Purinergic signalling and disorders of the central nervous system

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Abstract | Purines have key roles in neurotransmission and neuromodulation, with their effects being mediated by the purine and pyrimidine receptor subfamilies, P1, P2X and P2Y. Recently, purinergic mechanisms and specific receptor subtypes have been shown to be involved in various pathological conditions including brain trauma and ischaemia, neurodegenerative diseases involving neuroimmune and neuroinflammatory reactions, as well as in neuropsychiatric diseases, including depression and schizophrenia. This article reviews the role of purinergic signalling in CNS disorders, highlighting specific purinergic receptor subtypes, most notably A_{2A}, $P2X_4$ and $P2X_7$, that might be therapeutically targeted for the treatment of these conditions.

Taenia coli
The three separate longitudinal ribbons of smooth muscle on the outside of the ascending transverse, descending and sigmoid colons.

The concept of purinergic neurotransmission was proposed in 1972 (REF. 1), after it was shown that adenosine 5′-triphosphate (ATP) was a transmitter in non-adrenergic, non-cholinergic (NANC) inhibitory nerves supplying the guinea-pig taenia coli[2]. Later it was shown that ATP was a cotransmitter in sympathetic and parasympathetic nerves[3] and it is now recognized that ATP is a cotransmitter in all nerve types in both the peripheral and central nervous systems[4]. Separate membrane receptors for adenosine (ADO) and ATP were identified in 1978 and called P1 and P2 receptors, respectively[5]. P2 receptors were then divided into P2X and P2Y receptors on the basis of pharmacology[6] and molecular cloning[7]. Currently, 4 subtypes of P1 receptor, 7 subtypes of ionotropic P2X receptors, many of which can form heteromultimers and homomultimers, and 8 subtypes of metabotropic P2Y receptors are known to exist[8–10].

Since 1992, there has been an explosion of interest in purinergic neurotransmission and neuromodulation in different regions of the brain and spinal cord[4,11]. The various purinergic receptor subtypes are widely distributed throughout the central nervous system (CNS) (FIG. 1, TABLE 1) and they control local network behaviours by regulating the balance between the release and effects of ATP, ADO and ectonucleotidases on synaptic transmission[12]. Multiple purinergic receptors have also been identified on glial cells, including astrocytes, oligodendrocytes and microglia[13] (TABLE 1) and important purinergic mechanisms involving neuron–glial cell interactions have been described[14,15]. Astrocytes are intimately associated with neurons and, through their extensive contacts with synapses, they are able to regulate synaptic transmission (BOX 1). In addition to providing physiological modulatory actions, glial cells are also involved in neurological disorders and psychiatric states.

Most studies of the extracellular actions of ATP have been concerned with the short-term events that occur in neurotransmission and neuromodulation in the CNS, and the involvement of purinergic signalling in these processes is now well established. However, purines and pyrimidines can also have potent long-term (trophic) roles in cell proliferation and growth, as well as in disease and cytotoxicity[16]. ATP can act as a growth and trophic factor, altering the development of neurons[17] and glia[18] by regulating two important second messengers: cytoplasmic Ca^{2+} and cyclic adenosine monophosphate (cAMP). Moreover, the release of ATP by neural activity provides a mechanism that links functional activity in neural circuits to growth and differentiation of cells in the nervous system. Different effects, such as mitogenesis and apoptosis, might be induced depending on the functional state of glial cells, the expression of selective receptor subtypes, ectoenzymes controlling the availability of ATP and ADO and the presence of multiple receptors on the same cells.

Although it was originally thought that apart from the ATP released from nerves, the main source of ATP to act on P2 receptors was damaged or dying cells, it is now known that ATP is released from many cell types, including glial cells, in response to mechanical deformation, hypoxia or some agents (such as acetylcholine, ATP and thrombin) which do not damage the cell. However, there is active debate about the precise transport mechanism(s) involved in ATP release (BOX 2).

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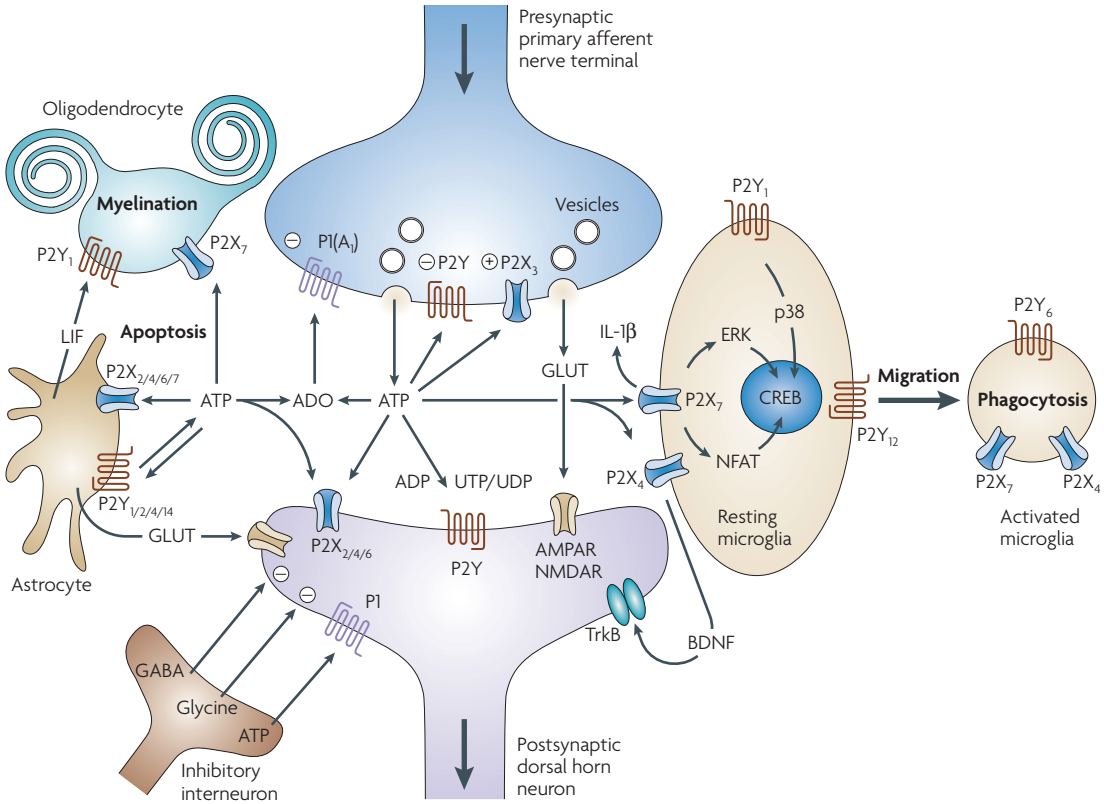

Figure 1 | **Purinergic signalling in the spinal cord.** Presynaptic primary afferent nerve terminals in the dorsal horn of the spinal cord are depicted releasing both glutamate (GLUT) and ATP as cotransmitters by exocytosis. The released ATP acts postsynaptically on $P2X_{2/4/6}$ and on various P2Y receptor subtypes activated by ADP, UTP and UDP, as well as ATP. Glutamate acts postsynaptically on α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptors (AMPARs) and/or *N*-methyl-D-aspartate receptors (NMDARs). ATP is broken down by ectonucleotidase to adenosine (ADO), which acts as a presynaptic inhibitory modulator through $P1(A_1)$ receptors, but ATP itself can act presynaptically either to inhibit the release of transmitter through P2Y receptors or to enhance the release of glutamate through $P2X_3$ receptors. ATP is also released from astrocytes (and probably also from microglia) together with glutamate to participate in glial–neuron interactions. Both P2X and P2Y receptor subtypes are expressed by astrocytes. Leukaemia inhibiting factor (LIF) released by astrocytes in response to ATP promotes myelination in oligodendrocytes and re-myelination through $P2Y_1$ receptors. $P2X_7$ receptors on oligodendrocytes mediate apoptosis. Resting microglia express $P2X_4$ and $P2X_7$ receptors involved in neuropathic pain. ATP, through $P2X_7$ receptors, promotes IL-1β release. Occupation of $P2X_4$ receptors leads to release of brain-derived neurotrophic factor (BDNF) to act on TrkB receptors expressed by neurons in the pain pathway. Occupation of $P2X_7$ receptors leads, through ERK and/or nuclear factor of activated T cells (NFAT), to activation of the transcription factor cAMP response element-binding protein (CREB), whereas $P2Y_1$ receptors also activate CREB, but through p38 signalling. $P2Y_{12}$ receptors on resting microglia mediate cell migration after injury, whereas $P2Y_6$ receptors that are expressed on the activated amoeboid microglia mediate phagocytosis of debris at the site of damage. Inhibitory interneurons that corelease γ-aminobutyric acid (GABA), glycine and ATP modulate the nociceptive pathway.

