

Purinergic signalling in the nervous system: an overview

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Purinergic receptors, represented by several families, are arguably the most abundant receptors in living organisms and appeared early in evolution. After slow acceptance, purinergic signalling in both peripheral and central nervous systems is a rapidly expanding field. Here, we emphasize purinergic co-transmission, mechanisms of release and breakdown of ATP, ion channel and G-protein-coupled-receptor subtypes for purines and pyrimidines, the role of purines and pyrimidines in neuronal communication and interactions of this system with other transmitter systems. We also highlight recent data involving purinergic signalling in pathological conditions, including pain, trauma, ischaemia, epilepsy, migraine, psychiatric disorders and drug addiction, which we expect will lead to the development of therapeutic strategies for these disorders with novel mechanisms of action.

Introduction

Intercellular signalling mediated by purines is present early in evolution and, therefore, is a widespread route for cell-to-cell communication. The concept of purinergic neurotransmission was introduced in 1972 [1] after it was shown that 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 recognized 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) [3] (Table 1). 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 neurons and glia [3]. It is now well established that ATP both acts as a fast excitatory neurotransmitter and has potent long-term (trophic) roles in cell proliferation, growth and development, and in disease and cytotoxicity [5,6].

We aim to present a broad, comprehensive overview of purinergic signalling. First, we introduce the series of more

focused articles to follow and, second, we provide an overview to the many neuroscientists who are still unaware of the importance of the rapidly growing field of purinergic signalling in the nervous system.

Storage, release and extracellular hydrolysis of ATP and related nucleotides

ATP and other nucleotides are taken up by and stored in secretory and synaptic vesicles. As revealed recently, accumulation of ATP into vesicles (Figure 1) can be mediated by a Cl⁻-dependent vesicular nucleotide transporter (VNUT), which belongs to the SLC17 anion transporter family, which includes also vesicular glutamate transporters [7]. VNUT preferentially recognizes ATP, GTP and ADP and is inhibited by diATP. This transporter is highly expressed in the brain and was allocated by immunocytochemistry to chromaffin granules and subpopulations of astrocytes. Further experiments need to clarify whether VNUT is also associated with synaptic vesicles and whether VNUT co-localizes with other vesicular neurotransmitter transporters. ATP is probably present in every synaptic and/or secretory vesicle, although at different concentrations, and can be co-stored and co-released with other neurotransmitters (e.g. γ -aminobutyric acid [GABA], noradrenaline or glutamate; Table 1). Some neuronal terminals (e.g. in the medial habenula and in the cortex) might contain pools of ATP-only vesicles [8,9].

At present, it is unclear whether various physiological nucleotide-receptor agonists (e.g. ATP, ADP, UTP, UDP, UDP sugars and NAD⁺) are released by common mechanisms. There is compelling evidence for exocytotic neuronal vesicular release of ATP [9] (Figure 1), and recent studies also support a vesicular release of ATP from astrocytes [10], which perhaps involves lysosomes [11]. Evidence has been provided for additional mechanisms of nucleotide release (Figure 1), including ATP-binding cassette transporters, connexin or pannexin hemichannels, plasmalemmal voltage-dependent anion channels, in addition to P2X₇ receptors [8,12,13]. Hemichannels have been implicated in ischemia-induced neuronal ATP release [14] and in ATP-mediated intercellular communication in taste buds [15]. Mechanisms of release of UTP and of UDP sugars are less

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Table 1. ATP as a co-transmitter in peripheral and central nervous systems^a.

Neuron type	Co-transmitters	Refs
Peripheral nervous system		
Sympathetic nerves	ATP + NA + NPY	[128]
Parasympathetic nerves	ATP + ACh + VIP	[129]
Sensory-motor	ATP + CGRP + SP	[130]
NANC enteric nerves	ATP + NO + VIP	[131]
Motor nerves (in early development)	ATP + ACh	[132]
Central nervous system		
Cortex, caudate nucleus	ATP + ACh	[133]
Hypothalamus, locus ceruleus	ATP + NA	[134]
Hypothalamus, dorsal horn, retina	ATP + GABA	[135]
Mesolimbic system	ATP + DA	[136]
Hippocampus, dorsal horn	ATP + glutamate	[137]

^aAbbreviations: ACh, acetylcholine; ATP, adenosine 5'-triphosphate; CGRP, calcitonin gene-related peptide; DA, dopamine; GABA, γ -aminobutyric acid; NA, nor-adrenaline; NANC, non-adrenergic, non-cholinergic; NO, nitric oxide; NPY, neuropeptide Y; SP, substance P; VIP, vasoactive polypeptide. Compiled from Ref. [3].

clear, but evidence for a regulated release of the P2Y₁₄-receptor agonist UDP glucose has been provided [16].

