Purinergic Signalling and Neurological Diseases: An Update

Running title: Purines and brain disorders
Purinergic Signalling and Neurological Diseases: Update

By

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Abstract: Purinergic signalling, i.e. ATP as an extracellular signalling molecule and cotransmitter in both peripheral and central neurons, is involved in the physiology of neurotransmission and neuromodulation. Receptors for purines have been cloned and characterised, including 4 subtypes of the P1(adenosine) receptor family, 7 subtypes of the P2X ion channel nucleotide receptor family and 8 subtypes of the P2Y G protein-coupled nucleotide receptor family. The roles of purinergic signalling in diseases of the central nervous system and the potential use of purinergic compounds for their treatment are attracting increasing attention. In this review, the focus is on the findings reported in recent papers and reviews to update knowledge in this field about the involvement of purinergic signalling in Alzheimer’s, Parkinson’s and Huntington’s diseases, multiple sclerosis, amyotrophic lateral sclerosis, degeneration and regeneration after brain injury, stroke, ischaemia, inflammation, migraine, epilepsy, psychiatric disorders, schizophrenia, bipolar disorder, autism, addiction, sleep disorders and brain tumours. The use in particular of P2X7 receptor antagonists for the treatment of neurodegenerative diseases, cancer, depression, stroke and ischaemia, A2A receptor antagonists for Parkinson’s disease and agonists for brain injury and depression and P2X3 receptor antagonist for migraine and seizures have been recommended. P2Y receptors have also been claimed to be involved in some central nervous disorders.

Keywords: Alzheimer’s, Parkinson’s, MS, Neuroprotection, Depression, Schizophrenia, Addiction, Epilepsy
1. INTRODUCTION

The concept of purinergic signalling, i.e. adenosine 5’-triphosphate (ATP) as an extracellular signalling molecule, was proposed in 1972 [1]. ATP was later shown to be a cotransmitter with established neurotransmitters in both the peripheral and central nervous systems [2, 3]. Receptor families for ATP (P2) and to its breakdown product adenosine (P1) were recognised in 1987 [4]. In the early 1990’s, P1 and P2 receptor subtypes were cloned and characterised (see [5]): four subtypes of P1 receptors (A1, A2A, A2B and A3); seven subtypes of P2X ion channel receptors (P2X1-7) and eight P2Y G protein-coupled receptors (P2Y1, 2, 4, 6, 11, 12, 13 and 14) are recognised (see [6]).

Reviews have been published previously about purinergic signalling and neurological disorders [7-15]. However, this field is currently attracting much attention, so this review is an update of recent findings.

2. NEURODEGENERATIVE DISEASES

Neurodegeneration is the basis of a wide range of central nervous system (CNS) diseases, involving both neurons and glial cells and inflammation [16] and there is recognition of the participation of purinergic signalling (see [9, 10, 17]). A recent review focuses on the supportive and detrimental roles of P2Y receptors in neurodegeneration [18]. Another review considers nucleotide salvage deficiencies and DNA damage in neurodegenerative diseases [19]. A third review focuses on the neuromodulatory actions mediated by P2X receptors in neurodegenerative diseases [20]. The role of the P2Y-like receptor, GPR17, in CNS remyelination is gaining attention as a novel reparative approach to neurodegenerative diseases [15].

2.1. Alzheimer’s disease

Alzheimer’s disease is characterised by progressive cognitive impairment, initially with prominent deficits in short term memory. There has been much interest in recent years about the potential of purinergic drugs for the treatment of Alzheimer’s disease. Previous studies suggested that both P2X7 and P2Y4 receptor antagonists are potential therapeutic targets for the treatment of Alzheimer’s disease [8, 21]. This has received support in recent reviews [20, 22-24]. It has been reported that β-amyloid increases release of ATP which, via P2X receptors, potentiates excitatory synaptic activity; these effects were blocked by P2X antagonists [25]. It has been claimed that adenosine A3 receptor agonists suppress amyloid-β protein precursor internalization and amyloid-β generation [26]. Hippocampal adenosine A2A receptor up-regulation has been reported to be necessary to trigger memory dysfunction in Alzheimer's disease [27].

