Purinergic Signalling in the CNS

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Abstract: Purinergic neurotransmission, involving release of ATP as an efferent neurotransmitter was first proposed in 1972. Later it was recognised as a cotransmitter in peripheral nerves and more recently as a cotransmitter with glutamate, noradrenaline, GABA, acetylcholine and dopamine in the CNS. Both ion channel and G protein-coupled receptors for purines and pyrimidines are widely expressed in the brain and spinal cord. They mediate both fast signalling in neurotransmission and neuromodulation and long-term (trophic) signalling in cell proliferation, differentiation and death. Purinergic signalling is prominent in neuron-glial cell interactions. Purinergic signalling has been implicated in learning and memory, locomotor activity and feeding behaviour. There is increasing interest in the involvement of purinergic signalling in the pathophysiology of the CNS, including trauma, ischaemia, epilepsy, neurodegenerative diseases, neuropsychiatric and mood disorders.

Keywords: ATP, adenosine, cotransmission, epilepsy, glia, memory, neurodegenerative diseases, purinoceptors, sleep.

INTRODUCTION

The concept of purinergic neurotransmission was born in 1972 [1], after it was shown that adenosine 5'-triphosphate (ATP) was a transmitter in non-adrenergic, non-cholinergic inhibitory nerves in the guinea-pig taenia coli. Subsequently ATP was identified as a co-transmitter in sympathetic and parasympathetic nerves [2] and it is now recognised that ATP acts as either sole transmitter or a co-transmitter in most nerves in both the peripheral nervous system and central nervous system (CNS) (see [3]). Since 1992, there has been an explosion of interest in purinergic transmission in the different regions of the brain and spinal cord [3, 4]. Various purinergic receptor subtypes have been shown to be widely distributed throughout the CNS being present in neurones and glia (see [3]). It is now well established that ATP acts both as a fast excitatory neurotransmitter or neuromodulator and has potent long-term (trophic) roles in cell proliferation, differentiation and death in development and regeneration, as well as in disease [5, 6].

Purinergic receptors were first defined in 1976 [7] and 2 years later a basis for distinguishing two types of purinoceptor, identified as P1 and P2 (for adenosine and ATP/adenosine diphosphate [ADP], respectively) was proposed [7]. At about the same time, two subtypes of the P1 (adenosine) receptor were recognised [8, 9], but it was not until 1985 that a proposal suggesting a pharmacological basis for distinguishing two types of P2 receptor (P2X and P2Y) was made [10]. A year later, two further P2 purinoceptor subtypes were identified, namely, a P2T receptor selective for ADP on platelets and a P2Z receptor on macrophages [11]. Further subtypes followed, perhaps the most important being the P2U receptor, which could recognize pyrimidines such as uridine 5'-triphosphate (UTP) as well as ATP [12]. Abbracchio and Burnstock [13], on the basis of studies of transduction mechanisms [14] and the cloning of nucleotide receptors [15-18], proposed that purinoceptors should belong to two major families: a P2X family of ligand-gated ion channel receptors and a P2Y family of G protein-coupled receptors. This nomenclature has been widely adopted and currently seven P2X subunits and eight P2Y receptor subtypes are recognised, including receptors that are sensitive to pyrimidines as well as purines (see [19]).

There is compelling evidence for exocytotic neuronal vesicular release of ATP [20] and recent studies also support a vesicular release of ATP from astrocytes [21, 22], perhaps involving lysosomes [23]. Evidence has been provided for additional mechanisms of nucleotide release, including ATP-binding cassette transporters, connexin or pannexin hemichannels, plasmalemmal voltage-dependent anion channels, as well as $P2X_7$ receptors [24, 25]. After release, ATP and other nucleotides undergo rapid enzymatic degradation by ectonucleotidases, which is functionally important as ATP metabolites act as physiological ligands for various purinergic receptors [6]. Ectonucleotidases include the E-NTPDases (ecto-nucleoside triphosphate diphosphohydrolases), E-NPPs (ecto-nucleotide pyrophosphatase /phosphodiesterases), alkaline phosphatases and ecto-5´-nucleotidase. Although generally adenosine is produced by ectoenzymatic breakdown of ATP, there may be subpopulations of neurones and/or astrocytes that release adenosine directly [26].