After release, ATP and other nucleotides undergo rapid enzymatic degradation by ectonucleotidases, which is functionally important because ATP metabolites act as physiological ligands for various purinergic receptors (Figure 1). The ectonucleotidases not only control the lifetime of nucleotide ligands but, by degrading or interconverting the originally released ligands, they also produce

ligands for additional P2 receptors and nucleosides (Figure 1; Table 2) [17]. All ectonucleotidase families hitherto identified are expressed in the brain. These include the E-NTPDases (ectonucleoside triphosphate diphosphohydrolases), E-NPPs (ectonucleotide pyrophosphatase and/or phosphodiesterases), alkaline phosphatases and ecto-5'-nucleotidase [17]. Individual enzymes differ in substrate specificity and product formation. E-NTPDases and E-NPPs hydrolyze ATP and ADP to AMP, which is further hydrolyzed to adenosine by ecto-5'-nucleotidase. Alkaline phosphatases equally hydrolyse nucleoside tri, di and monophosphates. Dinucleoside polyphosphates, NAD⁺ and UDP sugars are substrates solely for E-NPPs. Besides the catabolic pathways, nucleotide interconverting enzymes exist for nucleotide rephosphorylation and extracellular synthesis of ATP (e.g. ectonucleoside diphosphate kinase and adenylate kinase). Although usually adenosine is produced by ectoenzymatic breakdown of ATP, there might be subpopulations of neurons and/or astrocytes that release adenosine directly [18].

Receptors for purines and pyrimidines

Separate membrane receptors for adenosine (P1 receptors) and ATP (P2 receptors) were recognized in 1978 and, later, P2 receptors were divided into ionotropic P2X and metabotropic P2Y receptors on the basis of mechanism of action, pharmacology and molecular cloning [19–23] (Figure 1).