2.2. Parkinson’s disease

The main focus has been on the involvement of adenosine A2A receptors in Parkinson’s disease and their interactions with dopamine receptors, although P2X7 receptor antagonists have also been implicated in this disease [28, 29]. A2A receptor antagonists have been recommended as symptomatic treatment for Parkinson’s disease [30, 31]. More recent papers have implicated the involvement of ATP-sensitive potassium channels (K_{ATP}) in Parkinson’s disease [32]. A role for P2X1 receptors in Parkinson’s pathogenesis has also been reported, perhaps identifying a new therapeutic target [33, 34]. Clinical trials for the use of istradefylline, an A2A antagonist, have been undertaken and it was suggested that it could be an efficacy and safety augmentation drug added on to levodopa or other existing anti-Parkinson’s therapies [35]. Later studies have supported this strategy
A2A receptor blockade prevented rotenone-induced motor impairment in a rat model of parkinsonism and this was taken to reinforce the potential use of A2A receptor antagonists for the treatment of Parkinson's patients [38].

2.3. Huntington’s disease
Earlier studies showed that A2A receptor agonists had beneficial effects for Huntington’s disease and that P2X7 receptor antagonists prevented neuronal apoptosis and attenuated body weight loss and motor co-ordination effect deficit in Huntington’s patients (see [29]). It has been reported that inactivation of adenosine A2A receptors reverses working memory deficits at early stages of Huntington's disease models and it was suggested that A2A receptor antagonists would be novel targets to reverse cognitive deficits in Huntington’s patients [39]. In an earlier publication, adenosine A1 receptor agonists were also considered as a therapeutic target for this disease [40, 41].

2.4. Multiple sclerosis (MS)
Both P2X7 receptors and the P2Y-like GPR17 receptor were shown to be involved in MS [29, 42]. Upregulation of ecto-5'-nucleotidase (CD73) during experimental autoimmune encephalomyelitis, a model of MS, has been reported [43].

2.5. Amyotrophic lateral sclerosis (ALS)
The involvement of A2A, P2X4 and P2X7 receptors in ALS has been described (see [44]). A1 as well as A2A adenosine receptor subtypes have also been implicated in ALS [45]. Preconditioning with latrepirdine, an adenosine 5'-monophosphate-activated protein kinase activator, has beneficial effects on the progression of the disease in the SOD1 mouse model of ALS [46]. Activation of A2A receptors facilitates neuromuscular transmission in the pre-symptomatic phase of the SOD1 ALS mice, but not in the symptomatic phase [47].

3. BRAIN TRAUMA, INCLUDING STROKE, ISCHAEMIA AND INFLAMMATION
3.1. Brain injury
Traumatic brain injury results in cell death, inflammation and neurologic dysfunction in patients. The roles of purinergic signalling in neurodegeneration following brain injury have been reviewed [29, 48]. Brain stab injury alters ectonucleotidase activities and nucleotide levels in serum [49, 50]. P2X7 receptors play a critical role in radiation-induced brain injury, suggesting that P2X7 receptor antagonists might be effective for the treatment of patients with radiation injury [51]. Evidence has been presented to suggest that astrocytic p-connexin 43 promotes neuronal autophagy via activation of P2X7 receptors in the hippocampus, promoting repair of brain injury-induced cognitive deficits [52]. Neuroblasts migrate to sites of brain damage and activation of upregulated P2Y1 receptors contribute to this event [53]. A2A receptor activation has been recommended for the treatment of brain injury and subsequent neuroinflammation [54]. A2A receptors on bone marrow-derived cells are modulators of white matter lesions induced by chronic cerebral hypoperfusion [55].

3.2. Stroke
It has been proposed that P2X7 receptor antagonists would be effective to treat the occurrence of post-stroke pain and may be beneficial for the recovery of patients following stroke [56]. A beneficial role for P2X7 receptor activation in acute ischaemic stroke has been reported, by limiting the early oedema formation and possibly by modulating glial responses [57].