Purinergic signalling might also be involved in various behavioural pathways, but apart from brainstem control of autonomic functions[4], relatively few studies on this exist. ATP coreleased with glutamate induces long-term potentiation in CA1 neurons that are associated with learning and memory[19,20]. The hypnotic/sedative (somnogenic) actions of ADO are well known as are the central stimulant actions of methylxanthine antagonists[21]. ADO also exerts central inhibitory effects on spontaneous locomotor activity in rodents, which is antagonised by caffeine. A_{2A} receptors in the nucleus accumbens mediate this locomotor depression[22]. $P2X_2$ receptors are expressed by all hypothalamic hypocretin/orexin neurons, where they might be involved in arousal and wakefulness[23], and in the cerebellum, where they appear to be associated with motor learning and coordination[24]. In the striatum, extracellular ATP and ADO are involved in the regulation of the feeding-associated mesolimbic neuronal activity in an antagonistic manner[25] and increased hypothalamic $P2Y_1$ receptor expression is associated with enhanced food intake[26].

This Review will discuss the involvement of purinergic signalling and specific receptor subtypes in the pathophysiology of CNS disorders. It will emphasize the most recent findings in this field and focus on the therapeutic potential of targeting specific purinergic receptors to treat such conditions.

CNS injury

Trauma. Cellular damage can result in the release of large amounts of ATP into the extracellular environment, which might be important for triggering cellular responses to trauma[27]. Mechanical strain also causes ATP release from cortical astrocytes, leading to protein kinase B (AKT) activation. Interestingly, the P2 receptor antagonist, pyridoxal phosphate-6-azophenyl-2-4-disulphonic acid (PPADS) can attenuate this AKT activation[28]. Such activation of purinergic receptors coupled to protein kinase cascades regulates the expression of genes involved in long-term, trophic actions[29]. For example, trauma-induced activation of purinergic signalling in astrocytes via $P2Y_4$ receptors stimulates the synthesis and release of thrombospondin-1, an extracellular matrix molecule that induces synapse formation during development and might have a role in CNS repair and remodelling after injury[30].

In vivo, ATP released from astrocytes is essential for mediating the injury-induced defensive responses of microglia[31], establishing a potential barrier between the healthy and injured tissue[32]. However, in some cases, ATP might also contribute to the pathophysiology initiated after trauma[33]. Following brain trauma, activated $P2Y_{12}$ and probably $P2X_4$ receptors[34,35] stimulate the migration and chemotaxis of resting microglia to the site of damage, where they become transformed into the activated amoeboid form; an effect that is replicated by ATP[36] (Supplementary information S1 (figure)). In addition, $P2Y_6$ receptors are upregulated to limit secondary damage by mediating the phagocytosis of debris[37]. Accumulation of $P2X_4$ receptor-positive microglia and macrophages following experimental traumatic brain injury and spinal cord injury has been described[38]. Activated microglia also show significant changes in $P2X_7$ receptor expression, which have an important role in controlling microglial proliferation and death[39,40].

Following neuronal injury, ATP can also act in combination with fibroblast, epidermal and platelet-derived growth factors, as well as nerve growth factor (NGF) from both neurons and glial cells[16] to stimulate astrocyte proliferation, contributing to the process of reactive astrogliosis and to hypertrophic/hyperplastic responses[29]. P2 receptors stimulate the signal transducer and activator of transcription 3 (STAT3), suggesting that P2 receptor/STAT3 signalling could have an important role in astrocyte proliferation and reactive astrogliosis[41]. P2Y receptors mediate reactive astrogliosis, via induction of cyclooxygenase-2 (COX2), and P2Y receptor antagonists might counteract excessive COX2 activation in both acute and chronic neurological disease[42].

Cerebellar lesions result in upregulation of $P2X_1$ and $P2X_2$ receptors in precerebellar nuclei[43], and stab wound injury in the nucleus accumbens leads to increased expression of several subtypes of P2X and P2Y receptors[27]. A novel mechanism for inhibition of apoptosis in neuroprotection implicates parallel, interacting systems involving extracellular ATP acting through $P2Y_2$ receptors and neurotrophin acting through TrkA receptors[44]. It has also been claimed that $P2Y_2$ receptors activate neuroprotective mechanisms in astrocytes[45].

ATP released during trauma acts through P2 receptors to inhibit the release of the cytotoxic excitatory transmitter glutamate, but also stimulates the release of the inhibitory transmitter γ-aminobutyric acid (GABA) from hippocampal nerves, thus serving a protective role[46]. The number of $P2Y_1$ receptor-positive neurons and glial cells in the rat nucleus accumbens has been shown to be significantly increased after injury[47]. Oligodendrocytes can be killed by ATP, as well as by glutamate, released from damaged brain tissue in trauma injury or stroke, probably through $P2X_7$ receptors[48].

A number of studies illustrate potential therapeutic strategies that might be adopted following trauma (see TABLES 2,3). A role for $P2X_7$ receptors, which are highly expressed on spinal cord neurons, in mediating spinal cord injury has been proposed[49].

Cerebral ischaemia. Ischaemia can produce and exacerbate many serious insults to the CNS, including stroke and paralysis. ADO has an important protective role against ischaemic damage in the brain[50], although ATP, rather than ADO has been claimed to accelerate recovery from hypoxic/hypoglycaemic perturbation through a P2 receptor[51,52]. After transient forebrain ischaemia, ectonucleotidase is upregulated and there is an increased release of purines into cerebral cortical perfusates[53]. Upregulation of $P2X_2$ and $P2X_4$ receptors in cell cultures of hippocampus, cortex and striatum is associated with ischaemic cell death and was prevented by P2 receptor antagonists[54]. Rapid ischaemic release of ADO occurs via a Ca^{2+}-independent mechanism, with a subsequent, Ca^{2+}-dependent release of ATP only during anoxic depolarisation, suggesting that the release of these purines is governed by distinct temporal and mechanistic processes[55].

Following ischaemia, $P2X_7$ receptors are upregulated on neurons and glial cells in rat cerebral cortex[56,57], and become hypersensitive in cerebrocortical cell cultures[58], although earlier studies showed that deletion of $P2X_7$ receptors (knockout mice) and/or treatment with the $P2X_7$ antagonist KN62 had little effect on ischaemic cell death[59]. Microglial $P2X_4$ and $P2X_7$ receptors might be involved in cortical damage produced by oxygen and/or glucose deprivation[60] and activation of P2X receptors contributes to the ischaemia-induced facilitation of glutamate release[61].

Cortical spreading depression releases ATP into the extracellular space in rat cortex and the subsequent activation of P2Y receptors makes a major contribution to the induction of ischaemic tolerance in the brain[62,63]. The transcription cofactor, LMO4, is a rapidly induced downstream effector of ATP signalling that promotes neuron survival following hypoxia[64]. Pretreatment with cerebrocrast, a 1,4-dihydropyridine derivative, is claimed to prevent ischaemic brain damage and promote ATP production in brain cells[65].