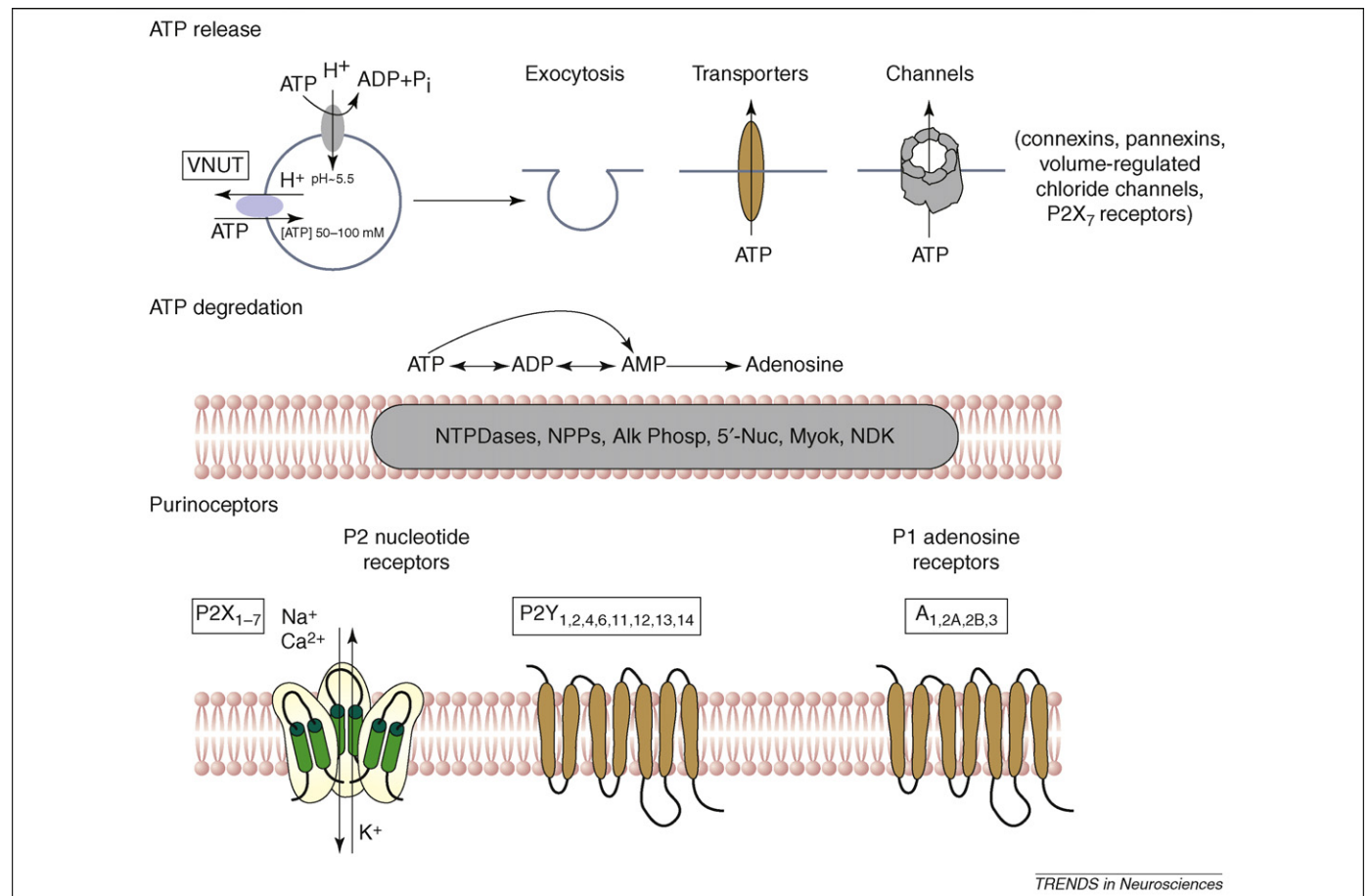


Figure 1. Mechanisms of ATP release, degradation and reception. Abbreviations: Alk Phos, alkaline phosphatase; Myok, myokinase (adenylate kinase); NDK, nucleoside diphosphate kinase; NPPs, nucleotide pyrophosphatase and/or phosphodiesterases; 5'-Nuc, 5'-nucleotidase; VNUT, vesicular nucleotide transporter.

Table 2. Agonist profile of P2 receptors^a

Receptor subtype	Endogenous agonist (human)	Selected antagonist
P2X ₁₋₆ ^b	ATP	
P2X ₁	ATP	TNP-ATP, NF023, NF449, R01
P2X ₂	ATP	NF778
P2X ₃	ATP	A317491, TNP-ATP, R03, RN-1838
P2X ₄	ATP	No antagonists
P2X ₅	ATP	PPADS, suramin (non-selective)
P2X ₆	ATP	No antagonists
P2X ₇	ATP	KN62, O-ATP, Coomassie BBG, RN-6189, A-740003, A-438079
P2Y ₁	ADP	MRS 2179
P2Y ₂	ATP, UTP (equipotent)	AR-C126313
P2Y ₄	UTP	Reactive Blue 2
P2Y ₆	UDP	MRS 2578
P2Y ₁₁	ATP, NAD ⁺ , NAADP ⁺	Reactive Blue 2, NF 157
P2Y ₁₂	ADP	CT 50547
P2Y ₁₃	ADP	MRS 2211
P2Y ₁₄	UDP glucose UDP galactose	No antagonists

^aAbbreviations: NAADP⁺, nicotinic acid adenine dinucleotide phosphate; O-ATP, oxidised ATP. Diadenosine polyphosphates are active at subtypes of P2X and P2Y receptors. Table modified from Ref. [127].

^bIndividual homo and heterooligomers are differentially modified by extracellular pH and differ in Ca²⁺ permeability and desensitization in response to ATP.

Purinoreceptors might be the most abundant receptors in mammalian tissues, as indeed they can be found in all types of cells including those of neural origin [24] (Figures 2 and 3).

P1 purinergic receptors

The four adenosine receptors A₁, A_{2A}, A_{2B} and A₃ are G-protein coupled (Figure 1). Typically, A₁ and A₃ couple to the G_{i/o} family of G proteins inhibiting cyclic AMP (cAMP) production, whereas A_{2A} and A_{2B} stimulate cAMP production via G_s. Depending on the cell type, other G-protein combinations have been revealed, and all adenosine receptors were shown to activate at least one subfamily of mitogen-activated protein kinases [25]. Accordingly, in the CNS, adenosine exerts a multitude of functions, including modulation of neural and glial functions, of neuron–glia signalling or of neural development [26,27]. Furthermore, adenosine plays an important part in the control of the innate and adaptive immune systems, and dysregulation of the adenosine system is involved in pathologies ranging from epilepsy to neurodegenerative disorders and psychiatric conditions [28]. The actions of adenosine are often antagonistic or synergistic with ATP, but this aspect will not be emphasized here.