3.3. Ischaemia

Extracellular concentrations of ATP and adenosine in the brain increase dramatically during ischaemia that activate both P1 and P2 receptors [58]. Evidence has been presented showing that P2X4 receptors expressed on microglia are involved in the post-ischaemic inflammation in brain ischaemic injury (see [59]). Hypoxic-ischaemic brain injury triggers an inward current and increase in intracellular Ca\(^{2+}\) in oligodendrocytes, which is partly mediated by P2X7 receptors (see [60]). Neuronal K\(_{\text{ATP}}\) channels were reported to mediate hypoxic preconditioning and reduce subsequent neonatal hypoxic-ischaemic brain injury and it was suggested that K\(_{\text{ATP}}\) channel openers may have therapeutic possibilities [61]. P2X7 antagonists or blockade of pannexin-1 channels attenuates post-ischaemic brain damage [62, 63]. Intranasal administration of guanosine reduced brain damage induced by ischaemia in rats [64].

Both A\(_{2A}\) receptor agonists and antagonists have been claimed to be protective against ischaemic brain injury (see [65]). In a recent paper, A\(_{1}\) receptors were reported to contribute to immune regulation after neonatal hypoxic ischaemic brain injury [66].

3.4. Inflammation

Recent reviews summarise the importance of inflammation in the responses to brain injury and disease, including bacterial infection and neurological disorders and the involvement of P1 and P2 receptors [67, 68] and of P2X7 receptors in particular [69].

4. NEUROPROTECTION AND NEUROREGENERATION

4.1. Neuroprotection

Recent reviews explore this topic [17, 29, 70]. Both blockade of ATP activation of P2X and P2Y receptors and the action of adenosine, after ectoenzymatic breakdown of ATP released from cells in the CNS, are claimed to be neuroprotective. Activation of the pannexin 1/P2X7 receptor complex appears to play a role in the neuroprotective mechanism of ischaemic pre- and post-conditioning [63]. It has been suggested that dietary docosahexaenoic acid may act as a purinergic modulator via P2X receptors to protect against neurodegenerative diseases [71]. Neuroprotection has been claimed to be mediated by P2Y\(_{13}\) nucleotide receptors in neurons [72]. A recent study showed that microglia-mediated neuroprotection depends on ATP-activated P2X7 receptors and induction of tumour necrosis factor-\(\alpha\) release [73]. Blockade of P2X7 receptors provides neuroprotection to various neurological disorders, including stroke, traumatic brain injury and subarachnoid haemorrhage [74].

Recent reviews have focussed on the roles played by P2X4 and P2X7 receptors [75] and A\(_{2A}\) receptors [76] in neurodegeneration and neuroprotection.

4.2. Neuroregeneration
A recent review focussed on neuroregeneration is available [29]. Activation of P2Y₂ receptors promotes regeneration of both nerves and glial cells and the P2Y-like GPR17 receptor promotes regeneration of oligodendrocytes. Activation of neural stem cells results in neuroregeneration, probably via P2X4 and P2X7 receptors. Lysosome-mediated ATP exocytosis and calcium signalling appear to play a role in astrocytic responses and may benefit the optimization of scaffold design for CNS healing [77].

4.3. Migraine
The involvement of ATP in migraine was first considered in a vascular theory for this disorder (see [10, 78]). Later, the involvement of P2X3 receptors in brain areas that mediate nociception, such as the trigeminal nucleus and thalamus, was explored [79] and the interaction with P2Y₁ receptors in trigeminal neurons. The therapeutic potential of P2X7 antagonists for the treatment of migraine has also been proposed [80], as well as P2X3 and P2X2/3 receptor antagonists [81]. A review of the roles of purinergic signalling in the aetiology of migraine concluded that ‘purinergic receptors can be an excellent target for pharmacologists constructing new antimigraine therapeutics’ [82].