Neurodegenerative diseases

P2Y receptor antagonists have been proposed as potential neuroprotective agents in the cortex, hippocampus and cerebellum following neuronal death associated

Cortical spreading depression
A short lasting wave of depolarization that spreads through the cerebral cortex.

Table 1 | **Characteristics of purine and pyrimidine receptors**

Receptor		Main distribution	Main functions
P1 (adenosine)	A_1	Brain, spinal cord, testis, heart, autonomic nerve terminals	Prejunctional neuromodulation of neurotransmitter release; behavioural effects (sedation, anticonvulsive, anxiolytic); cardiac depression
	A_{2A}	Brain, heart, lungs, spleen	Facilitates neurotransmission; smooth muscle relaxation
	A_{2B}	Large intestine, bladder	Role in allergic and inflammatory disorders; vasodilatation
	A_3	Lung, liver, brain, testis, heart	Facilitates release of allergic mediators; cardio- & cytoprotective
P2X	$P2X_1$	Smooth muscle, platelets, cerebellum, dorsal horn spinal neurons	Smooth muscle contraction; platelet activation
	$P2X_2$	Smooth muscle, CNS, retina, chromaffin cells, autonomic and sensory ganglia	Sensory transmission & modulation of synaptic function
	$P2X_3$	Sensory neurons, NTS neurons, some sympathetic neurons	Mediates sensory transmission; facilitates glutamate release in CNS
	$P2X_4$	CNS, testis, colon	Modulates chronic inflammatory & neuropathic pain
	$P2X_5$	Proliferating cells in skin, gut, bladder, thymus, spinal cord	Inhibits proliferation & increases differentiation
	$P2X_6$	CNS, motor neurons in spinal cord	Functions as a heteromeric channel in combination with $P2X_2$ & $P2X_4$ subunits
	$P2X_7$	Apoptotic cells in, for example, immune system, pancreas and skin	Mediates apoptosis, cell proliferation & pro-inflammatory cytokine release
P2Y	$P2Y_1$	Epithelial and endothelial cells, platelets, immune cells, osteoclasts, glial cells	Smooth muscle relaxation & mitogenic actions; platelet shape change & aggregation; bone resorption
	$P2Y_2$	Immune cells, epithelial and endothelial cells, kidney tubules, osteoblasts, astrocytes	Vasodilatation through endothelium & vasoconstriction through smooth muscle; mitogenic actions; mediates surfactant secretion; epithelial cell Cl^- secretion; bone remodelling
	$P2Y_4$	Endothelial and epithelial cells, intestine, pituitary, brain (low levels in liver and bone marrow)	Regulates epithelial Cl^- transport; vasodilatation through endothelium; mitogenic actions
	$P2Y_6$	Some epithelial cells, placenta, T cells, thymus, spleen, kidney, activated microglia	NaCl secretion in colonic epithelium; role in epithelial proliferation
	$P2Y_{11}$	Spleen, intestine, brain, granulocytes	Role in maturation & migration of dendritic cells; granulocytic differentiation
	$P2Y_{12}$	Platelets, glial cells, spinal cord	Platelet aggregation; role in dense granule secretion
	$P2Y_{13}$	Spleen, brain, lymph nodes, bone marrow, liver, pancreas, heart	Function largely unknown, but present in both the immune system and brain
	$P2Y_{14}$	Placenta, adipose tissue, stomach, intestine, discrete brain regions, spleen, lung, heart, bone marrow, peripheral immune cells	Chemoattractant receptor in bone marrow hematopoietic stem cells; dendritic cell activation

Abbreviations: CNS, central nervous system; NTS, nucleus tractus solarius.

with neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, Huntington's disease and amyotrophic lateral sclerosis (ALS)[66]. It has been suggested that adenine is involved in the control of Purkinje cell survival[67].

Microglia might help fight infection in the CNS, but over-stimulation of microglia could accelerate neuronal damage caused by neurodegenerative diseases that exhibit microglial proliferation and activation[68]; P2Y agonists might be a potential treatment for toxic immunoreactions. Cross-talk between neurons and mast cells has also been implicated in neurodegenerative diseases with an inflammatory and/or autoimmune component, such as Alzheimer's disease and multiple sclerosis (MS)[69].

Parkinson's disease. In Parkinson's disease there is a progressive loss of dopaminergic neurons of the substantia nigra pars compacta projecting to the striatum. The dopamine precursor L-3,4-dihydroxyphenylalanine

Box 1 | **Astrocyte purinergic signalling**

Following synaptic activity, neurotransmitters acting through glial metabotropic receptors induce the release of glutamate and adenosine 5′-triphosphate (ATP) from astrocytes[213]. By releasing ATP, which is converted in the extracellular space to adenosine, astrocytes exert powerful presynaptic inhibition of synaptic transmission. Rapid electrical signals in neuronal networks interact with slow modulatory signals provided by glia. *In vivo* studies have shown that neuronal networks are under the continuous modulatory control of astrocytes through both purinergic and *N*-methyl-D-aspartate (NMDA) receptor-dependent pathways. ATP enhances the release of glutamate and γ-aminobutyric acid (GABA) from nerve terminals through $P2X_1$, $P2X_3$, $P2X_{2/3}$ and $P2X_7$ receptors[214] and inhibits release through $P2Y_1$, $P2Y_2$ and $P2Y_4$ receptors[215].

(L-DOPA) is still the most commonly prescribed treatment for Parkinson's disease, but long-term treatment with L-DOPA often produces uncontrollable movements known as dyskinesia. Expression of A_{2A} receptors in the brain of patients with Parkinson's disease and dyskinesia is increased[70], and in a rat model of Parkinson's disease, A_1, dopamine D1 and glutamate mGlu5 receptors have been shown to interact during locomotion[71]. Specific A_{2A} receptor antagonists are currently being investigated for the treatment of Parkinson's disease[72].

Release of ATP from disrupted cells might cause cell death in neighbouring cells expressing $P2X_7$ receptors, leading to a necrotic volume increase, which has been implicated in the pathogenesis of Parkinson's disease[73]. Differing expression patterns of the P2 receptor subtypes in the dopaminergic system[74] and the facilitatory action of ATP and glutamate on the effect of taurine on osmolarity could influence the vulnerability of nigral dopaminergic cells in Parkinson's disease[75].

Several non-selective P2 agonists (for example, hydrolysis-resistant adenine and uracil nucleotide analogues) and antagonists (for example, suramin, PPADS, Reactive Blue 2 and Brilliant Blue G) have been available for some time, but it has been only recently that ligands displaying selectivity towards specific P2 receptor subtypes have been identified[8,76].

Alzheimer's disease. ATP release during neuronal excitation or injury can enhance the inflammatory effects of cytokines and prostaglandin E2 in astrocytes and might contribute to the chronic inflammation seen in Alzheimer's disease[77].

$P2X_7$ receptors are upregulated in the brain of patients with Alzheimer's disease and in animal models[78,79]. Stimulation of $P2X_7$ receptors on human macrophages and microglia enhanced the degenerative lesions observed in Alzheimer's disease[80]. $P2X_7$ receptors could therefore represent a therapeutic target for inflammatory responses seen in neurodegenerative disorders. Furthermore, the G51S purine nucleoside phosphorylase polymorphism is associated with a faster rate of cognitive decline in patients with Alzheimer's, highlighting the important role of purine metabolism in the progression of the disease[81].

$P2Y_1$ receptors are expressed on a number of structures that are characteristic of Alzheimer's disease, such as neurofibrillary tangles, neuritic plaques and neuropil threads[82], and $P2Y_2$ receptor activation might mediate a neuroprotective effect[83]. Abnormalities in Ca^{2+}-mediated signal transduction triggered by ATP in microglia from patients with Alzheimer's disease have been reported[84].

Hippocampal presynaptic A_1 receptors are decreased in Alzheimer's disease[85], but accumulate in neurodegenerative structures where they mediate both amyloid precursor protein processing and Tau phosphorylation and translocation[86].