PURINERGIC SIGNALLING IN THE CNS

The actions of adenosine in the CNS have been recognised for many years (see [27-30]). However, consideration of the role(s) of ATP in the CNS received less attention until more recently (see [4, 31-40]). In particular, fast purinergic synaptic transmission has been clearly identified in the brain

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[41]. It was first observed in the medial habenula [42] and has now been described in a number of other areas of the CNS, including spinal cord [43], locus coeruleus [44], hippocampus [45, 46] and somatic-sensory cortex [47]. Electron microscopic immunocytochemical studies support these functional experiments. Although adenosine, following ectoenzymatic breakdown of ATP, is the predominant, presynaptic modulator of transmitter release in the CNS (see [29]), ATP itself can also act presynaptically [48]. A strong case is made for coordinated purinergic regulatory systems in the CNS controlling local network behaviours by regulating the balance between the effects of ATP, adenosine and ectonucleotidases on synaptic transmission [49, 50].

ATP is present in high concentrations within the brain, varying from approximately 2mM/Kg in the cortex to 4mM/Kg in the putamen and hippocampus [51]. Much is now known about the breakdown of ATP released in the CNS [52]. Cortex and hippocampus synaptic membranes exhibit higher activities of NTPDase1 and NTPDase2 than cerebellum and medulla oblongata, while ecto-5'-nucleotidases and adenosine deaminase were found in most brain regions.

1. Purine Receptors in the CNS

In situ hybridisation of P2 receptor subtype mRNA and immunohistochemistry of receptor subtype proteins have been carried out in recent years to show wide, but heterogeneous distribution in the CNS of both P2X receptors [53-57] and P2Y receptors [33, 58, 59]. $P2X_2$, $P2X_4$ and $P2X_6$ receptors are widespread in the brain and often form heteromultimers. $P2X_1$ receptors are found in some regions such as cerebellum and $P2X_3$ receptors in the brain stem. $P2X_7$ receptors are probably largely pre-junctional. $P2Y_1$ receptors are also abundant and widespread in the brain. The hippocampus expresses all P2X receptor subtypes and $P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$ and $P2Y_{12}$ receptors.

Evidence has been presented that nucleotides can act synergistically with growth factors to regulate trophic events [60, 61]. However, a recent paper has shown that ATP can also stimulate neurite outgrowth from neuroblastoma cells independent of nerve growth factor [62].

2. Cotransmission

Evidence for purinergic cotransmission in the CNS has lagged behind that presented for purinergic cotransmission in the periphery (see [63]). However, in the last few years a number of such studies have been reported.

Release of ATP from synaptosomal preparations and slices from discrete areas of the rat and guinea-pig brain including cortex, hypothalamus, medulla, and habenula, has been measured [64-66]. In cortical synaptosomes, a proportion of the ATP appears to be coreleased with acetylcholine (ACh), and a smaller proportion with noradrenaline [67]. In preparations of affinity-purified cholinergic nerve terminals from the rat caudate nucleus, ATP and ACh are coreleased [68]. There is evidence for corelease of ATP with catecholamines from neurons in the locus coeruleus [69] and hypothalamus [66, 70]. Purinergic and adrenergic agonist synergism for vasopressin and oxytocin release from hypothalamic supraoptic neurons is consistent with ATP cotransmission in the hypothalamus [71]. Corelease of ATP with γ-amino butyric acid (GABA) has been demonstrated in the rabbit retina [72] and in dorsal horn and lateral hypothalamic neurons [73]. There is evidence for corelease of ATP with glutamate in the hippocampus [45] as well as widespread and pronounced modulatory effects of ATP on glutamatergic mechanisms [74]. A recent study has shown that in central neuronal terminals, ATP is primarily stored and released from a distinct pool of vesicles and that the release of ATP is not synchronized either with the cotransmitters GABA or glutamate [21]. Cooperativity between extracellular ATP and *N*-methyl-d-aspartate receptors in long-term potentiation induction in hippocampal CA1 neurons [75] is consistent with ATP/glutamate cotransmission. Colocalisation of functional nicotinic and ionotropic nucleotide receptors have also been identified in isolated cholinergic synaptic terminals in midbrain [76]. Interactions between $P2X_2$ and both $\alpha_4\beta_4$ and $\alpha_4\beta_2$ nicotinic receptor channels have been shown in oocyte expression studies [77].