4.4. Epileptic seizures
Early attention was on the role of P1 adenosine receptors in epileptic seizures, but more recently P2X4 and P2X7 receptor antagonists have been explored as neuroleptic agents [8, 83, 84]. P2Y₁₂ knockout mice exhibited reduced seizure-induced increases in microglial process numbers and worsened kainate-induced seizure behaviours [85]. It has been suggested that the antiepileptic effects of deep brain stimulation may be mediated by adenosine [86]. Experiments support a role for post-transcriptional regulation of the P2X7 receptors and it was suggested that therapeutic targeting of microRNA-22 may prevent inflammation and development of epilepsy [87]. Upregulated expression of P2X3 receptors was demonstrated in both epileptic humans and rats and it was suggested that P2X3 receptor antagonists may represent novel therapeutic targets for antiepileptic drugs [88]. Microelectrode biosensors have been used to measure the release of ATP and adenosine during ongoing epileptiform activity [89]. Increased levels of ATP were shown during epileptic seizures, which were not of neuronal origin [90]. An insightful Editorial was published recently about purinergic signalling-induced neuroinflammation in epilepsy [91].

5. PSYCHIATRIC DISORDERS
Antipsychotic drugs, such as haloperidol, chlorpromazine and fluspirilene, inhibited ATP-evoked responses mediated by P2X receptors. Such antipsychotic drugs have a therapeutic effect by suppressing dopaminergic hyperactivity through inhibition of P2X-mediated effects (see [8]). Haloperidol was shown to be an inhibitor of NTPDase and adenosine deaminase activities in zebrafish brain [92]. Reviews concerned with the roles of purinergic signalling in psychiatric disorders, including schizophrenia, bipolar disorder, depression and addiction, as well as autism (a developmental disorder), have been published [93-95]. A recent review raises the possibility that increases in adenosine concentrations in the brain by increasing activation of NTPDases, inhibition of adenosine deaminase and/or adenosine kinase, as well as the use of P1 receptor agonists, might be beneficial in therapy of psychiatric disorders [96].
5.1. Schizophrenia
Most attention has been paid to adenosine neuromodulation in schizophrenia (see [97, 98]). For example, deletion of A$_{2A}$ receptors from astrocytes disrupts glutamate homeostasis leading to psychomotor and cognitive impairment, relevant to schizophrenia [99]. However, there is also evidence that P2X7 receptors and pannexin 1 channels are involved (see [10, 100]).

5.2. Bipolar disorder
There is increasing evidence that P2X7 receptors, mediating neuroinflammation, via the activity of microglia, play a role in bipolar disorder (see [101-103]). Increased serum levels of uric acid have been demonstrated in different phases of bipolar disorder in patients [104] and those patients treated with lithium [105]. This implied that dysregulation of the purinergic system may occur during manic episodes and suggested that increased uric acid levels may be a useful marker for the manic phase of bipolar disorder. It has been claimed that purinergic modulators that lower uric acid levels could play a role in clinical practice [106].

5.3. Mood disorder
Reviews are available describing the roles of both P1 (A$_1$ and A$_{2A}$) and P2X7 receptors in mood disorders [9, 107-109].

Antidepressant and anticomulsive effects of P2X7 antagonists have been reported [110]. P2X2 receptors in the medial prefrontal cortex were shown to mediate the antidepressant-like effects of ATP released from astrocytes [111]. Brilliant blue G, a P2X7 receptors antagonist, had anti-inflammatory and antidepressant effects in mice after lipopolysaccharide administration [112]. The P2X7 receptor antagonist, A-804598, was shown to have an impact on the neuroimmune and behavioural consequences of stress [113]. A mutation causing increased K$_{ATP}$ channel activity was reported to lead to reduced anxiety in mice [114].

An A$_{2A}$ receptor antagonist was reported to have antidepressant activity [93]. It was claimed that creatine and ketamine had antidepressant effects, probably mediated by activation of A$_1$ and A$_{2A}$ receptors [115]. Caffeine, acting as an antagonist at A$_{2A}$ receptors, prevented depression and memory dysfunction triggered by chronic stress [116]. It was reported that striatal and extrastriatal A$_{2A}$ receptors in the forebrain regulate fear responses in mice [117]. Adenosine, via A$_{2A}$ receptors, increased interleukin 1β in the brain contributing to anxiety [118]. Administration of a derivative of adenosine, WS0701, alleviated anxiety and fear in a mouse model of posttraumatic stress disorder [119]. In the aetiology of anxiety disorders and addiction, the basolateral amygdala plays a critical role and adenosine, via activation of A$_{2A}$ receptors, facilitated basolateral amygdala pyramidal cell output [120].

