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P2X receptors as targets for the treatment of status epilepticus

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Prolonged seizures are amongst the most common neurological emergencies. Status epilepticus is a state of continuous seizures that is life-threatening and prompt termination of status epilepticus is critical to protect the brain from permanent damage. Frontline treatment comprises parenteral administration of anticonvulsants such as lorazepam that facilitate γ-amino butyric acid (GABA) transmission. Because status epilepticus can become refractory to anticonvulsants in a significant proportion of patients, drugs which act on different neurotransmitter systems may represent potential adjunctive treatments. P2X receptors are a class of ligand-gated ion channel activated by ATP that contributes to neuro- and glio-transmission. P2X receptors are expressed by both neurons and glia in various brain regions, including the hippocampus. Electrophysiology, pharmacology and genetic studies suggest certain P2X receptors are activated during pathologic brain activity. Expression of several members of the family including $P2X_2$, $P2X_4$, and $P2X_7$ receptors has been reported to be altered in the hippocampus following status epilepticus. Recent studies have shown that ligands of the $P2X_7$ receptor can have potent effects on seizure severity during status epilepticus and mice lacking this receptor display altered seizures in response to chemoconvulsants. Antagonists of the $P2X_7$ receptor also modulate neuronal death, microglial responses and neuroinflammatory signaling. Recent work also found altered neuronal injury and inflammation after status epilepticus in mice lacking the $P2X_4$ receptor. In summary, members of the P2X receptor family may serve important roles in the pathophysiology of status epilepticus and represent novel targets for seizure control and neuroprotection.

Keywords: anticonvulsant, ATP, epilepsy, hippocampus, interleukin-1β, microglia, neuroprotection

INTRODUCTION

Status epilepticus is a potentially devastating neurological condition of continuous seizures. Current treatments are often unsuccessful in achieving complete seizure suppression, particularly when delivered late, so novel targets must be identified. ATP-gated ion channels—P2X receptors—are an interesting new focus of status epilepticus research. The pleiotropic effects of P2X receptor activation, including neuromodulation under conditions of excessive neuronal firing and indirect effects on excitability via control of neuroinflammation and gliosis offer a "multi-targeting" mode of action that may be particularly well suited to suppressing both the immediate pathologic brain activity and its downstream consequences. This review summarizes recent work on P2X receptors in status epilepticus, with particular emphasis on the $P2X_7$ receptor ($P2X_7R$), and speculates on the potential of these receptors as future drug targets for seizure control.

STATUS EPILEPTICUS AND LIMITATIONS OF CURRENT TREATMENT

Status epilepticus is a state of continuous seizures, with an annual incidence ranging from 10 to 86 per 100,000 individuals (Chen and Wasterlain, 2006). Status epilepticus is traditionally defined as seizures lasting 30 min or more, but the current operational definition is clinical or electrographic seizures lasting beyond 5 min (Brophy et al., 2012). Status epilepticus represents a neurological emergency that is associated with profound morbidity and mortality. In humans and animal models, status epilepticus results in selective neuronal loss and gliosis, particularly within the hippocampus, as well as cognitive deficits and lasting hyper-excitability (Lowenstein, 2005; Chen and Wasterlain, 2006). Status epilepticus may result from metabolic disturbances, infection, drug toxicity or withdrawal, and non-compliance with the taking of anti-epileptic drugs (AEDs) (Brophy et al., 2012). Identifying the underlying cause of status epilepticus and treating it appropriately is paramount to alleviating the condition (Shorvon, 2011).

Pharmacological treatment of status epilepticus has been reviewed elsewhere (Lowenstein, 2005; Chen and Wasterlain, 2006) and new guidelines were recently published (Brophy et al., 2012). Initial therapy is to provide parenteral benzodiazepines such as lorazepam. Where benzodiazepines fail to control seizures, second-line therapy is usually with certain AEDs,

including phenytoin. If both groups of drug fail and status epilepticus has become refractory, treatment options include ongoing intravenous combinations of the above or alternative treatments including hypothermia (Brophy et al., 2012). Recent work supports the use of the *N*-methyl-D-aspartate (NMDA) receptor antagonist ketamine for refractory status epilepticus (Synowiec et al., 2013).

ANIMAL MODELS OF STATUS EPILEPTICUS

Animal models of status epilepticus have been critical for understanding the pathophysiology and treatment of status epilepticus. During status epilepticus there is a failure of the normal mechanisms for seizure termination, such as build-up of the anticonvulsant adenosine, acidosis or ion channel block. Other changes also accompany status epilepticus, including internalization of receptors for the inhibitory neurotransmitter γ-amino butyric acid (GABA) and externalization of receptors for the excitatory neurotransmitter glutamate (Wasterlain and Chen, 2006; Loscher, 2009). This is thought to underlie the development of benzodiazepine resistance which is common in status epilepticus. Optimal therapy is still lacking and there remains a need to identify other targets.