Huntington's disease. A_2 receptors localized on striatal output neurons are dramatically decreased in Huntington's disease[87] and blockade of A_{2A} receptors prevents electroencephalogram (EEG) and motor abnormalities in a rat model of the disease[88]. There is also a decrease of striatal A_1 and A_{2A} receptors in hyperkinetic neurodegenerative movement disorder, which is common in patients with Huntington's disease[89]. In a mouse model of Huntington's disease, a transient increase in A_{2A} expression in early postnatal development is followed by a decrease[90,91]. Changes in P2X receptor-mediated neurotransmission in cortico-striatal projections have been found in two different transgenic models of Huntington's disease[92].

Amyotrophic lateral sclerosis. Potentiation of $P2X_4$ receptors by the anti-parasite medication ivermectin (22,23-dihydroavermectin B_{1a} + 22,23-dihydroavermectin B_{1b}) extends the life span of the transgenic superoxide dismutase 1 (SOD1) mouse model of ALS[93]. Increased expression of $P2X_1$ receptors on axotomized facial motor neurons was impaired in SOD1-G93A-mutant mice after injury[94], perhaps due to the SOD1 mutation interfering with injury-elicited $P2X_1$ activation. This finding suggests that the release of ATP from mutant motor neurons is altered after damage.

Diabetes. Diabetic neuropathy includes central neuropathic complications, such as decreased cognitive performance accompanied by modifications of hippocampal morphology and plasticity[95,96]. It was recently shown that synaptic ATP signalling is depressed in streptozotocin-induced diabetic rats[97] and that the density of $P2X_{3/6/7}$ and $P2Y_{2/6/11}$ receptors was decreased in hippocampal nerve terminals compared with controls. Changes in prejunctional modulation of neurotransmission in the hippocampus of streptozotocin-induced diabetic rats was also shown, namely, downregulation of inhibitory A_1 receptors and upregulation of facilitatory A_{2A} receptors.

Neuroimmune and neuroinflammatory disorders

Contrary to the earlier view that the brain is an immunologically privileged organ unable to mediate an inflammatory response, immuno-mediated reactions do occur in the brain. The CNS can undergo all the typical changes induced by inflammation, activate endogenous inflammatory cells and generate inflammatory mediators[98]. Neurons are surrounded by a dense population of support cells,

Box 2 | **Mechanisms of ATP release**

There is compelling evidence for exocytotic vesicular release of adenosine 5′-triphosphate (ATP) from nerves[216], but for ATP release from non-neuronal cells various other transport mechanisms have been proposed, including ATP-binding cassette transporters, connexin or pannexin hemichannels, and possibly plasmalemmal voltage-dependent anion channels[4,217]. Surprisingly, exocytotic vesicular release of ATP has been shown in endothelial cells, urothelial cells and osteoblasts. Recent papers have also shown vesicular, glutamate-triggered ATP transport from astrocytes[218], which might involve lysosomes[219]; although ATP transport through connexin hemichannels has also been described[220]. Adenosine is largely produced by the ectoenzymatic breakdown of ATP released from neurons, but it is possible that some subpopulations of neurons and/or astrocytes release adenosine directly[221,222]. Extracellular breakdown of ATP released from neurons and non-neuronal cells occurs through ectoenzymes, but the details of the signalling pathway(s) are yet to be determined[223].

astroglia, oligodendroglia and microglia, and biochemical information is exchanged between them. Microglia are immune cells and share all the roles of macrophages in the periphery. Indeed, microglia have a key protective role in CNS trauma and infection and also have roles in regeneration and CNS malfunction. Microglia release several factors that affect neural functions including cytokines, chemokines, growth factors, ATP and activated oxygen and nitrogen species.

Purinergic signalling, involving ATP released from both neurons and glial cells and its breakdown product ADO, appear to have a major role in the neuroimmune and neuroinflammatory events involving microglia[99,100], including neuropathic pain[101,102]. ATP potently activates nuclear factor of activated T cells (NFAT), a central transcription factor involved in cytokine gene expression and could represent a novel mechanism by which extracellular ATP can modulate early inflammatory gene expression within the nervous and immune system[103]. A later paper suggested that $P2X_7$ receptors mediate the phosphorylation of cAMP response-element binding protein (CREB), a putative inhibitory transcription factor in microglia, indicating that ATP might be an endogenous inhibitor or neuroprotective molecule that decreases the inflammatory capacity of microglia[104].

Microglia often need priming by proinflammatory factors such as interleukin (IL)-1β, which is implicated in neurodegeneration, to generate a full immune response. Secretion of IL-1β is the end result of a chain of intracellular events occurring within a multi-molecular structure called the 'inflammasome'[105]. Inflammasome activation and IL-1β release is regulated by various factors, including extracellular ATP acting through $P2X_7$ receptors[106]. ATP also increases 2-arachidonoylglycerol (2-AG) production through $P2X_7$ receptors on microglial cells and because prolonged increases in 2-AG levels in brain parenchyma are thought to orchestrate neuroinflammation, $P2X_7$ receptors could be a target for therapy aimed at controlling exacerbated neuroinflammation. Microglial $P2X_7$ receptors are activated by purines to release inflammatory cytokines such as IL-1β, IL-6 and tumour necrosis factor (TNF)-α[107]. Activated microglia can also act as scavenger cells that induce apoptosis in damaged neurons by releasing toxic factors, including nitric oxide, and then take up the debris by phagocytosis[32]. The $P2X_7$ receptor is involved in the formation of multinucleated giant macrophage-derived cells, a hallmark of chronic inflammatory reactions[108]. Lysophosphatidylcholine, an inflammatory phospholipid, might regulate microglial function by enhancing the sensitivity of $P2X_7$ receptors[109]. Indeed, a recent report showed that prion infection is associated with hypersensitivity of $P2X_7$ receptors in microglia[110].

Expression of the $P2X_4$ receptor by lesion-activated microglia during formalin-induced inflammatory pain has also been reported[111]. Activation of microglial cells by pro-inflammatory bacterial lipopolysaccharide leads to a transient increase in ivermectin-sensitive $P2X_4$ receptor currents, whereas dominant $P2X_7$ receptor currents remain largely unaffected; both receptor subtypes contribute to neuroinflammatory mechanisms and pathologies[112].

Astrocytes can sense the severity of damage in the CNS from the ATP that is released from damaged cells and can modulate the TNF-α-mediated inflammatory response, depending on the extracellular ATP concentration and the type of astrocyte P2 receptor that is activated[113]. Thus, micromolar ATP activation of P2Y receptors might boost a moderate inflammatory response, whereas millimolar ATP activation of P2X receptors might prevent the perpetuation of a comparatively large inflammatory response, perhaps by inducing apoptosis. Protein kinase B/AKT is a key signalling molecule that regulates cell survival, growth and metabolism and inhibits apoptosis[28]. $P2X_7$ receptor activation in astrocytes increases chemokine monocyte chemoattractant protein 1 (MCP1) expression through mitogen-activated protein kinase (MAPK) signalling, and it has been suggested that regulation of MCP1 in astrocytes by ATP could be important in mediating communication with haematopoietic inflammatory cells[114].

Multiple sclerosis. P2 receptors on oligodendrocytic progenitor cells regulate migration, proliferation and differentiation of these cells[115]. In MS lesions of autopsied brain tissue, $P2X_7$ receptors were detected on reactive astrocytes, whereas in cultured astrocytes, $P2X_7$ receptor stimulation increased the production of nitric oxide synthase activity[116]. Interferon-β (IFN-β) has beneficial effects in remitting/relapsing MS, perhaps by preventing astrocyte apoptosis; the levels of apyrase and 5′-nucleotidase increased in synaptosomes from the cerebral cortex of rats that were experimentally demyelinated with ethidium bromide and treated with IFN-β[117], indicating that IFN-β might interfere with the metabolism of purines.

