, Cre-LoxP).
4. To date, the majority of studies investigating a role for P2 receptors in epilepsy have been performed in rodent models of seizures, including acute seizures, such as Ptz or maximal electroshock, and models mimicking TLE, such as KA- or pilocarpine-induced status epilepticus. Taking in consideration that epilepsy is a heterogeneous group of epilepsy syndromes with many etiologies, the analysis of a possible role of P2 receptors should be extended to other disease models such as models of acquired epilepsy (eg, TBI) or genetic models.
5. None of the P2 receptor–based treatments have resulted in complete cessation of seizures; however, promising data have suggested P2 receptor–based treatments as adjunctive treatment for drug-refractory epilepsy potentiating effects of anticonvulsants (eg, P2X₇ antagonism boosting effects of lorazepam in the IA KA mouse model¹¹⁹). Importantly, new treatments are unlikely to be applied as monotherapies, and

Table 1 In vivo studies demonstrating the impact of P2-receptor targeting in seizures and epilepsy (selected examples)

Acute seizures						
Receptor	Seizure type	Model	Approach	Effects on neuropathology, seizures, and epilepsy	Effects on inflammation	Study
P2X ₄	Status epilepticus	IP KA (dose 8–22 mg/kg) Mice, sex not specified	P2X ₄ KO mice.	Neuroprotection in the CA1 area of the hippocampus. No effects on behavioral seizures.	Impaired microglial function (eg, cell recruitment and upregulation of voltage-dependent potassium channels) in P2X ₄ -KO mice.	142
P2X ₇	Status epilepticus	IP KA (25 mg/kg), IP pilocarpine (150, 175, 200, 225, or 250 mg/kg), IP picrotoxin (5 mg/kg) Mice, male	P2X ₇ KO mice. P2X ₇ antagonists: OxATP (5 mM), A438079 (10 μM), A740003 (10 μM) delivered over 1 week via osmotic minipump before seizure induction.	P2X ₇ deletion and blockade increased pilocarpine-induced seizure susceptibility via nonglutamatergic and non-GABAergic transmission. No effects on seizures in KA or picrotoxin models.	Not studied.	205
	Status epilepticus	IP pilocarpine (380 mg/kg) Rats, male	P2X ₇ agonist: BzATP (5 mM, ICV) given 1 week prior to pilocarpine injection. P2X ₇ antagonists: OxATP (5 mM), A740003 (5 mM) and A438079 (10 μM), via osmotic minipump 1 week prior to pilocarpine injection.	BzATP attenuated SE-induced neuronal damage. OxATP-, A438079, and A740003 increased neuronal death. Effect on seizures was not studied.	BzATP increased TNFα immunoreactivity in dentate granule cells, while OxATP decreased it. Reduced astroglial death and reduced infiltration of neutrophils mediated via P2X ₇ antagonism.	206–208
	Status epilepticus	IA KA (3 μg) Mice, male	P2X ₇ KO mice. P2X ₇ agonist: BzATP (0.1 nmol, ICV) given 10 minutes prior to KA injection. P2X ₇ antagonists: A438079 (1.75 nmol, ICV), BBG (1 nmol, ICV) given 10 minutes prior to KA injection and during SE. P2X ₇ R antibody (0.7 mg/mL, ICV) given 10 minutes prior to KA injection.	Increased seizure severity after P2X ₇ -agonist treatment. P2X ₇ antagonists/P2X ₇ R KO reduced seizures and neuroprotection in hippocampus and cortex.	P2X ₇ antagonists blocked IL1β and reduced the number of activated microglia after SE.	119, 188
	Status epilepticus	IM coriaria lactone (40 mg/kg) Rats, male	P2X ₇ antagonists: BBG (1, 5, 10 μg, ICV), A740003, A740003 (10 μM, ICV), A438079 (10 μM, ICV), A740003 (10 μM, ICV), given prior to KA injection.	P2X ₇ antagonism reduced neuronal damage, seizures.	P2X ₇ antagonism reduced inflammation (astrogliosis and microgliosis).	197

(Continued)

Table 1 (Continued).