There is indirect evidence supporting the possibility that dopamine and ATP are cotransmitters in the CNS [78]. After cerebellar lesions in rats producing axotomy of mossy and climbing fibre systems, nitrergic and purinergic systems were activated with similar time courses on pre-cerebellar stations [79]. This raises the possibility that, as in a subpopulation of neurons in the gut, nitric oxide and ATP are co-transmitters.

3. Glial Cells

Multiple P1 and P2 receptor subtypes are expressed by astrocytes, oligodendrocytes and microglia (see [57]). Adenosine stimulates glutamate release from astrocytes *via* A_{2A} receptors [80]. A_3 receptors mediate chemokine CCL2 synthesis in cultured mouse astrocytes [81]. Astrocytes in the cortex and cerebellum express $P2Y_{13}$ as well as $P2Y_1$ and $P2X_2$ receptors [82]. Astrocytes and microglia express many purinergic receptor subtypes, but as with myelinating glia, the patterns of expression are complex and can change with physiological and developmental conditions. Many glial cells co-express multiple types of P1 and P2 receptors, but there can be considerable heterogeneity in expression patterns among individual cells. NTPDase2 is the dominant ectonucleotidase expressed by rat astrocytes [83].

ATP participates in both short-term calcium signalling events and in long-term proliferation, differentiation and death of glia [84]. Both adenosine and ATP induce astroglial cell proliferation and the formation of reactive astrocytes [85]. ATP and basic fibroblast growth factor (bFGF) signals merge at the mitogen-activated protein kinase cascade, and this integration may underlie the synergistic interactions of ATP and bFGF in astrocytes. Activation of adenosine A_{2B} receptors in astroglioma cells has been shown to increase interleukin-6 (IL-6) mRNA and IL-6 protein synthesis. Blockade of A_{2A} receptors prevents bFGF-induced reactive astrogliosis in rat striated primary astrocytes [86]. Extracellular nucleotide signalling has also been identified in adult neural stem cells [87].

Release of ATP through connexin hemichannels in astrocytes has been reported [88], although vesicular release has also been described [89, 90]. It has also been suggested that $P2X_7$ receptor pores may directly mediate efflux of cytosolic ATP, glutamate and GABA from glial cells in the CNS [91].

Calcium rises in rat cortical astrocytes are mediated by $P2Y_1$ and $P2X_7$ receptors, but additional P2 receptors ($P2X_2$, $P2X_4$ $P2X_5$, $P2Y_2$, $P2Y_4$ and $P2Y_{14}$) may also contribute [92]. Another study has shown that cultured astrocytes are able to release UTP either at rest or following hypoxia and that $P2Y_2$ receptor mRNA increased by 2-fold during glucose-oxygen deprivation [93]. $P2Y_2$ and $P2Y_4$ receptors are strongly expressed in glial endfeet apposed to blood vessel walls [94, 95].

4. Neuron-Glial Interactions

Purinergic signalling is emerging as a major means of integrating functional activity between neurons, glial and vascular cells in the CNS. These interactions mediate effects of neural activity, in development and in association with neurodegeneration, myelination, inflammation and cancer (see [5, 96]).

New findings from purinergic research began to converge with glial research as it became more widely appreciated that ATP was co-released from synaptic vesicles and thus accessible to perisynaptic glia, while ATP released from glial cells could also act on neurons. This common currency for cell-cell communication opened the possibility of an intercellular signalling system that could unite glia and neurons functionally.

BEHAVIOURAL STUDIES

While the involvement of purinergic signalling in neurotransmission and neuromodulation in the CNS is now well established, there are relatively few studies of the involvement of purinergic signalling in behavioural pathways, apart from brainstem control of autonomic functions, although behavioural changes have been reported in pathological situations (see [3]).

ATP and adenosine are involved in mechanisms of synaptic plasticity and memory formation [97, 98]. The hypnotic/sedative (somnogenic) actions of adenosine are well known as are the central stimulant actions of methylxanthine antagonists (see [99-101]). Adenosine, acting through A_1 receptors, is an endogenous, homeostatic sleep factor, mediating the sleepiness that follows prolonged wakefulness. The basal forebrain as well as neurons in the cholinergic laterodorsal tegmental nuclei are essential areas for mediating the sleep inducing effects of adenosine by inhibition of wake-promoting neurons [102]. It has been suggested that adenosine may promote sleep by blocking inhibitory inputs on ventrolateral preoptic area sleep-active neurons [103]. A_{2A} receptors in the subarachnoid space below the rostral forebrain, activating cells in the nucleus accumbens that increase activity of ventrolateral preoptic area neurons, may also play a role in the somnogenic effect of adenosine [104].