Neuronal pathology is an early feature of MS and the animal model of experimental autoimmune encephalomyelitis (EAE). Lesional accumulation of P2X receptors on macrophages in rat CNS during EAE has

been described[118]. $P2X_7$ expression is elevated in seemingly normal axon tracts in patients with MS and ATP can kill oligodendrocytes by activating $P2X_7$ receptors. Mice deficient in $P2X_7$ receptors are more susceptible to EAE than wild-type mice and show enhanced inflammation in the CNS[119].

NTPase1, an ectonucleotidase that degrades ATP to AMP, is expressed by immuno-suppressive regulatory T cells (Treg) cells. Patients with the remitting/relapsing form of MS have strikingly reduced numbers of NTPDase1-positive Treg cells, suggesting that purines might be involved[120]. A regulatory role of $P2Y_1$ receptor signalling in oligodendrocyte progenitor cells has been observed and it has been suggested that ATP released in high amounts under inflammatory conditions might act on $P2Y_1$ receptors to influence the remyelination processes in MS[115].

Epileptic seizures

Epilepsy affects approximately 1% of the population worldwide and recurring seizures have devastating behavioural, social and occupational consequences, damaging the brain and increasing pre-existing neurological deficits. Current anticonvulsant drugs and complementary therapies are not sufficient to control seizures in about a third of epileptic patients, so there is an urgent need for improved treatments. Epilepsy is often accompanied by massive glial cell proliferation, but the role of these cells in seizures and epilepsy is still unclear.

Microinjection of ATP analogues into the prepiriform cortex induces generalized motor seizures suggesting that P2X receptor antagonists might have potential as neuroleptic agents[121]. Epileptiform activity in the CA3 region of rat hippocampal slices is modulated by adenine nucleotides, probably acting through an excitatory P2X receptor[122]. The hippocampus of chronic epileptic rats shows abnormal responses to ATP associated with increased expression of $P2X_7$ receptors, which are substantially upregulated in chronic pilocarpine-induced epilepsy in rats (perhaps in microglia) and might participate in the pathophysiology of temporal lobe epilepsy[123]. In a study of kainate-provoked seizures, enhanced immunoreactivity of the $P2X_7$ receptor was observed in microglia as they changed from the resting to the activated state[124]. The amount of extracellular ATP detected in hippocampal slices following electrical stimulation of Schaffer collaterals was significantly greater in mice with an inherited susceptibility to audiogenic seizures[125], this is perhaps associated with reduced brain Ca^{2+}-ATPase activity. Uridine is released during epileptic activity and might act as an inhibitory neuromodulator[126], although the underlying mechanism is not known. Increased hydrolysis of ATP occurs in rat hippocampal slices after seizures induced by quinolinic acid[127]. There is a decrease of presynaptic P2X receptors in the hippocampus of rats that have suffered a convulsive period, which might be associated with the development of seizures and/or neurodegeneration during epilepsy[128]. Glutamate released from astrocytes by ATP has also been implicated in epileptogenesis[129].

P1 (ADO) receptors might also have a role in epileptic seizures[130,131]. Decreased extracellular ADO levels and altered A_1 and P2 receptor activation caused by hypercapnia in hippocampal slices provide a plausible mechanism for hyperventilation-induced epileptic seizures in vulnerable humans[132]. A lower density of P1 (A_1) receptors in the nucleus reticularis thalami in rats with genetic absence epilepsy has been reported[133].

Neuropsychiatric disorders

Mood and motivation: depression and anxiety. Reduced adenosinergic activity is involved in mania and aggressive behaviour[134] and A_{2A} receptors have been implicated in panic disorder[135]. ADO has been reported to interact with two potent mood regulators: the psychotomimetic phencyclidine and with alcohol[136]. Striatal A_{2A} receptors appear to be important mediators of the molecular and behavioural sequelae following administration of the antipsychotic drug haloperidol[137]. There is selective attenuation of psychostimulant-induced behavioural responses in mice lacking A_{2A} receptors[138]. Caffeine is probably the most widely used psychologically active drug for many psychomotor variables[139].

Purinergic stimulation via inosine and hypoxanthine can produce an anxiety response that is related to the benzodiazepine receptor[140]. Mice lacking the A_1 receptor showed signs of increased anxiety[141] whereas stimulation of $P2Y_1$ receptors in the dorsomedial hypothalamus had anxiolytic-like effects[142]. Chronically administered guanosine has anxiolytic effects in mice that are perhaps associated with modulation of glutamatergic excitation[143], although receptors for guanosine have not been identified yet.

An antidepressant effect of ADO has been reported in mice, apparently involving A_1 and A_{2A} receptors[144]. Major depressive illness is associated with significant elevation in the density of microglia and in circulatory levels of pro-inflammatory cytokines[145]. The $P2X_7$ receptor gene has been shown to be involved in both major depressive illness[146] and bipolar affective disorders[147]. Electroconvulsive therapy is considered one of the most effective treatments for major depression[148]. The possibility that high levels of ATP are released with electroconvulsive therapy does not appear to have been considered.

The inhibitory action of dilazep (a nucleoside transport inhibitor) on clonidine-induced aggressive behaviour was mostly attributed to central purinoceptor stimulation[149]. Suramin blocked the conditioned fear response in a rat model, suggesting that P2 receptors might be involved in fear behaviour[150]. A_1 receptor activation selectively impairs the acquisition of fear conditioning in rats[151]. An A_{2A} receptor genetic polymorphism has been implicated in panic disorder. P2 receptors of the mesolimbic-mesocortical system, probably of the $P2Y_1$ subtype, are involved in the release of transmitters such as dopamine and glutamate, which are responsible for the generation and pattern of behaviour after motivation-related stimuli[152]. Evidence from A_{2A} receptor knockout mice suggests that A_{2A} receptors are involved in goal-directed behaviour[153]. Glial P1 receptors have also been implicated in mood disorders[154].

Hypercapnia
Abnormally high levels of carbon dioxide in the blood.

Genetic absence epilepsy
A type of epilepsy with non-convulsive seizures.

Panic disorder
An anxiety disorder characterized by recurrent inappropriate and sudden attacks of fear.

Schizophrenia. The involvement of ATP receptors in schizophrenia has been discussed in relation to reports that antipsychotic drugs such as haloperidol, chlorpromazine and fluspirilene inhibit ATP-evoked responses mediated by P2X receptors[155]. It was suggested that ATP might facilitate dopaminergic neurotransmission and that various antipsychotic drugs suppress dopaminergic hyperactivity through inhibition of P2X receptor-mediated effects. ADO might also contribute to the pathophysiology of schizophrenia[156] and ADO–dopamine interactions in the ventral striatum have been implicated[157]. A_{2A} and D_2 receptor heterooligomerization has been postulated[158]. A hypothesis in which dysfunction of purinergic signalling (for example, decreased ATPase activity in erythrocytes, leading to increased levels of ATP and decreased ADO) could lead to schizophrenia has been put forward[159]. Striatal A_{2A} receptors are upregulated in schizophrenia[160] and transgenic overexpression of ADO kinase in brain might lead to altered sensitivity to the psychomimetic drugs that are commonly used to treat this disorder[161].

Alcohol and drug addiction. Addiction is a chronic relapsing neurological disorder in which ADO A_{2A}, P2X and P2Y receptors have been implicated[4]. For example, specific involvement of A_{2A} receptors in the addictive properties of cannabinoids has been reported. The lack of A_{2A} receptors in knockout mice diminishes the addictive-reinforcing efficacy of cocaine. A number of studies have suggested that opioids can modulate ADO signalling. Morphine has been shown to release purines in brain and spinal cord, and opioid analgesia can be at least partially antagonised by P1 ADO receptor antagonists. In animals withdrawn from chronic treatment with either morphine or cocaine, there are persistent increases in extracellular ADO in the ventral tegmental region, a brain region that is intimately involved in the rewarding effects of these drugs. Heroin administration seems to enhance the catabolism of ADO in the brain by increasing ADO deaminase. $P2Y_1$ receptors were upregulated in both astrocytes and neurons in the striatum and nucleus accumbens of rats treated for 5 days with amphetamine[162].