	Focal, generalized, and generalized tonic-clonic	Timed IV Ptz infusion test (1% Ptz 2 mL/min), MES-T, and 6 Hz electroshock-induced seizures (0.2 ms square pulse at 6 Hz for 3 seconds) Mice, male	<i>P2X₇</i> antagonists: BBG (50–200 mg/kg, IP) given 30 minutes before seizure induction.	Reduced seizures during 6 Hz test (focal seizure) via BBG. No significant anticonvulsive effects of BBG in IV Ptz and MES-T test (generalized and generalized tonic-clonic seizures).	Not studied.	204
	Absence seizures	Male WAG/Rij rats (inbred strain with genetic absence epilepsy) Rats, male	<i>P2X₇</i> agonist: B ₂ ATP (50 µg and 100 µg, ICV). <i>P2X₇</i> antagonists: A438079 (20 µg and 40 µg, ICV).	No effects of <i>P2X₇</i> agonists or antagonists on spike-wave discharges.	Not studied.	10
	Acute (tonic-clonic)	MES-T (inusoidal pulses 4–14 mA, 50 Hz, 0.2 seconds' duration) and SC Ptz-T (87 mg/kg) Mice, male	<i>P2X₇</i> antagonists: JNJ47965567 (15 or 30 mg/kg, IP), AFCS128 (25 or 50 mg/kg, IP), BBG (50 mg/kg, IP), tanshinone (30 mg/kg, IP), given prior to induction of seizures.	No effects on acute seizures of <i>P2X₇</i> antagonism when given alone. Reduced seizure severity when <i>P2X₇</i> antagonists were given in combination with carbamazepine.	Not studied.	203
P2Y₁	Status epilepticus	IP KA (10 mg/kg) intrahippocampal injection of quinolinic acid (1 µL at 0.2 µL/min) Rats, male	<i>P2Y₁</i> antagonist: MRS2500 (1 nmol, ICV) given prior to KA injection.	<i>P2Y₁</i> antagonist-mediated neuroprotection, but no impact on seizure severity.	Not studied.	225
	Status epilepticus	IA KA (3 µg/2 µL) and IP pilocarpine (340 mg/kg) Mice, male	<i>P2Y₁</i> antagonist: MRS2500 (1 nmol, ICV) given prior to KA and during SE. <i>P2Y₁</i> agonist: MRS2365 (0.3 and 1 nmol, ICV) given prior to KA and during SE. <i>P2Y₁</i> KO mice.	<i>P2Y₁</i> KO mice: Increase in seizure severity during SE and neurodegeneration. <i>Pretreatment:</i> <i>P2Y₁</i> agonist reduced seizure severity and protected brain from damage; <i>P2Y₁</i> antagonism increased seizure severity and brain damage. <i>Treatment during SE:</i> <i>P2Y₁</i> agonist increased seizure severity; <i>P2Y₁</i> antagonism decreased seizure severity and brain damage (hippocampus and cortex).	<i>P2Y₁</i> KO mice: Reduced astrogliosis. <i>Pretreatment:</i> <i>P2Y₁</i> agonist reduced astrogliosis and <i>P2Y₁</i> antagonism increased astrogliosis. <i>Treatment during SE:</i> <i>P2Y₁</i> agonist increased astrogliosis and <i>P2Y₁</i> antagonist decreased astrogliosis.	194, 196

(Continued)

Table I (Continued).