The most common animal models of status epilepticus use a chemoconvulsant or neurotoxin which is systemically administered or injected directly into the brain. Status epilepticus can also be triggered via electrical stimulation of the brain (e.g., perforant pathway, amygdala). Each has advantages and disadvantages, which have been reviewed elsewhere (Sperk, 1994; Loscher, 2002; Curia et al., 2008). To date, only the pilocarpine and kainic acid models have been used to investigate P2X modulation *in vivo*. Pilocarpine is a cholinergic agonist which produces status epilepticus and a pattern of hippocampal damage similar to that observed in epilepsy patients with mesial temporal sclerosis. However, induction of status epilepticus by pilocarpine appears to be secondary to peripheral immune responses and opening of the blood-brain barrier and the model is associated with high mortality, inter-animal variability in hippocampal pathology, and neuronal injury caused by ischemic as well as excitotoxic mechanisms (Fabene et al., 2007; Marchi et al., 2009). While the use of systemic kainic acid is also associated with variable hippocampal pathology, triggering status epilepticus using an intracerebral (e.g., intra-amygdala) injection of kainic acid produces a highly consistent focal and unilateral hippocampal lesion, with minimal mortality and reliable onset of spontaneous seizures (Li et al., 2008; Mouri et al., 2008; Liu et al., 2013). Such differences are important in critical evaluation of some of the conflicting findings with P2X ligands and genetically-modified mice.

ATP-GATED RECEPTORS; POTENTIAL TARGETS FOR SEIZURE CONTROL?

ATP acts as a neurotransmitter/co-transmitter in the CNS where it has important neuromodulatory and trophic effects (Burnstock, 2008). ATP is released from neurons and glia in response to neuronal activity via exocytosis as well as through alternative routes, including hemichannels and other mechanisms (Lazarowski et al., 2003; Dale and Frenguelli, 2009). ATP can also accumulate because of release from damaged cells. Convulsive activity produces an overall reduction in brain ATP levels but intense activation of neuronal pathways also triggers ATP release (Dale and Frenguelli, 2009). Once released, ATP acts on ionotropic P2X and metabotropic P2Y receptors, and produces a mixture of excitatory and inhibitory effects [for review, see Burnstock (2007); Abbracchio et al. (2009)]. The other major class of purinoceptor, P1 receptors, is activated by adenosine. Adenosine is a potent anticonvulsant, and its important contribution to seizure control has recently been reviewed (Boison, 2013a,b).

P2X receptors are all ATP-gated ion channels, usually composed of heterotrimers of individual subunits encoded by seven different genes (*P2rx1-7*) that gate fast depolarizing sodium (Na^+) and calcium (Ca^{2+}) entry. A number of additional properties are attributed to P2X receptors. Extended activation of certain P2X receptors leads to the formation of a large pore with permeability to small molecules. This is best understood for the $P2X_7R$, in which the response has been linked to a direct cytolytic effect, but other members of the P2X family may also form such channels (Surprenant et al., 1996; Di Virgilio et al., 1998; Virginio et al., 1999). The pore is not necessarily cytolytic, however, and there is controversy over whether the pore is instead formed by adjacent pannexin-1 channels (Duan et al., 2003; Pelegrin and Surprenant, 2006; Iglesias et al., 2008). The $P2X_7R$ has a number of other distinct characteristics. There is a large intracellular domain that enables it to directly interact with downstream pathways, including structural proteins (Kim et al., 2001). The receptor generally does not form heterotrimers and is found as a homotrimer, although recent work suggests it can interact with $P2X_4R$ in some cells (Craigie et al., 2013). The $P2X_7R$ also has low affinity for ATP, requiring mM levels for activation (Gever et al., 2006; Skaper et al., 2010). The implication is that the $P2X_7R$ is not activated under physiological conditions. The necessary conditions to generate sufficient extracellular ATP to activate $P2X_7R$ activation might include after cell lysis (e.g., neuronal necrosis due to excitotoxicity) or pathologic brain activity such as during prolonged or repeated brief seizures. Another feature of the $P2X_7R$ is that repeated agonist application under certain conditions results in sensitization and increased inward currents (Chessell et al., 1998; North and Surprenant, 2000; Armstrong et al., 2002).