The central inhibitory effects of adenosine on spontaneous locomotor activity of rodents and antagonism by caffeine have been known for some time (e.g. [105, 106]). Later A_{2A} receptors on the nucleus accumbens were shown to mediate locomotor depression [107]. Modulation of striatal A_1 and A_2 receptor-mediated activity induces rotational behaviour in response to dopaminergic stimulation in intact rats [108]. Interactions between adenosine and L-type Ca^{2+} channels in the locomotor activity of rat were demonstrated [109]. A predominant role for A_1 receptors in the motor-activity effects of acutely administered caffeine in rats has been reported [110]. A combination of A_1 and A_{2A} receptor blocking agents induces caffeine-like spontaneous locomotor activity in mice [111]. It has been reported that ATP continuously modulates the cerebellar circuit by increasing the inhibitory input to Purkinje neurons, probably *via* $P2X_5$ and $P2Y_2$ and/or $P2Y_4$ receptor subtypes, thus decreasing the main cerebellar output activity, which contributes to locomotor coordination [112]. $P2X_2$ receptor immunoreactivity in the cerebellum was demonstrated and claimed to be consistent with a role for extracellular ATP acting as a fast transmitter in motor learning and coordination of movement [113].

Adenosine given centrally can result in a decrease in food intake [114]. In the striatum, extracellular ATP and adenosine are involved in the regulation of the feeding-associated mesolimbic neuronal activity in an antagonistic manner [115]. It has been reported that feeding behaviour relies on tonic activation of A_{2A} receptors in the nucleus accumbens in rats [116]. NTPDase3 and 5′-ectonucleotidase regulate the levels of adenosine involved in feeding behaviour in rat brain [117]. Enhanced food intake after stimulation of hypothalamic $P2Y_1$ receptors in rats has been described [118]. Both adenosine and ATP have been implicated in mood and motivation behaviour [119-122].

PURINERGIC PATHOPHYSIOLOGY IN THE CNS, INCLUDING EPILEPSY

There is a rapidly growing literature about the involvement of purinergic signalling in most disorders of the CNS, such as neurodegeneration diseases, including Alzheimer's, Parkinson's and Huntington's diseases and multiple sclerosis, cerebral ischaemia, migraine, neuropsychiatric and mood disorders (see [123] and Fig. **1**).

The particular focus of this Special Issue is purinergic signalling in epilepsy. 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 treatments that prevent development and control epilepsy better. Epilepsy is often accompanied by massive glial cell proliferation, although the role of these cells in seizures and epilepsy is still unclear.

Both P1 and P2 receptors have been implicated in epilepsy (see [124-127]). Microinjection of ATP analogs into the prepiriform cortex induces generalized motor seizures, suggesting that P2X receptor antagonists may have potential as neuroleptic agents [125]. Epileptiform activity in the CA3 region of rat hippocampal slices is modulated by adenine nucleotides, probably acting *via* excitatory P2X receptors [128]. 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 may participate in the pathophysiology of temporal lobe epilepsy [129]. In a study of kainate-provoked seizures, enhanced immunoreactivity of the $P2X_7$ receptor was observed in microglia as they are changed from the resting to the activated state [130]. The amount of extracellular