P2Y₁₂	Status epilepticus	IP KA (18–22 mg/kg) and ICV KA (0.12–0.18 µg) Mice, male and female	P2Y ₁₂ KO mice.	Increased seizure phenotype in P2Y ₁₂ -deficient mice.	Reduced hippocampal microglial processes toward neurons.	160
Epilepsy						
Receptor	Model	Approach	Effects on seizures and epilepsy	Effects on inflammation	Study	
P2X₃	IP Ptz (30 mg/kg) Mice, male	P2X ₃ antagonist: NF110 (20 nM, 40 nM, and 60 nM, ICV) given from day 1 until the end of the study (day 42).	Not studied.	Reduced proinflammatory cytokines, oxidative stress, and mitochondrial dysfunction.	221	
P2X ₇	IP Ptz kindling (35 mg/kg) Rats, male	P2X ₇ antagonists: JNJ47965567 (30 mg/kg, IP), AFC5128 (50 mg/kg, IP), BBG (50 mg/kg, IP), tanshinone (30 mg/kg, IP) given prior to Ptz injection.	AFC5128 and JNJ47965567 showed a significant and long-lasting delay in kindling development.	AFC5128 and JNJ47965567 reduced IBA1 and GFAP immunoreactivity in the hippocampus.	203	
	IP Ptz kindling (30 mg/kg) Rats, sex not specified	P2X ₇ antagonist: BBG (15 and 30 mg/kg, IP) given 30 minutes before Ptz injection.	Reduced generalized tonic-clonic seizures (kindling seizures). Improved motor performance and cognitive deficits.	Not studied.	209	
	IP pilocarpine (370 mg/kg) Rats, male	P2X ₇ antagonists: AZ10606120 (3 µg, ICV), BBG (50 mg/kg, IP), one injection per day for 4 days post-SE.	P2X ₇ antagonisms increased seizure frequency and seizure severity during epilepsy.	Not studied.	213	
	IP pilocarpine (370 mg/kg) Rats, male	P2X ₇ -targeting siRNA (ICV) given 6 hours post-SE.	P2X ₇ -targeting siRNA treatment led to neuroprotection in hippocampus, reduced edema, and reduced mortality following SE. In addition, P2X ₇ suppression delayed seizure onset and seizure numbers during chronic epilepsy.	Not studied.	210	
	Intrahippocampal KA (4 µg) Rats, male	P2X ₇ antagonist: BBG (2 nM, ICV) given 30 minutes prior to induction of SE.	Pretreatment with linagliptin/BBG reduced seizure severity, improved spatial memory, and reduced neuronal cell death and aberrant mossy-fiber sprouting.	BBG/linagliptin therapy decreased astrogliosis.	211	

(Continued)

Table 1 (Continued).

	Timed IV Ptz-infusion test (1% Ptz 2 mL/min), MES-T, and 6 Hz electroshock-induced seizures (0.2 ms square pulse at 6 Hz for 3 seconds) Mice, male	<i>P2X₇</i> antagonists: BBG (25–100 mg/kg, IP) given for 7 consecutive days.	Reduced seizures during 6 Hz test (focal seizure) via BBG. No significant anticonvulsive effects of BBG in IV Ptz or MES-T (generalized and generalized tonic-clonic seizures).	Not studied.	204
P2Y₁	IA KA (3 µg) Mice, male	<i>P2Y₁</i> antagonist: MRS2500 (1 nmol, ICV) given 4 and 24 hours post-SE.	<i>P2Y₁</i> antagonism delayed epilepsy development.	Not studied.	196
	Rapid kindling model (biphasic square current pulses [1 ms] for 10 s at 50 Hz, delivered ten times per day every 20 minutes) Rats, male	<i>P2Y₁</i> antagonist: MRS2179 (10 µM, ICV) given prior to and after kindling.	Not studied.	MRS2179 decreased astroglial Ca ²⁺ oscillations by reducing the frequency of slow Ca ²⁺ transients, thereby restoring the balance between slow and fast Ca ²⁺ transients.	153, 226, 228
Epilepsy					
Receptor	Model	Approach	Effects on seizures and epilepsy	Effects on inflammation	Study
P2X₇	Multiple low-dose IP KA (22.2±2.02 mg/kg) Rats, male	<i>P2X₇</i> antagonist: JNJ47965567 (0.6 g/kg/2mL, osmotic minipump) given during a week.	Decreased seizure severity, but no changes in the total number of seizures.	<i>P2X₇</i> antagonist did not alter microglia activation or astrogliosis.	214
	IA KA (3 µg) Mice, male	<i>P2X₇</i> antagonist: JNJ47965567 (30 mg/kg, IP) given 10 days post-SE for 5 days.	Reduced seizure frequency during treatment and for an additional 5 days posttreatment.	Decreased inflammation (astrogliosis and microgliosis).	192
P2Y₁	IA KA (3 µg) Mice, male	<i>P2X₇</i> antagonist: MRS2500 (1 nmol, ICV) given 10 days post-SE for 5 days.	Reduced spontaneous seizures, delaying epilepsy development during treatment.	Not studied.	196
P2Y₁₃	Brain tissue from patients with mesial temporal lobe epilepsy	<i>P2Y₁</i> antagonist: MRS2500 (10 µM). <i>P2Y₁₃</i> antagonist: MRS2211 (10 µM).	Not studied.	Joint application of <i>P2Y₁</i> and <i>P2Y₁₃</i> antagonists suppressed retraction of microglial processes in human tissue from epilepsy patients.	171

Abbreviations: ADP, adenosine diphosphate; BBG, Brilliant Blue G; BzATP, 2'(3')-O-(4-benzoylbenzoyl)adenosine-5'-triphosphate; IA, intraamygdala; IM intramuscular; IP, intraperitoneal; ICV, intracerebroventricular; IV, intravenous; KA, kainic acid; KO, knockout; MES-T, maximal electroshock seizure–threshold test; Ptz-T, pentylenetetrazol seizure–threshold test; SE, status epilepticus.