Distribution of the P2X receptors has been previously reviewed, although characterization of their exact subunit composition in different tissues and cells is not yet complete (Norenberg and Illes, 2000; North and Surprenant, 2000; Gever et al., 2006). P2X receptors are found on neurons, where they may localize to both pre- and post-synaptic sites, and on non-neuronal cell types. The main subtypes expressed in the brain, including neurons in the hippocampus, are P2X2, 4 and 6 although P2X1, 3 and 5 receptor transcript and/or immunoreactivity has also been reported in the hippocampus (Papp et al., 2004; Dona et al., 2009; Engel et al., 2012a; Ulmann et al., 2013). The $P2X_7R$ was originally cloned from rat brain but considerable controversy has surrounded the exact localization in the brain. Early studies reported only microglial expression of the $P2X_7R$ in the adult brain (Collo et al., 1997). A number of groups have since reported $P2X_7R$ expression in neurons, including in the hippocampus (Deuchars et al., 2001; Armstrong et al., 2002; Dona

et al., 2009; Engel et al., 2012a). However, the specificity of the antibodies used in those studies was questioned by the finding of widespread immunostaining with different antibodies in $P2X_7R$ knockout mice (Sim et al., 2004). Although that study concluded that the $P2X_7R$ was not expressed at appreciable levels in neurons of the hippocampus, other studies have identified the mRNA for $P2X_7R$ in neurons in the rodent hippocampus (Yu et al., 2008). New evidence for constitutive expression of the $P2X_7R$ in hippocampal neurons has come from studies of mice expressing enhanced green fluorescent protein (EGFP) under the control of the $P2X_7R$ promoter (Engel et al., 2012a,b; Jimenez-Pacheco et al., 2013). In the normal mouse brain, EGFP is seen in few dentate granule neurons and also in certain populations of neurons in the neocortex (Engel et al., 2012a,b; Jimenez-Pacheco et al., 2013).

The other major cell type expressing $P2X_7R$ in the brain is microglia (Collo et al., 1997; Rappold et al., 2006; Dona et al., 2009). Oligodendrocytes also express $P2X_7R$ (Matute et al., 2007; Yu et al., 2008). Although expression of $P2X_7R$ has been reported in cultured astrocytes (Duan et al., 2003), there is limited evidence for such expression *in vivo* (Yu et al., 2008; Engel et al., 2012a; Jimenez-Pacheco et al., 2013). Thus, multiple members of the P2X receptor family are expressed in brain where they may exert important modulatory effects on neuro- and glio-transmission (Khakh, 2001; Abbracchio et al., 2009).

EXPRESSIONAL RESPONSE OF P2X RECEPTORS FOLLOWING STATUS EPILEPTICUS

Injury to the brain produces widespread changes to the expression of P2X receptors (Burnstock, 2008). Following status epilepticus, there is a prominent increase in $P2X_7R$ immunoreactivity and functional responses in microglia (Rappold et al., 2006; Avignone et al., 2008). Protein levels of the $P2X_7R$ measured by immunoblotting also increase after status epilepticus in the hippocampus and neocortex, including in the synaptodendritic compartment (Dona et al., 2009; Engel et al., 2012a; Jimenez-Pacheco et al., 2013). Transcript levels of $P2X_7R$ are increased in hippocampal neurons, particularly granule neurons, and microglia after status epilepticus (Avignone et al., 2008; Engel et al., 2012a). There has not been convincing *in vivo* evidence of changes to $P2X_7R$ expression in astrocytes or oligodendrocytes after status epilepticus (Rappold et al., 2006; Engel et al., 2012a; Jimenez-Pacheco et al., 2013).

There is less data on expressional responses of other P2X receptors after status epilepticus. Down-regulation of $P2X_2R$ has been reported after status epilepticus (Engel et al., 2012a) and $P2X_2R$ expression is also decreased in seizure-sensitive gerbils (Kang et al., 2003). For the $P2X_4R$, studies have reported both up- and down-regulation in the hippocampus after status epilepticus (Avignone et al., 2008; Dona et al., 2009). The $P2X_4R$ was recently reported to be up-regulated on hippocampal microglia after status epilepticus in rats (Ulmann et al., 2013) but is expressed at lower levels in the seizure-sensitive gerbil (Kang et al., 2003). Hippocampal protein levels of P2X1, 3, and 5 receptors, as measured by immunoblotting, were all unchanged after status epilepticus (Engel et al., 2012a). Thus, status epilepticus produces select changes to levels of P2X receptors which are likely to result in altered responsiveness of glia and neurons to ATP signaling in the brain. A summary of status epilepticus-induced changes to P2X receptor expression is provided in **Table 1**.