Although ethanol is probably the oldest and most widely used psychoactive drug, the cellular mechanisms by which it affects the nervous system are poorly understood. Some insights in relation to purinergic P2 receptor signalling have emerged in recent years[163]. Ethanol inhibits P2X receptor-mediated responses of dorsal root ganglion neurons by an allosteric mechanism. In the case of $P2X_4$ receptors, ethanol inhibition is altered by mutation of histidine 241 in the rat. Furthermore, ethanol differentially affects ATP-gated $P2X_3$ and $P2X_4$ receptor subtypes expressed in *Xenopus* oocytes. Finally, A_1 receptor activation has been shown to mediate ethanol-induced inhibition of stimulated glutamate release in the hippocampus of near-term fetal guinea-pig.

Neuropathic pain

ATP released locally can initiate pain pathways through $P2X_3$ and $P2X_{2/3}$ receptors located on sensory fibres in visceral organs, the tongue and the skin[164,165]. $P2X_3$ receptors — probably those located on primary afferent nerve terminals in the inner lamina II of the spinal cord (FIG. 2) — also have a significant role in neuropathic and inflammatory pain[166,167]. $P2X_2$, $P2X_4$ and $P2X_6$ receptors have been located on dorsal horn neurons relaying nociceptive information further along the pain pathway[168]. In addition, ATP coreleased with GABA in spinal interneurons is probably involved in modulation of nociceptive pathways[169]. Importantly, it has been shown that $P2X_7$ receptors in microglia are also involved in neuropathic pain, although the underlying mechanisms involving both $P2X_4$ and $P2X_7$ receptors are still not clear. P2X receptor activation in the spinal cord might also elicit allodynia, with $P2X_4$ receptor upregulation in spinal cord microglia having a crucial role [170]. These observations have led to an explosion of work focused on purinergic signalling in neuropathic pain[165,171–173].

Following the initial discovery by Tsuda *et al.*[170], there have been a number of papers investigating the role of $P2X_4$ receptors in spinal microglia in neuropathic pain[172]. Brain-derived neurotrophic factor (BDNF) is released from microglia by the stimulation of $P2X_4$ receptors that affect anion reversal potentials in spinal lamina I neurons[174]. The increased levels of spinal fibronectin following peripheral nerve injury have been implicated in the upregulation of microglial $P2X_4$ receptors [175]. Ligands for the innate immune system sensor: toll-like receptors, and the nucleotide-binding oligomerization domain 2 receptors stimulate microglial $P2X_4$ receptor upregulation, suggesting that microglia sense the activation of an inflammatory response using multiple recognition systems[176]. Recently, LYN, a member of SRC-family kinases, was also shown to have an important role in the pathogenesis of neuropathic pain and could be a key mediator of nerve injury-induced upregulation of $P2X_4$ receptors (K. Inoue, personal communication). Enhancement of pain behaviour after nerve injury not only requires the P2X receptor, but also phospho38 (p38) MAPK[177]. ATP causes the activation of p38 or ERK1/2 MAPKs resulting in the release of TNF-α and IL-6. In rats exhibiting allodynia, the level of p38 was increased in microglia. Intraspinal administration of the p38 inhibitor, SB203580, suppressed allodynia, suggesting that neuropathic pain hypersensitivity depends on the activation of the p38 signalling pathway in microglia in the dorsal horn following peripheral nerve injury. Platelet activating factor, which is released from activated microglia, is a potent inducer of tactile allodynia and thermal hyperalgesia after intrathecal injection into the spinal cord, and it was suggested that this response is mediated by ATP[178]. The possible mechanisms that underlie the role of $P2X_4$ receptors in neuropathic pain and the involvement of inflammatory cytokines have been reviewed in REFS 179,180.

The $P2X_7$ receptor, by regulating IL-1β production, also has a role in the development of neuropathic and inflammatory pain[181]. Data from $P2X_4$ and $P2X_7$ receptor knockout animals indicate that they share a common pain phenotype, although this phenotype appears to be conferred through different mechanisms[182]. One report suggests that $P2X_4$ and $P2X_7$

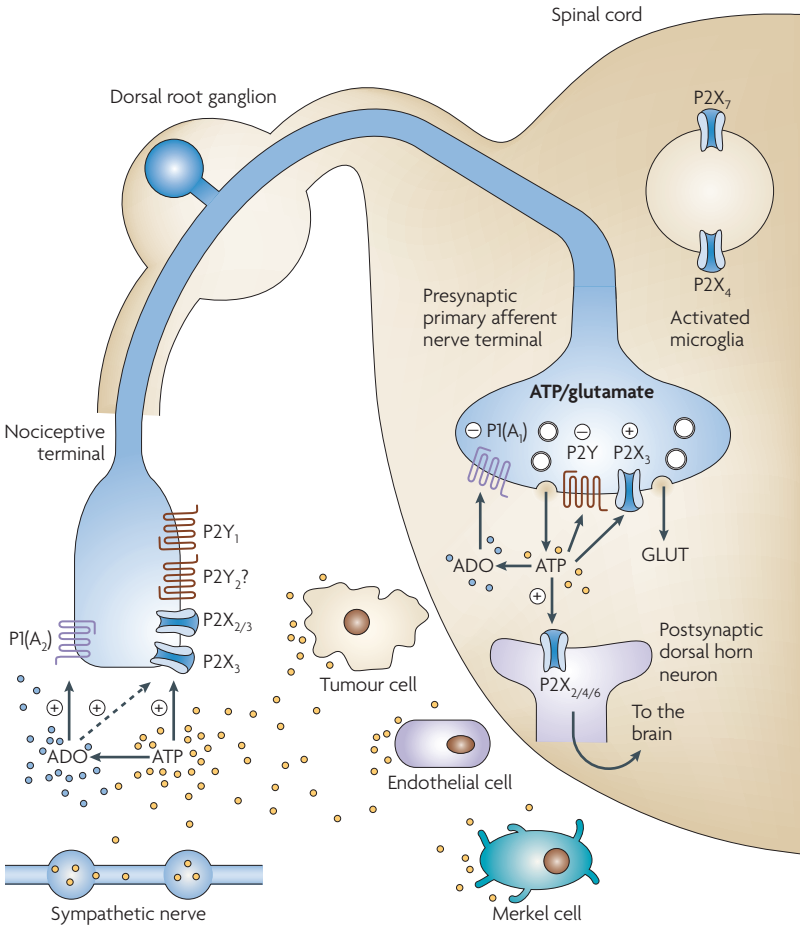

Figure 2 | **Hypothetical schematic of the roles of purine nucleotides and nucleosides in pain pathways.** At sensory nerve terminals in the periphery, $P2X_3$ and $P2X_{2/3}$ receptors have been identified as the main P2X purinoceptors, although more recent studies have also shown expression of $P2Y_1$ and possibly $P2Y_2$ receptors on a subpopulation of $P2X_3$ receptor-immunopositive fibres. Other known P2X receptor subtypes (1-7) are also expressed at low levels in dorsal root ganglia. Although less potent than ATP, adenosine (ADO) also appears to act on sensory terminals, probably directly through $P1(A_2)$ receptors; however, it also acts synergistically (dashed line) to potentiate $P2X_{2/3}$ receptor activation; 5-hydroxytryptamine, capsaicin and protons might also have this effect. At synapses in sensory pathways in the CNS, ATP appears to act postsynaptically through $P2X_2$, $P2X_4$ and/or $P2X_6$ receptor subtypes, perhaps as heteromultimers, and after breakdown to ADO, it acts as a presynaptic inhibitor of transmission through $P1(A_1)$ receptors. ATP and glutamate are cotransmitters in primary afferent central nerve terminals (see FIG. 1). $P2X_3$ receptors on the central projections of primary afferent neurons in lamina II of the dorsal horn mediate facilitation of glutamate and probably also ATP release. $P2X_4$ and $P2X_7$ receptors on activated microglia have been implicated in neuropathic pain. Sources of ATP acting on $P2X_3$ and $P2X_{2/3}$ receptors on sensory terminals include sympathetic nerves, endothelial, Merkel and tumour cells. Yellow dots, molecules of ATP; blue dots, molecules of ADO. Modified, with permission, from REF. 224 © (1996) Elsevier.