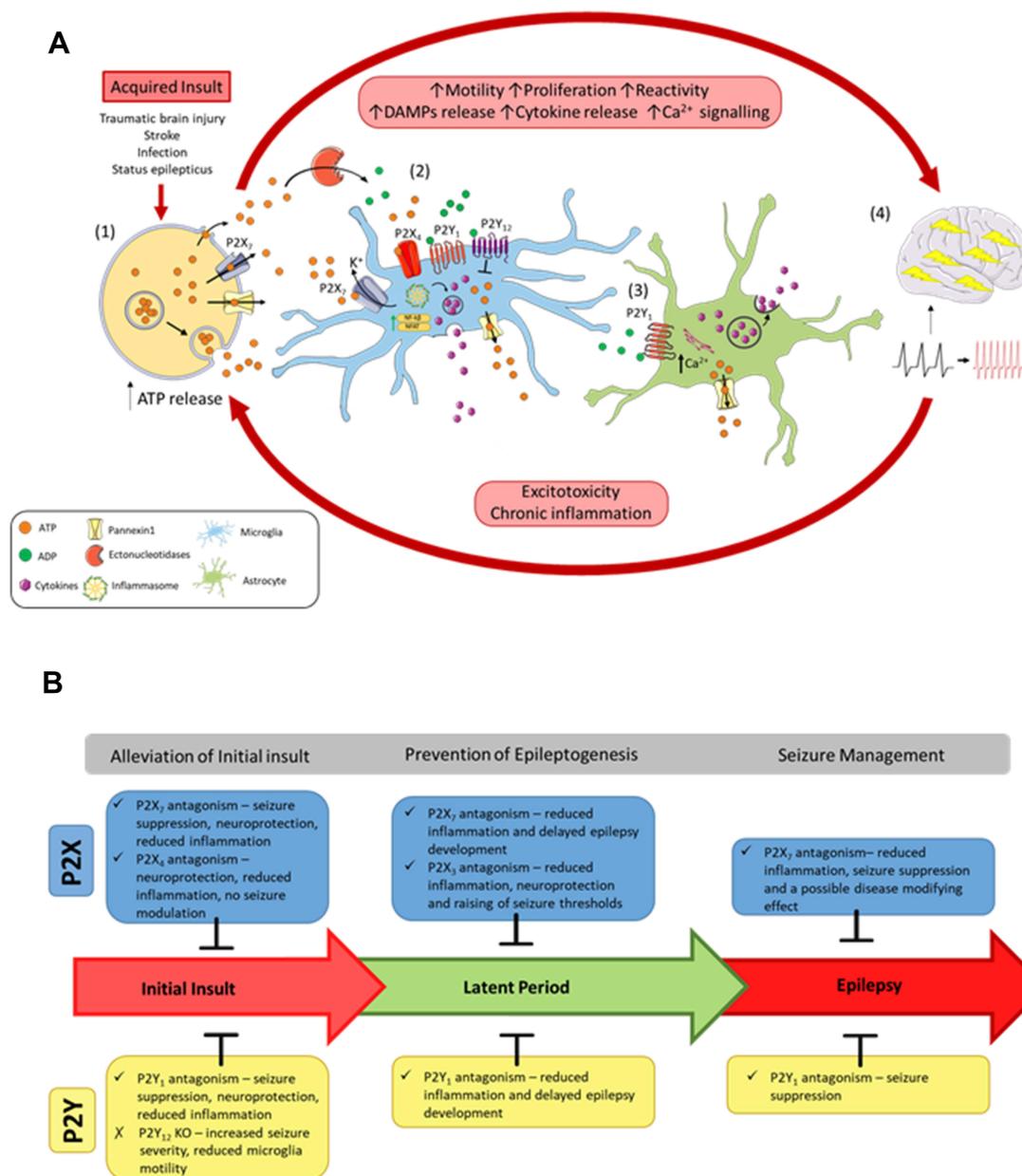


Figure 1 The vicious cycle of inflammation and purinergic signaling underlying epilepsy progression. **(A)** Following an acquired initial insult to the brain, (1) ATP is released from cells via exocytotic mechanisms, through leakage across damaged membranes, and through purinergic channels, such as P2X₇ receptors and pannexin 1. Once released, ATP activates P2 receptors or is metabolized by ectonucleotidases into different breakdown products, such as ADP. ATP and ADP act on the P2 receptors to induce motility, proliferation, and reactivity of microglia and astrocytes. (2) Activation of P2X receptors on microglia via ATP induces inflammasome activation and upregulation of inflammatory transcription factors (NFκB and NFAT), leading to cytokine release. Activation of P2Y₁ also induces cytokine release. However, the P2Y₁₂ receptor acts to oppose these proinflammatory cascades. P2 receptor activation also stimulates release of ATP from microglia. (3) Activation of P2Y₁ on astrocytes releases Ca²⁺ from the endoplasmic reticulum, leading to further cytokine release and ATP release. Acting as a paracrine-signaling molecule, ATP can potentiate these inflammatory cascades. (4) An increase in inflammation of the brain and large amounts of ATP release increases the excitability of the brain, resulting in seizures and epilepsy progression. Chronic inflammation and excitotoxicity induced by seizures leads to further ATP release and epilepsy progression. **(B)** Effects of blocking P2 receptors at the different stages of acquired epilepsy progression following an initial insult to the brain (eg, status epilepticus).

are most likely used as adjunctive therapy in combination with ASDs already in clinical use. Therefore, future studies should target P2 receptors in combination with ASDs to identify better options for seizure control during pharmacoresistant epilepsy.

6. Studies to date have analyzed P2 receptors separately; however, this receptor family is constituted of P2X and P2Y receptors, which can have synergistic effects on each other. Multitargeting approaches should be carried out in future studies.

For example, inhibition of both P2Y₁ and P2X₇ at the same time may improve their effectiveness against seizures and delay or stop the progression of epilepsy, possibly improving previously reported disease-modifying effects.¹⁹²