ROLE OF P2X RECEPTORS IN BRAIN EXCITABILITY

Under physiological circumstances, P2X gated currents at synapses are thought to be small and not uniformly detected (North, 2002; Khakh and North, 2006). Intracellular recordings have estimated the ATP-dependent fast excitatory component to comprise 5–20% of the total synaptic current in CA1 pyramidal cells (Pankratov et al., 2002). The real significance of P2X receptor-mediated current may be to facilitate Ca^{2+} entry into cells. Together with the presynaptic location of certain P2X receptors, this implicates them in control of neurotransmitter release (Sperlagh et al., 2007). The distribution of P2X receptors at synapses—particularly at the periphery of the post-synaptic density—suggests their contribution becomes more important under conditions of intense neuronal activity (Khakh and North, 2006).

A number of studies have investigated the effects of P2X receptor activation or blockade on hippocampal excitability and there is evidence for both pro- and anti-excitatory consequences. An excitatory effect of the P2X agonist α, β-meATP was found in rat hippocampal slices (Ross et al., 1998) and hippocampal slices from seizure-prone mice release more extracellular ATP when stimulated (Wieraszko and Seyfried, 1989). In contrast, blockade of post-synaptic P2X receptors was observed to facilitate long-term potentiation, suggesting some P2X receptor functions restrict certain aspects of synaptic plasticity (Pankratov et al., 2002). $P2X_2R$ are present on the presynaptic terminals of CA3 axons (Schaffer collaterals) that terminate on inhibitory interneurons in the CA1 subfield and are thought to function as a physiological brake on excessive neuronal activity (Khakh and North, 2006). Activation of $P2X_2R$ enhances release of excitatory neurotransmitter onto CA1 interneurons, which in turn increases release of inhibitory neurotransmitter to reduce excitatory drive onto CA1 pyramidal neurons (Khakh et al., 2003). Notably, down-regulation of the $P2X_2R$ has been reported in models of status epilepticus, suggesting loss of this receptor might represent a novel "channelopathy" (Engel et al., 2012a).

$P2X_7R$ also mediate effects on hippocampal excitability. Stimulation of $P2X_7R$ present on the presynaptic terminals of mossy fibers reduced excitatory field potentials recorded in the CA3 subfield (Armstrong et al., 2002). These data are consistent with a model whereby pre-synaptic $P2X_7R$ are activated during high-level neuronal excitability and function to reduce further release of glutamate from mossy fiber terminals (Armstrong et al., 2002). The $P2X_7R$ effect to decrease transmitter release probability at mossy fiber synapses is therefore the opposite of what was found for pre-synaptically-located $P2X_2R$, which enhanced transmitter release probability, but functionally these actions are synergistic, to limit over-excitation within the hippocampus. These results are also consistent with the main effects of ATP being presynaptic, not post-synaptic in the hippocampus (Khakh et al., 2003). Other recent work in a model of recurrent epileptiform activity found a small effect of $P2X_7R$ antagonists against slow

Table 1 | P2X receptors in status epilepticus.

	Expression in seizure-relevant brain regions	Expressional response to status epilepticus	Effect of agonists/antagonists or knockout on status epilepticus, seizure-induced cell death and inflammation
$P2X_1$	Hippocampus[a] Cortex[b]	*Hippocampus* No change (W)[c] Up-regulated (qPCR)[d] *Cortex* not studied	Not studied
$P2X_2$	Hippocampus[e,f] Cortex[e]	*Hippocampus* Decreased (W)[c] No change (W)[g] *Cortex* not studied	Not studied
$P2X_3$	Hippocampus[h] Cortex[h]	*Hippocampus* No change (W)[3] *Cortex* not studied	Not studied
$P2X_4$	Hippocampus[f,i,j] Cortex[i,j]	*Hippocampus* Increased (W, IH)[k] No change (W)[c,g] Up-regulated (qPCR)[d] *Cortex* not studied	*$P2X_4$ knock-out mice:* Decreased seizure-induced cell death (i.p. KA)[k] No effect on seizures (i.p. KA)[k] Decreased inflammation and microglia density (i.p. KA)[k] No change in IL-1β levels (i.p. KA)[k]
$P2X_5$	Hippocampus[l] Cortex[l]	*Hippocampus:* No change (W)[c] *Cortex* not studied	Not studied
$P2X_6$	Hippocampus[f,i] Cortex [i]	Not studied	Not studied
$P2X_7$	Hippocampus[c,m,n] Cortex[n,o]	*Hippocampus:* Increased (W, GFP)[c,g] Up-regulated (qPCR)[d] Increased (IH)[p] *Cortex:* Increased (W, GFP)[o]	*Agonists (BzATP):* Increased seizures (i.a. KA)[c] No effect on seizures (Pilo)[q] Increased microglia activation (Pilo)[r] Increase in astrocyte loss (Pilo)[s] Increased TNF-α immunoreactivity (Pilo)[t] Decreased seizure-induced cell death (Pilo)[t] *$P2X_7$R knock-out mouse:* Decreased seizures (i.a. KA)[c] Increased seizures (Pilo)[q] No effect on seizures (i.p. KA and i.p. Pic)[q] *Antagonists (A-43, A-74, BBG, OxATP, IgG-$P2X_7$):* Decreased seizures (i.a. KA)[c,o] Increased seizures (Pilo)[q] Decreased seizure-induced cell death (i.a. KA)[c] Increased seizure-induced cell death (Pilo)[t] Decreased astrocyte loss (Pilo)[s] Decreased microglia activation (i.a. KA and Pilo)[c,r] Decreased Il-1β levels (i.a. KA)[c]