5.4. Autism
Both adenosine and suramin, a non-selective antagonist of ATP, have been claimed to reverse autism-like behaviours [121-123].

5.5. Addiction
There is experimental evidence that targeting A$_{2A}$ receptors may offer innovative translational strategies for combating drug addiction [124]. It is generally accepted that behavioural sensitization in animals provides a
model for drug craving believed to underlie addiction in humans and sensitization to amphetamine was interrupted by P2Y1 receptor blockade in the mesocortico-limbic dopaminergic system [125]. Striatopallidal A2A receptor signalling in the dorsomedial striatum may be a therapeutic target to reverse abnormal habit formation associated with drug addiction [126]. Caffeine, an adenosine receptor antagonist, potentiates the addictive effects of drugs of abuse, including cocaine, amphetamine derivatives and energy drinks with alcohol [127].

5.5.1. Opiates
Treatment of rodents with the P2X7 receptor antagonist, 2',3'-O-(2,4,6-trinitrophenyl) adenosine 5'-triphosphate (TNP-ATP), diminished opioid tolerance, in part by inhibiting excitatory amino acid release [128]. Opiate-induced changes in brain adenosine levels may explain some of the neurobehavioral features associated with opiate addiction and withdrawal [129]. Heroin administration may enhance the catabolism and inhibit the anabolism of purine nucleotides in the brain [130].

5.5.2. Cocaine
Adenosine, acting via A2A receptors, regulated addiction induced by cocaine [131]. Enhancement of dopamine function via A2A receptor blockade was suggested for reducing the neurobehavioral deficits associated with chronic cocaine addiction [132]. Females have a higher sensitivity and thus higher vulnerability to cocaine abuse compared to males; treatment with adenosine receptor antagonists was identified as a possible treatment [133]. Cocaine self-administration differentially affects A2A-D2 receptor-receptor interactions in the dorsal striatum, which would contribute to the development of compulsive drug seeking [134].

5.5.3. Methamphetamine
Methamphetamine transiently increased the expression of the ATP-binding cassette transporter ABCB1 [135]. Striatal A2A and glutamate (mGlu5) receptor interactions modulate the psychomotor and drug-seeking effects of methamphetamine [136]. Methamphetamine self-administration produces selective alterations in adenosine receptor expression in the nucleus accumbens and stimulation of adenosine receptors reduces several behavioural indices of methamphetamine addiction [137]. A2A receptor antagonism in dorsomedial striatum reduces methamphetamine-induced deficits [138]. Adenosine A2A receptors are needed to integrate rewarding and motivational behaviours of methamphetamine [139].

5.5.4. Nicotine
Treatment with A2A receptor agonists can help counteract the nicotine addiction [140].

5.5.5. Alcohol
A2A receptors were suggested to be a potential target for the treatment of alcohol abuse [141, 142]. A review focussed on the regulation of adenosine signalling in striatal circuits in alcohol addiction is available [143]. A1 receptor signalling may play a role in regulating basolateral amygdala excitability, contributing to the pathophysiology of alcohol addiction [144]. P2X4 receptors have been claimed to modulate synaptic signalling associated with alcohol addiction [145]. A recent review about alcohol addiction includes consideration of the role of adenosine [146].
6. NEUROPATHIC PAIN
Evidence for the involvement of both P1 and P2 receptors in neuropathic pain has been reviewed [9, 147-149]. The discovery by Inoue and colleagues that antagonists to P2X4 and P2X7 receptors on microglia were effective against neuropathic pain was particularly important (see [150, 151]). P1 and P2Y receptors have also been shown to be involved (see [152]). A3 receptor agonists prevent the development of neuropathic pain [153]. Glial P2Y2 receptors have been proposed as potential targets for the management of trigeminal-related pain [154]. An interaction between pannexin 1 and P2X7 receptors has been implicated in chronic pain [155]. The expression levels of P2X1 and P2X5 receptors increased in neuropathic pain [156].