Reactive hyperaemia
A transient increase in blood flow following ischaemia.

Cerebral vascular vasospasm
A sudden constriction of blood vessels in the brain.

receptors form heteromultimers[183], which probably have a different pharmacological profile from the homomultimer receptors.

In contrast to the P2X receptors, activation of UTP-sensitive $P2Y_2$ and/or $P2Y_4$ receptors and the UDP-sensitive $P2Y_6$ receptor, inhibit spinal pain transmission[184]. $P2Y_1$ and $P2Y_4$ receptors were identified in sensory neurons, in a subpopulation of which $P2X_3$ receptors were also expressed[185]. P2Y receptors are expressed on the sensory ganglia, neurons in the dorsal spinal cord and in glial cells[186]. The rostral ventromedial medulla serves as a crucial link in bulbo-spinal nociceptive modulation and it has been suggested that on-cells preferentially express P2X receptors, whereas off-cells express P2Y receptors in this region[187]. Activation of P2Y receptors inhibits $P2X_3$ receptor channels through G protein-dependent facilitation of their desensitisation[188].

A number of studies have demonstrated the therapeutic potential of modulating specific P2X receptor subtypes to treat neuropathic pain (TABLE 2). Intrathecal administration of ATP produces long-lasting allodynia, most probably through $P2X_{2/3}$ receptors[189]. The involvement of spinal $P2X_2$ and $P2X_3$ receptors in neuropathic pain in a mouse model of chronic constriction injury has been claimed[190]. A recent study suggests that $P2X_3$/$P2X_{2/3}$ receptor-dependent cytosolic phospholipase A_2 ($cPLA_2$) activity in primary sensory neurons is a key event in neuropathic pain and that $cPLA_2$ might also be a potential drug target[191]. It is claimed that sensitisation of $P2X_3$ receptors, rather than a change in ATP release, is responsible for neuropathic pain and allodynia[192]. There are data suggesting that the $P2X_3$ and $P2X_{2/3}$ receptor antagonism that reduces inflammatory hyperalgesia and chemogenic nociception is mediated by the spinal opioid system[193]. The role of $P2X_3$ receptors in acute pain, inflammatory pain, chronic neuropathic pain, migraine and cancer pain is reviewed in REF. 194.

As neuropathic pain and allodynia are abolished in both $P2X_4$ and $P2X_7$ knockout mice, there is great interest in finding selective antagonists that might be suitable for therapeutic development. Recent reviews on the role of $P2X_7$ receptors in pain and inflammation highlight the potential therapeutic benefit of $P2X_7$ receptor modulation[195,196]. Antidepressants have been shown to be effective in relieving neuropathic pain[197] and preliminary clinical studies with paroxetine, which antagonises $P2X_4$ receptors in transfected cells, suggest that it is effective against chronic pain. Classical antidepressants, such as sertraline and clomipramine, have also been shown to inhibit extracellular breakdown of ATP thereby modulating ATP and ADO levels in the synaptic cleft[198]. There is still an urgent need to understand the various mechanisms underlying the successful application of $P2X_3$, $P2X_4$ and $P2X_7$ receptor antagonists for the treatment of pain, neurodegenerative disorders and trauma. For a review of the recent progress in the development of ADO receptor ligands as anti-inflammatory agents, readers are referred to REF. 199.

Migraine

The involvement of ATP in migraine was first suspected in conjunction with the vascular theory of this disorder, which proposes that ATP is released from endothelial cells during the reactive hyperaemia that is associated with pain following cerebral vascular vasospasm (that is not associated with pain)[200]. More recently, $P2X_3$ receptor involvement in neuronal dysfunction in brain areas that mediate nociception, such as the trigeminal nucleus and

the thalamus, have been considered[201,202]. $P2X_3$ receptors are the only ligand-gated channels known to be expressed exclusively by a subset of trigeminal and spinal sensory neurons[203]. The interaction of $P2Y_1$ receptors on trigeminal neurons with $P2X_3$ receptors after sensitization with algogenic stimuli (for example, NGF, BDNF or bradykinin) has been proposed and could also represent a new potential target for anti-migraine drugs[204]. Slow upregulation of nociceptive $P2X_3$ receptors on trigeminal neurons by calcitonin gene-related peptide (CGRP) has been demonstrated[202]. In an *in vivo* model of mouse trigeminal pain, anti-NGF treatment suppressed responses evoked by $P2X_3$ receptor activation[205]. However, the effect of adding NGF on $P2X_3$ receptor-mediated currents was shown not to be mediated by NGF-induced CGRP release.

Evidence for the possible role of ADO in migraine has been reviewed in REF. 206. Plasma ADO has been observed to rise during migraine attacks and ADO has been reported to trigger migraine attacks. Conversely, dipyridamole, an ADO uptake inhibitor, can increase migraine attack frequency. A_1 receptor stimulation has also been considered for migraine treatment[207] and it has been claimed that A_{2A} receptor gene variation might contribute to the pathogenesis of migraine[208].

Future directions and therapeutic strategies

There is compelling evidence that purinergic signalling has an important role in CNS disorders[4,172] and considerable effort has been made to synthesize therapeutic purinergic antagonists (TABLES 2,3). Besides being important pharmacological tools for the characterization of the pathophysiological roles of P2X and P2Y receptors in native systems, there is agreement that such ligands might represent new therapeutic entities of potential interest in various human diseases. In my opinion, the most promising areas for purinergic drug discovery at present are for the treatment of visceral pain with $P2X_3$/$P2X_{2/3}$ receptor antagonists and neuropathic and inflammatory pain with $P2X_7$ antagonists. A_{2A} antagonists are promising for the treatment of Parkinson's disease. Interest in $P2X_7$ antagonists, and to a lesser extent $P2Y_2$ agonists, for the treatment of other neurodegenerative diseases is also gaining ground. Other purinergic therapeutic strategies that are being explored include A_1 agonists and A_{2A} antagonists for the treatment of epilepsy, and the use of $P2X_7$ antagonists for the treatment of brain and spinal cord trauma and neuroinflammation. Areas of potential interest, although largely unexplored at present, include the treatment of depression and anxiety with A_1 and A_{2A} agonists and $P2Y_1$ antagonists. A_{2A} receptor antagonists are also being investigated for the treatment of schizophrenia. The design and synthesis of selective P2Y ligands has been greatly aided by the development of three-dimensional structures of these receptors, structure-activity relationships, mutagenesis and homology modelling studies based on the crystallization of the GPCR rhodopsin[8]. Detailed three-dimensional structures of P2X receptors have not yet been proposed due to the lack

Table 2 | **Agonists and antagonists of P1 receptors and potential therapeutic strategies for CNS disorders**

Receptor	Selective agonists	Selective antagonists	Diseases and potential therapeutic strategies
P1 (non-specific)	ADO	Theophylline, caffeine	• ADO is neuroprotective[225] • Caffeine is effective against Alzheimer's and Parkinson's disease[226] • Ectonucleotidase inhibition reduces glioma progression[227]
A_1	CPA, CCPA, S-ENBA, GR79236, CVT-510	DPCPX (8.5), MRS1754, N-0840, WRC-0571	• ADO attenuates glioblastoma growth[228]; reduces epileptic seizures[229], has antidepressant[143] and anticonvulsant[230] effects • Agonists might be candidates for antimigraine drugs[207] • Paeoniflorin activates receptors attenuating neuroinflammation and dopaminergic neurodegeneration in Parkinson's disease[231] • Propentofylline and AIT-082 enhance memory in Alzheimer's disease[232]
A_{2A}	CGS 21680, HENECA, ATL-146e, CVT-3146	ZM241385 (9.0), SCH58261 (7.9-9.5), KF17837, KW-6002	• Agonists have antidepressant effects[143]; reduce long-term injury after spinal trauma[233] • Antagonists are used to treat Parkinson's disease[72], motor abnormalities in Huntington's disease[88], protect motor neurons in amyotrophic lateral sclerosis[234]and treat schizophrenia[235]. They are also anticonvulsive[230] and can be used to manage drug addiction[235] • KW-6002 enhances motor & motivational responses[236]
A_{2B}	Bay60-6583, MRS3997	MRS1754 (8.7), MRS1706 (8.4), PSB1115 (7.7), Enprofylline, MRS2029-F20,	No information available
A_3	2-Cl-IB-MECA, IB-MECA, DBXRM, VT160	MRS1220 (8.8), VUF5574 (8.4), MRS1523 (7.7), MRS1191 (7.0), L-268605, VUF8504	No information available

CNS, central nervous system. For definitions of agonists and antagonists see Supplementary information S3 (box).