A-43, A-438079; A-74, A-740003; BzATP, 2′,3′-O-(4-benzoylbenzoyl)-adenosine 5′-triphosphate; OxATP, oxidised ATP GFP, $P2X_7$R-GFP-reporter mouse; i.a. KA., intra-amygdala KA; i.p. KA, intra-peritoneal KA; Pilo, Pilocarpine; i.p. Pic, intra-peritoneal Picrotoxin; IgG-$P2X_7$, anti-$P2X_7$ antibodies; Il-1β, interleukin-1beta; qPCR, quantitative polymerase chain reaction; IH, Immunohistochemistry; TNF-α, Tumor necrosis factor alpha; W, Western blot.

[a] Cavaliere et al., 2007; [b] Lalo et al., 2008; [c] Engel et al., 2012a; [d] Avignone et al., 2008; [e] Kanjhan et al., 1999; [f] Rubio and Soto, 2001; [g] Dona et al., 2009; [h] Seguela et al., 1996; [i] Collo et al., 1996; [j] Le et al., 1998; [k] Ulmann et al., 2013; [l] Guo et al., 2008; [m] Armstrong et al., 2002; [n] Yu et al., 2008; [o] Jimenez-Pacheco et al., 2013; [p] Rappold et al., 2006; [q] Kim and Kang, 2011; [r] Choi et al., 2012; [s] Kim et al., 2011a; [t] Kim et al., 2011b.

field potentials induced by potassium-bicuculline treatment of rat cortical slices (Klaft et al., 2012).

Overall, the properties of the P2X system—activation under high levels of neuronal activity—are particularly relevant to status epilepticus and raise the prospect of a class of receptor that when targeted may influence pathologic brain activity while leaving normal neurotransmission largely unaffected. Nevertheless, until very recently, no study had directly investigated the effect of ligands acting at P2X receptors on status epilepticus.

In vivo STUDIES WITH $P2X_7R$ LIGANDS IN STATUS EPILEPTICUS

There has been significant interest in $P2X_7R$ ligands as therapeutics for neurological conditions (Skaper et al., 2010). The leading clinical application of $P2X_7R$ receptor antagonists is for treatment of neuropathic pain but there are indications in acute neurologic injuries. For example, $P2X_7R$ antagonists have been reported to reduce injury or inflammation following intracerebral hemorrhage (Chen et al., 2013) and global ischemia (Yu et al., 2013). In focal cerebral ischemia, protective effects were reported in some studies (Melani et al., 2006; Arbeloa et al., 2012) but not others (Le Feuvre et al., 2003). Also of interest, $P2X_7R$ agonists have been shown to trigger a protective state, a form of "chemical preconditioning," that rendered cardiac tissue resistant to subsequent ischemia (Vessey et al., 2011). Protective effects have also been reported for $P2X_7R$ antagonists in models of neurodegeneration, including Huntington's disease (Diaz-Hernandez et al., 2009), Parkinson's disease (Marcellino et al., 2010), amyotrophic lateral sclerosis (Cervetto et al., 2013) and Alzheimer's disease (Diaz-Hernandez et al., 2012; Murphy et al., 2012).