7. The lack of reliable biomarkers to predict seizures and/or the development of epilepsy remains an important gap in the field. Blood-based inflammation markers have been suggested as strong candidates for the diagnosis of epilepsy.²² Of note, a recent study showed that blood-purine levels correlated with seizure severity in mice and were elevated in humans with epilepsy, demonstrating their diagnostic potential.²²⁹ Future studies should be designed to establish the diagnostic and prognostic potential of purinergic signaling for epilepsy and whether we can stratify patients according to the need for P2 receptor-based treatment.

Abbreviations

ADK, adenosine kinase; ADP, adenosine-5'-diphosphate; AMP, adenosine monophosphate; ASDs, antiseizure drugs; ATP, adenosine 5'-triphosphate; BBB, blood-brain barrier; BBG, Brilliant Blue G; BzATP, 2,3-*O*-(4-benzoylbenzoyl) ATP; cAMP, cyclic AMP; CNS, central nervous system; DAMPs, damage-associated molecular patterns; GABA, γ -aminobutyric acid; GFAP, glial fibrillary acidic protein; HMGB1, high-mobility group box 1; ILAE, International League Against Epilepsy; IL, interleukin; IRF5, interferon regulatory factor; IA, intraamygdala; IP, intraperitoneal; IV, intravenous; NFAT, nuclear factor of activated T cells; NF κ B, Nuclear factor κ light-chain enhancer of activated B cells; NMDA, *N*-methyl-D-aspartate; NR2B, *N*-methyl-D-aspartate receptor subtype 2B; NLRP3, NLR family, pyrin domain containing 3; OxATP, oxidized ATP; P2XRs, P2X receptors; P2Ys, P2Y receptors; PDI, protein disulfide isomerase; PI3K, phosphatidylinositol-3-kinase; Ptz, pentylentetrazol; STAT3, signal transducer and activator of transcription 3; TLE, temporal lobe epilepsy; TLR4, Toll-like receptor 4; TNF, tumor necrosis factor; TBI, traumatic brain injury; VNUT, vesicular nucleotide transporter.

Funding

This work was supported by funding from Science Foundation Ireland (17/CDA/4708 and 16/RC/3948, cofunded under the European Regional Development

Fund and by FutureNeuro industry partners) and the Irish Research Council (Government of Ireland Postdoctoral Fellowship Programme, GOIPD/2020/865).

Disclosure

The authors declare that they have no competing interests in this work.

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