Algogenic stimuli
A pain-producing stimuli.

of a suitable protein template. Moreover, development of selective P2X receptor ligands has been complicated by the presence of heteromultimeric P2X receptors displaying unique pharmacology[9]. Selective non-nucleotide antagonists have been reported for $P2Y_{1,2,6,12,13}$ and $P2X_{2/3}$, $P2X_3$ and $P2X_7$ receptors[76] (Supplementary information S2 (table)). Initially $P2X_3$ and $P2X_{2/3}$ receptors were targeted because they were shown to

Table 3 | **Agonists and antagonists of P2 receptors and potential therapeutic strategies for CNS disorders**

Receptor	Selective agonists	Selective antagonists	Diseases and potential therapeutic strategies
P2 (non-specific)	ATP, UTP, UDP	Suramin, PPADS	• ATP improves locomotion after trauma[237] • ATP-$MgCl_2$ protects the spinal cord from secondary injury after trauma & protects the brain following ischaemia[238] • Suramin & PPADS are neuroprotective against ischaemia[239], whereas ATP accelerates recovery following hypoxia / hypoglycaemia[51] • PPADS is neuroprotective against NMDA-induced toxicity[240] • Ap_4A might protect against cerebral ischaemia in stroke[241] • P2Y receptor activation induces ischaemic tolerance in brain[62] • P2Y antagonists are beneficial in acute & chronic neurological disease[42] • Apyrase reduces glioblastoma growth[242]
$P2X_1$	L-β,γ-meATP, α,β-meATP	TNP-ATP (8.9), IP_5I (8.5), NF023 (6.7), NF449 (6.3)	No information available
$P2X_2$	None	None	No information available
$P2X_3$	α,β-meATP	TNP-ATP (8.9), A317491 (7.5), RO3 (7.5)	• A317491 reduces chronic inflammatory & neuropathic pain & tactile allodynia[170] • Antagonists might be candidates for antimigraine drugs[243]
$P2X_4$	None	benzofuro-1,4-diazepin-2-ones	• Antagonists proposed for treatment of neuropathic pain & allodynia[170]
$P2X_5$	None	None	No information available
$P2X_6$	None	None	No information available
$P2X_7$	BzATP	Brilliant blue G (8.0), decavanadate (7.4), KN62, A438079 6.9)	• o-ATP improves function in trauma area[49] & relieves inflammatory pain[244] • Antagonists (& nicotinamide) reduce demyelination in EAE[48] and control neuroinflammation[245] • A438079 (REF. 195) & A74003 reduce neuropathic pain & allodynia[246,247]
$P2Y_1$	2-MeSADP, ADPβS, MRS2365,	MRS2500 (8.8), MRS2279 (8.0), MRS2179 (7.0), PIT (6.8)	• Agonists cause anxiolytic-like effects[141] • Antagonists blocking Ca^{2+} waves in astrocytes are possible new treatments for epilepsy[128]
$P2Y_2$	UTPγS, Ap_4A, INS 37217, INS365, 2-thio-UTP	AR-C126313	• Agonists mediate neuroprotection[44]; have analgesic effects & activate neuroprotective mechanisms in astrocytes[183]
$P2Y_4$	UTPγS, Up_4U	ATP (6.2)	• Agonists might be beneficial in CNS repair and remodelling after injury[30]
$P2Y_6$	UDP, 2-phenacyl-UDP, UDPβS	MRS2578 (7.4)	• Agonists have analgesic effects, mediate phagocytosis of debris at site of damage in brain trauma[37]
$P2Y_{11}$	ARC67085, NAD^+, $NAADP^+$, NF546	NF157, 5′-AMPS, NF340	No information available
$P2Y_{12}$	ADP, 2-MeSADP	ATP, ARL-66096, CT50547, Cangrelor (AR-C69931MX), INS49266, AZD6140, PSB0413	No information available
$P2Y_{13}$	None	MRS2211	No information available
$P2Y_{14}$	MRS2690, UDP glucose, UDP-galactose	None	No information available

CNS, central nervous system; EAE, experimental autoimmune encephalitis; NMDA, *N*-methyl-*D*-aspartate; PPADS, phosphate-6-azophenyl-2-4-disulphonic acid. For definitions of agonists and antagonists see Supplementary information S3 (box).

be localized on primary afferent nociceptive neurons and some useful drugs were developed as selective antagonists, notably 2′,3′-O-(2,4,6-trinitrophenyl)-ATP (TNP-ATP). Roche have recently developed some small molecules (RO3 and derivatives) that are orally bioavailable and stable *in vivo*; these are currently in clinical trial[209]. There has been promising development of $P2X_7$ antagonists in the past year, notably the Abbott compound A438079 (REF. 196). However, finding antagonists for the $P2X_4$ receptor has been more problematic. So far, suramin, PPADS and Reactive Blue 2 are non-selective antagonists for $P2X_4$ receptors.

Lithium and valproate are commonly prescribed mood stabilising drugs that have a neuroprotective effect against neuronal death produced by extracellular ATP[210], and it has been suggested that these drugs could be used for the prophylaxis and/or treatment of neurodegenerative diseases involving increases in ATP, such as brain trauma and ischaemia. Acute and chronic morphine administration can increase the catabolism of purine nucleotides in the brain and this persists for a period after withdrawal, suggesting that the relationship between nucleotide metabolism and opioid addiction might be worth further exploration[211].

In summary, although current developments are promising, the use of purinergic agents as CNS therapeutics has yet to reach the clinic. In general, therapeutic developments are limited by the lack of selective agents that can be used *in vivo*, although the use of A_{2A} antagonists are well advanced in the clinic for the treatment of Parkinson's disease. There are no publications describing clinical evaluations of P2 receptor antagonists and related purinergic compounds for the relief of pain, although clinical trials for some compounds are in progress and reduced pain sensation was noted in a suramin Phase I cancer clinical trial[212]. The emerging information about P2 receptor subtype-selective antagonists is promising and they could prove to be useful pharmacological tools for preclinical studies. However, medicinal chemists are urged to try to develop antagonists that are small molecules with aqueous solubility for oral administration, are stable *in vivo* and can penetrate the blood–brain barrier.

The chemistry of ATP in the extracellular environment is dynamic and complex, and more must be learned about the extracellular biochemistry and enzymes that regulate the synthesis and degradation of ATP outside the cell. The activity of ectonucleotidases in subcellular domains and how these enzymes change during development, disease and physiological state are still not known in sufficient detail. The development of selective inhibitors for the different subtypes of ectonucleotidases would be a valuable step forward.

Whereas it is now apparent that many different cell types release ATP physiologically in response to mechanical distortion, hypoxia and various agents, we still await a clear understanding of the mechanisms that underlie ATP transport. Hopefully, once this is achieved, agents that enhance or inhibit ATP release will be developed as effective therapeutics.

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DATABASES

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