$P2X_7R$ antagonists have been reported to produce potent anticonvulsant effects in some, but not all models of status epilepticus (see **Table 1**). Studies by the authors demonstrated that a central (intracerebroventricular) injection the $P2X_7R$ antagonists BBG or A-438079 resulted in as much as a 50% reduction in electrographic seizures during status epilepticus triggered by intra-amygdala microinjection of kainic acid in mice (Engel et al., 2012a; Jimenez-Pacheco et al., 2013). Behavioral convulsions were also reported to be reduced in mice treated with A-438079 prior to status epilepticus (Jimenez-Pacheco et al., 2013). Experiments using the "Pfizer" $P2X_7R$ knockout mice (Solle et al., 2001) supported these pharmacological studies, with seizure severity reduced compared to wild-type animals. Further complementing these findings, intracerebroventricular injection of a $P2X_7R$ blocking antibody suppressed seizures while BzATP, a $P2X_7R$ agonist, exacerbated seizures in the model (Engel et al., 2012a). Analysis of the hippocampus and neocortex of mice pre-treated with $P2X_7R$ antagonists found reductions in neuronal death, microgliosis and interleukin-1β (Engel et al., 2012a; Jimenez-Pacheco et al., 2013). Treatment of mice with $P2X_7R$ antagonists 20 min after triggering status epilepticus - a more clinically-relevant scenario—also reduced seizure severity and protected the hippocampus (Engel et al., 2012a). Finally, injection of A-438079 1 h after status epilepticus began, at a time when sensitivity to lorazepam was reduced, also had modest seizure-suppressive effects (Engel et al., 2012a). This finding is important since it supports the possible use of P2X ligands as adjunctive treatments for status epilepticus alongside frontline drugs such as lorazepam.

These findings add complexity to the pathophysiological functions assigned to the $P2X_7R$ in the brain. *In vitro* data had supported the $P2X_7R$ as a "physiological brake" on overexcitation (Armstrong et al., 2002) but these *in vivo* data indicate blocking the $P2X_7R$ reduces hyper-excitation. It will be important to establish mechanisms that account for the observed *in vivo* effects of $P2X_7R$ antagonists against status epilepticus. Direct effects of $P2X_7R$ on neuronal activity may behave differently *in vivo* during status epilepticus. For example, the pre-synaptic $P2X_7R$ thought present on mossy fibers facilitating rather than opposing glutamate release, as demonstrated for $P2X_2R$ (Khakh et al., 2003). Also, perhaps blocking $P2X_7R$ on glia (e.g., microglia) confers anti-excitatory effects that functionally supersede presynaptic effects limiting transmitter release. As always, findings based mainly on pharmacology require careful consideration of the specificity of the ligands involved (Anderson and Nedergaard, 2006).

OTHER P2X RECEPTOR LIGANDS IN STATUS EPILEPTICUS

The only other member of the P2X family for which *in vivo* data exist in a model of status epilepticus is the $P2X_4R$. Mice lacking the $P2X_4R$ display a reduction in neuronal death after status epilepticus, although seizures themselves were not altered in these mice (Ulmann et al., 2013). Notably, while some inflammatory signaling was also reduced, the induction of interleukin-1β was not found to be different, supporting other work linking modulation of this pathway to the $P2X_7R$ (see below).

We can speculate that targeting other members of the P2X family would have seizure-modulating effects *in vivo*, although there are fewer ligands selective for the other P2X receptors. An obvious candidate would be an agonist of the $P2X_2R$. Activation of this receptor promotes inhibitory transmission within the hippocampus (Khakh and North, 2006). Delivery of such a ligand might enhance endogenous mechanisms of seizure suppression.

GLIA-RELATED FUNCTIONS OF THE $P2X_7R$ IN STATUS EPILEPTICUS

The immediacy of the seizure-suppressive effects of $P2X_7R$ antagonists implies a direct action on neurons, for which there is supporting evidence (Armstrong et al., 2002; Engel et al., 2012a,b). However, expression and activation of the $P2X_7R$ on glia may have important effects on excitability that influence the pathophysiology and outcome of status epilepticus. First, $P2X_7R$ activation has been associated with production of cytokines from microglia (Ferrari et al., 1996; Chakfe et al., 2002). In particular, activation of the $P2X_7R$ leads to processing and release of interleukin-1β, which is a potent pro-convulsive molecule implicated as a target for seizure control (Vezzani et al., 2010). Activated microglia also exacerbated excitotoxicity in hippocampal cultures, an effect shown to be $P2X_7R$-dependent (Bernardino et al., 2008). Thus, targeting $P2X_7R$ effects on microglia may reduce post-status epilepticus inflammation and susceptibility to excitotoxicity.

$P2X_7R$ have been implicated in certain trophic functions, including the activation and proliferation of microglia. This

serves both restorative and pathologic functions after status epilepticus, contributing to tissue repair but also releasing pro-inflammatory mediators which may contribute to hyper-excitability (Devinsky et al., 2013). Increased $P2X_7R$ expression and receptor activation was found to transform resting microglia to an activated state (Monif et al., 2009). Activation of the pore-forming function of the $P2X_7R$ was also found to be required for microglial proliferation (Monif et al., 2009). Consistent with this model, blockade of $P2X_7R$ reduces microglia activation after status epilepticus (Kim et al., 2009; Choi et al., 2012; Engel et al., 2012a). Targeting the pore-forming functions of the $P2X_7R$ may therefore be a novel approach to limit microglia responses following status epilepticus.