7. COGNITIVE IMPAIRMENT
Earlier papers reported the involvement of P1 receptors in the cognitive impairment associated with Alzheimer’s disease as well as physiological aging (see [9, 157]). It has been suggested that blockade of either A1 or A2A receptors may be potentially effective for the treatment of attention-deficit hyperactivity disorder. P2X7 receptors were also implicated. In a recent review, knowledge of the role of glial purinergic signalling in cognitive-related neurological diseases is discussed [158]. Activation of P2Y1 receptors in the medial prefrontal cortex impairs cognition [159, 160]. TNP-ATP, a selective antagonist at P2X1 and P2X3 receptors, was beneficial for the treatment of neonatal hypoxia-induced hypomyelination and cognitive decline ([161]; see also the review by Guzman and Gerevich [162]).

8. BRAIN TUMOURS
Neuroblastoma, a tumour in childhood, expresses P2X7 receptors, which appear to mediate proliferation [163]. In a later paper, it was reported that P2X7 receptors mediate modulation of myeloid-derived suppressor cell functions in the neuroblastoma microenvironment [164]. Data was presented to suggest that the P2X7 receptor is an upstream regulator of the main signalling pathways involved in neuroblastoma growth, metabolic activity and angiogenesis and it was suggested that it may be a promising therapeutic target for neuroblastoma treatment [165]. The effect of temozolomide, an antitumor agent, was potentiated by the antiproliferative actions of antagonists to A3, P2Y1 and P2X7 receptors [166].

P2X7 receptors were found to be over-expressed in human gliomas and antagonists resulted in a decrease in tumour cell number, suggesting that they may be a therapeutic target for human glioma proliferation [167]. CD38, a multifunctional enzyme, decreased intracellular levels of ATP and mediated reduction of C6 glioma cells and it was suggested that P2X receptors contributed to this action [168]. It was claimed that K_ATP channels are associated with cell proliferation and tumorigenesis of human glioma [169]. Extracellular nucleotides control glioma growth via P2X7 and P2Y6 receptor activation, resulting in secretion of inflammatory cytokines; growth was reduced with antagonists to these receptors [170]. A2A and P2X7 receptor activation is necessary for release of cytokines by macrophages exposed to glioma-conditioned medium; this was prevented by antagonists to these receptors [171]. BMS-536924, an ATP-competitive insulin-like growth factor receptor inhibitor, decreased viability and migration of glioma cells in vitro and suppressed tumour growth in vivo [172].

9. NEUROAIDS
It has been suggested that P2X4 receptors may be novel therapeutic targets for restricting synaptodendritic injury and neurodegeneration that accompanies neuroAIDS and opiate abuse [173]. Astrocytes mediate HIV-1 Tat-induced neuronal damage via P2X7 receptors and P2X receptor antagonists reduced HIV Tat-induced neuronal death, suggesting that it would be a novel target for therapeutic management of neuroAIDS [174].

10. SLEEP DISORDERS
Evidence was presented to suggest that P2Y11 receptors are associated with narcolepsy [175]. Endothelial dysfunction in cerebral arteries, due to reduced dilation to ATP, occurred in a rat model of obstructive sleep apnoea [176]. Adenosine was identified as one of the players in the regulation and maintenance of sleep-wake dependent changes in neural activities; dysregulation leads to sleep-wake disorders [177].

CONCLUSIONS
This review has aimed at updating information about the roles of purinergic signalling in a wide variety of disorders of the CNS. The use of P2X7 and A2A receptor antagonists for therapeutic treatment in particular is gaining attention.

LIST OF ABBREVIATIONS:
ATP - Adenosine 5'-triphosphate
ALS - Amyotrophic lateral sclerosis
CNS – Central nervous system
MS - Multiple sclerosis

CONFLICT OF INTEREST
The author confirms that this article content has no conflicts of interest.
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