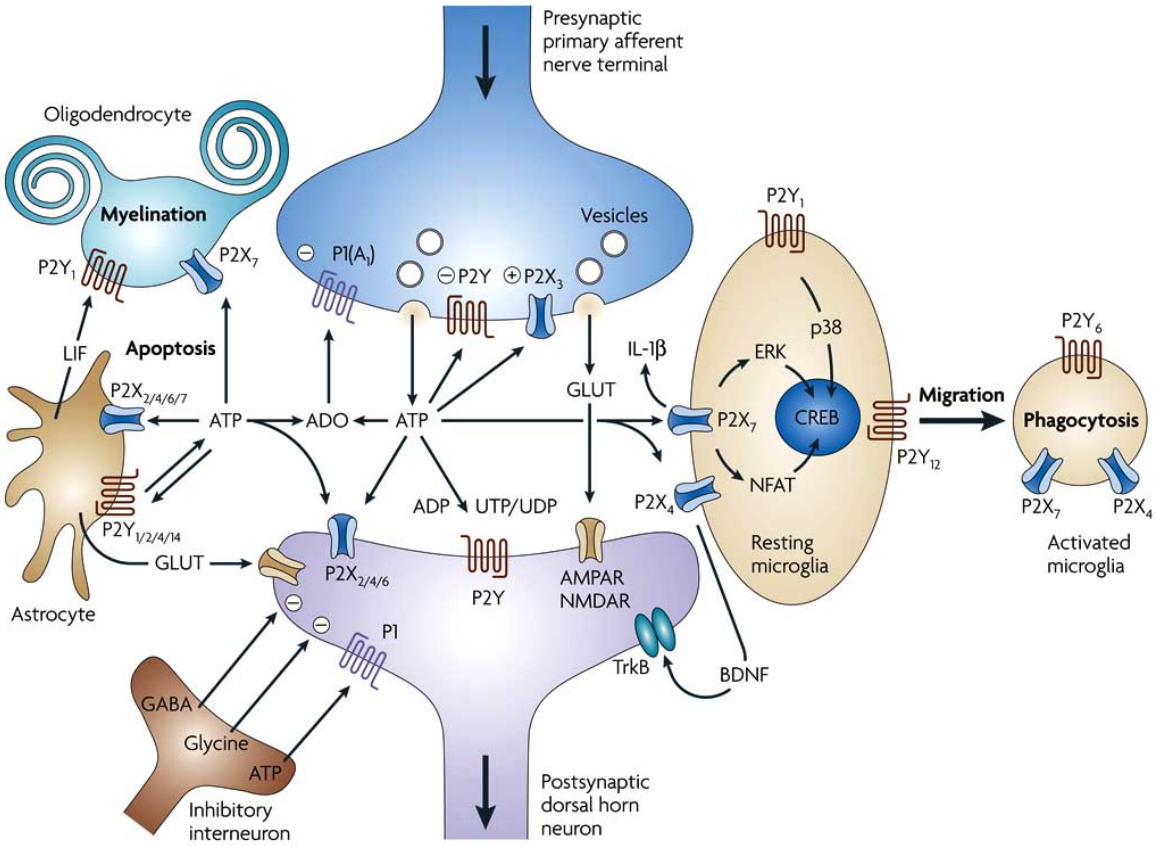

Fig. (1). Purinergic signalling in the spinal cord.
Presynaptic primary afferent nerve terminals in the dorsal horn of the spinal cord are depicted releasing both glutamate (GLUT) and ATP as cotransmitters by exocytosis. The released ATP acts postsynaptically on $P2X_{2/4/6}$ and on various P2Y receptor 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 remyelination 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. (Reproduced from [123] with Permission).

ATP detected in hippocampal slices following electrical stimulation of Schaffer collaterals was significantly greater in mice that have an inherited susceptibility to audiogenic seizures [131], perhaps associated with reduced brain Ca^{2+}-ATPase activity. Uridine is released during epileptic activity and may act as an inhibitory neuromodulator [132], although the underlying mechanism is not known. Increased hydrolysis of ATP occurs in rat hippocampal slices after seizures induced by quinolinic acid [133]. There is a decrease of presynaptic P2X receptors in the hippocampus of rats that have suffered a convulsive period, which may be associated with the development of seizures and/or of neurodegeneration during epilepsy [134]. Release of glutamate from astrocytes by ATP has been implicated in epileptogenesis [135].

P1 receptors have also been implicated in epileptic seizures [100, 124, 136-138]. Decreased extracellular adenosine 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 [139]. Adenosine, acting *via* A_1 receptors, reduces seizures in an experimental model of temporal lobe epilepsy induced by pilocarpine in rats [140]. A lower density of $P1(A_1)$ receptors in the nucleus reticularis thalami in rats with genetic absence epilepsy has been reported [141]. Several antiepileptic agents reduce the ability of astrocytes to transmit calcium waves, raising the possibility that purinergic receptor antagonists blocking intercellular calcium waves in astrocytes could offer new treatments for epileptic disorders.

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