Astrocytes represent another non-excitable cell involved in mediating the effects of ATP. There is *in vitro* evidence that $P2X_7R$ activation on astrocytes triggers glutamate release, perhaps directly through the channel/pore (Duan et al., 2003). Such $P2X_7R$-mediated glutamate release from astrocytes may contribute to astrocyte signaling, promote excitability or even excitotoxicity. $P2X_7R$ activation on astrocytes may also serve a cell-killing function. Injection of rats with the $P2X_7R$ agonist BzATP was found to reduce astrocyte numbers after status epilepticus and $P2X_7R$ antagonists prevented astrocyte death (Kim et al., 2009, 2011a). Thus, secondary effects of P2X7R ligands will need to be considered as modulation of astrocyte number or activation has profound effects on excitability in the brain (Boison, 2008). Oligodendrocytes are also sensitive to the toxic effects of ATP acting on $P2X_7R$ (Matute et al., 2007).

Finally, it has emerged that the $P2X_7R$ may promote axonal growth and branching in the hippocampus (Diaz-Hernandez et al., 2008). Studies have also reported that $P2X_7R$ antagonists improved recovery after spinal cord injury (Peng et al., 2009). Synaptic reorganization is long-recognized following status epilepticus and has been implicated in establishing recurrent excitatory circuits (e.g., mossy fiber sprouting) that may contribute to epileptogenesis or chronic epilepsy (Houser et al., 2012). The benefits from targeting the $P2X_7R$ could therefore extend well beyond the initial period of seizure activity. As with effects on astrocytes and microglia, these data suggest targeting the $P2X_7R$ may have pleiotropic effects and the timing of manipulations or site of targeting may be critical to obtain optimal therapeutic benefit.

Figure 1 summarizes the mechanisms of ATP release during seizures, the receptors upon which ATP may act, expressional changes, and some of the downstream effects of P2X receptor modulation relevant to the pathophysiology of status epilepticus.

LIMITATIONS OF TARGETING $P2X_7$ RECEPTORS

While there is significant support for a role for $P2X_7R$ in neuronal injury and/or glial activation, there are also conflicting findings. Excitotoxic injury has been reported to be unchanged in mice lacking $P2X_7R$ or in response to $P2X_7R$ antagonists (Le Feuvre et al., 2003). Recent work by Frenguelli's group found a limited role for P2X receptors in electrically-evoked seizure-like activity in rat hippocampal slices, and no effect of $P2X_7R$ antagonists on these events (Lopatar et al., 2011). Similarly, no effects of $P2X_7R$ antagonists on *in vitro* epileptiform activity were detected in acute cortical slices from epileptic rats (Klaft et al., 2012). Inter-species differences could be to blame and have been reported for the $P2X_7R$ (Chessell et al., 1998). Last, Kang's group reported that pilocarpine-induced seizures were in fact exacerbated in mice lacking $P2X_7R$ and in wild-type animals treated with $P2X_7R$ antagonists (Kim and Kang, 2011). These data are in sharp contrast to the findings with intra-amygdala kainic acid-induced

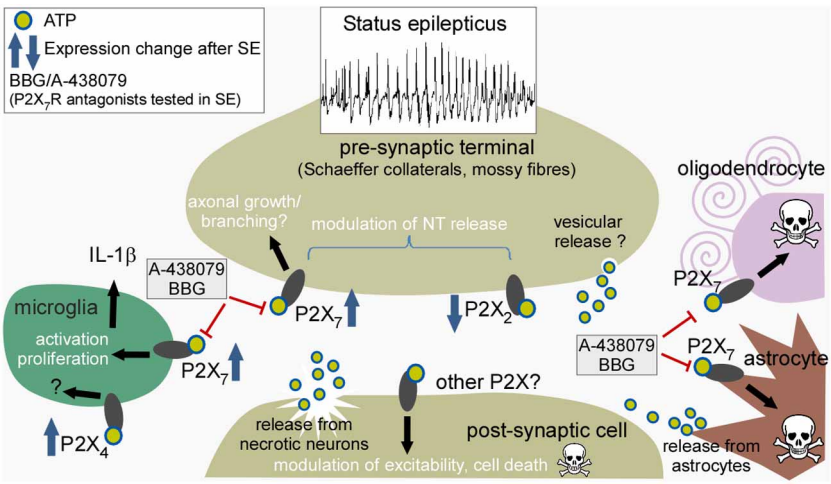

FIGURE 1 | Potential sites of action of ATP released during status epilepticus, expressional responses of individual P2X receptors, and consequences of receptor activation. Cartoon depicts the various different cell types reported to express P2X receptors and their presumed cellular locations. ATP is released during sustained neuronal activity and from damaged neurons to act pre- and post-synaptically on neurons, particularly targeting pre-synaptic receptors to modulate neurotransmitter release. ATP may also act on receptors of microglia to promote activation and release of interleukin-1β, and act on astrocytes and oligodendrocytes to trigger cell death. Drugs such as A-438079 and BBG have been reported to reduce seizures and gliosis after status epilepticus. SE, status epilepticus; IL-1β, interleukin-1β.

status epilepticus (Engel et al., 2012a; Jimenez-Pacheco et al., 2013). Although the reasons underlying these contradictory findings are uncertain they may relate to the specificity and dose of the ligands used, models or genetic tools. Indeed, the findings may be peculiar to the pilocarpine model because seizures induced by kainate or picrotoxin were not exacerbated in mice lacking $P2X_7R$ in that study (Kim and Kang, 2011). Another factor may be the variable influence of tissue responses affecting the pharmacological properties of the receptor. The $P2X_7R$ is inhibited by acidosis (North, 2002), which develops during status epilepticus (Ziemann et al., 2008). Does $P2X_7R$ blockade develop regardless of pharmacological antagonism in some models of status epilepticus? Further studies will be needed to resolve these complicated issues.

OTHER CONSIDERATIONS IN THE DEVELOPMENT OF P2X LIGANDS FOR STATUS EPILEPTICUS

New P2X receptor ligands have recently emerged while several of the known P2X antagonists show potentially suitable drug profiles. This includes the $P2X_7R$ antagonist A-438079, a relatively small molecule that crosses the blood-brain barrier following systemic delivery (McGaraughty et al., 2007). A recently developed $P2X_7R$ antagonist is also able to pass the blood brain barrier (Bhattacharya et al., 2013). More complete reviews on the potential of P2X receptor ligands as drugs can be found elsewhere (Burnstock, 2008; North and Jarvis, 2013).

Despite the need for new therapies, there has been disengagement of several of the major pharmaceutical companies from developing new anticonvulsants and treatments for epilepsy. It has been suggested that the identification of additional clinical applications (i.e., non-epileptic conditions) would make a potential anticonvulsant drug candidate significantly more attractive for development (Bialer and White, 2010). One example is with the effectiveness of certain AEDs for the treatment of pain. Notably, a key area of deployment of $P2X_7R$ antagonists is for the treatment of pain conditions (Trang et al., 2012; Alves et al., 2013).

REMAINING CHALLENGES

There are opportunities for the use of P2X receptor ligands in the control or prevention of seizures but significant challenges remain. Foremost, $P2X_7R$ antagonists need to be evaluated in other models because of the conflicting reports between kainic acid and pilocarpine models. These issues are not unique to status epilepticus; conflicting data on the $P2X_7R$ have emerged in the stroke field (Le Feuvre et al., 2003; Melani et al., 2006; Yanagisawa et al., 2008; Arbeloa et al., 2012). A genetic approach will be needed to confirm specific drug effects are lost in animals lacking the $P2X_7R$, although such studies are not without problems (Nicke et al., 2009; Masin et al., 2012).

Assuming $P2X_7R$ antagonists display consistent anticonvulsive profiles, work will be needed to explore dosing and route of delivery. Do these drugs suppress seizures when given systemically and are there off-target effects? Increased attention is needed on assessing the pleiotropic actions of these drugs, and consideration given to looking for effects on glia and inflammation, including long after the initial period of status epilepticus. Are anti-inflammatory effects of $P2X_7R$ antagonists countered by trophic effects that promote gliosis? P2X ligands may have effects against spontaneous seizures, which would raise their potential as future AEDs. P2X receptors serve important roles in neurodevelopment and ligands may also have potential to treat seizures in the developing brain, a condition currently poorly served by available treatments (Slaughter et al., 2013). It will also be important to determine the mechanism(s) controlling P2X receptor expression after status epilepticus. Indeed, efforts to "rescue" the post-status epilepticus decline in $P2X_2R$ levels could promote inhibitory transmission. Last, we lack relevant human data. Although there is evidence for altered expression of P2X receptors in human epilepsy (Jimenez-Pacheco et al., 2013), their expression and function in status epilepticus is unknown.

In summary, there is increasing evidence for a role for ATP and P2X receptors in seizure states such as status epilepticus and epilepsy. Certain P2X receptors may represent novel drug targets for seizure control. Targeting these receptors could provide frontline or adjunctive seizure suppression during status epilepticus, as well as influencing post-injury glial function that may help mitigate outcomes including epileptogenesis.

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