P2X receptors

P2X receptors are classical cationic ligand-operated channels that upon ATP binding open the pore permeable to Na⁺, K⁺ and Ca²⁺ (Figure 1; Table 2). The P2X receptors are trimers [29] formed from individual subunits encoded by seven distinct genes (designated P2X₁ to P2X₇ according to historical order of cloning [30,31]). Diversity of the P2X-

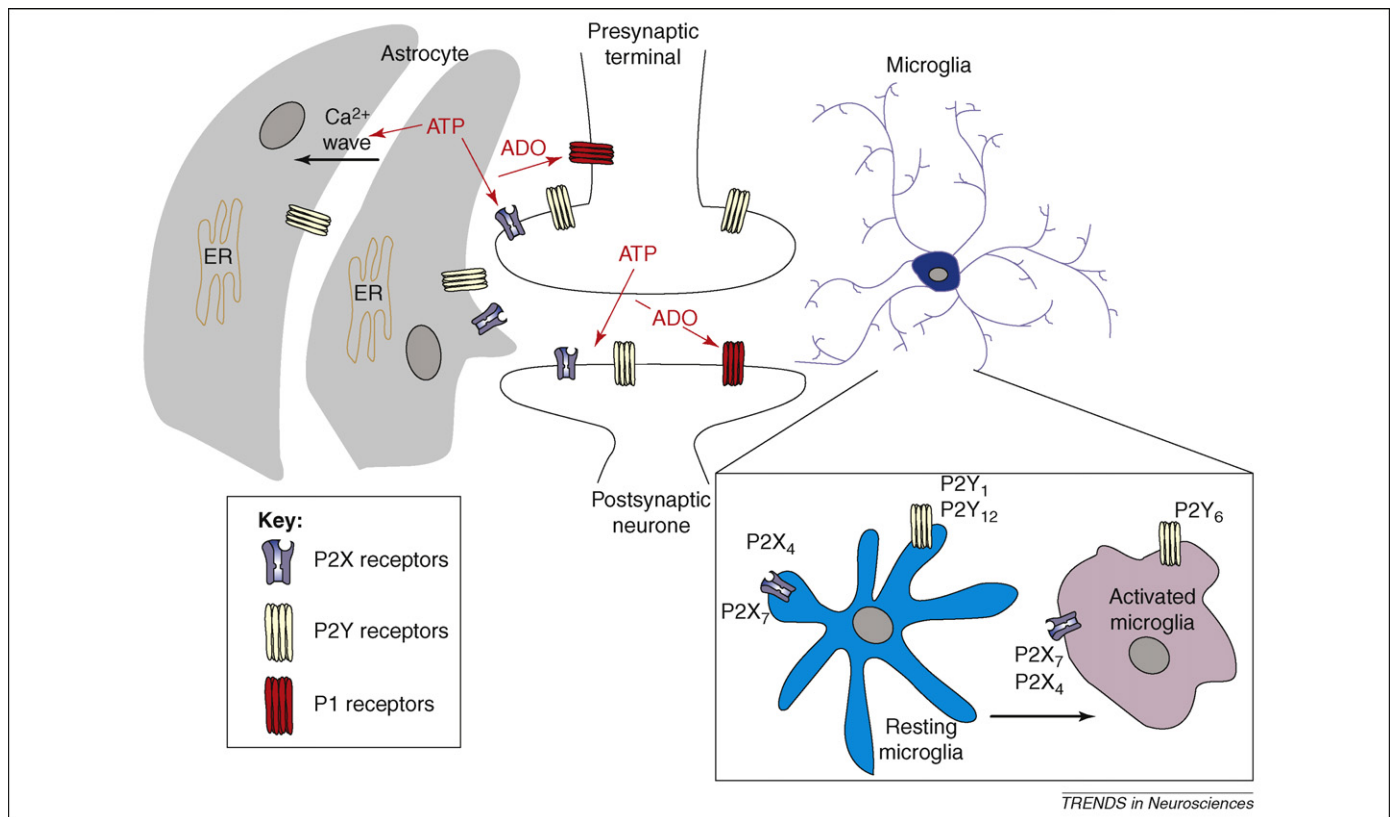


Figure 2. Nucleotide-mediated transmission in the CNS. ATP released on its own or co-released with other transmitters during synaptic activity stimulates P2 receptors localized on postsynaptic and presynaptic membranes and in astroglia. Simultaneously degradation of ATP produces adenosine, which acts on P1 receptors. ATP can be also released from astrocytes, where it is instrumental for both initiation and propagation of glial Ca²⁺ waves and for glial–neuronal signalling, which can be mediated either by ATP or by adenosine. Purinoreceptors expressed in microglial cells control their activation and their expression might, in turn, be modified during this activation process. Abbreviations: ADO, adenosine; ER, endoplasmic reticulum.

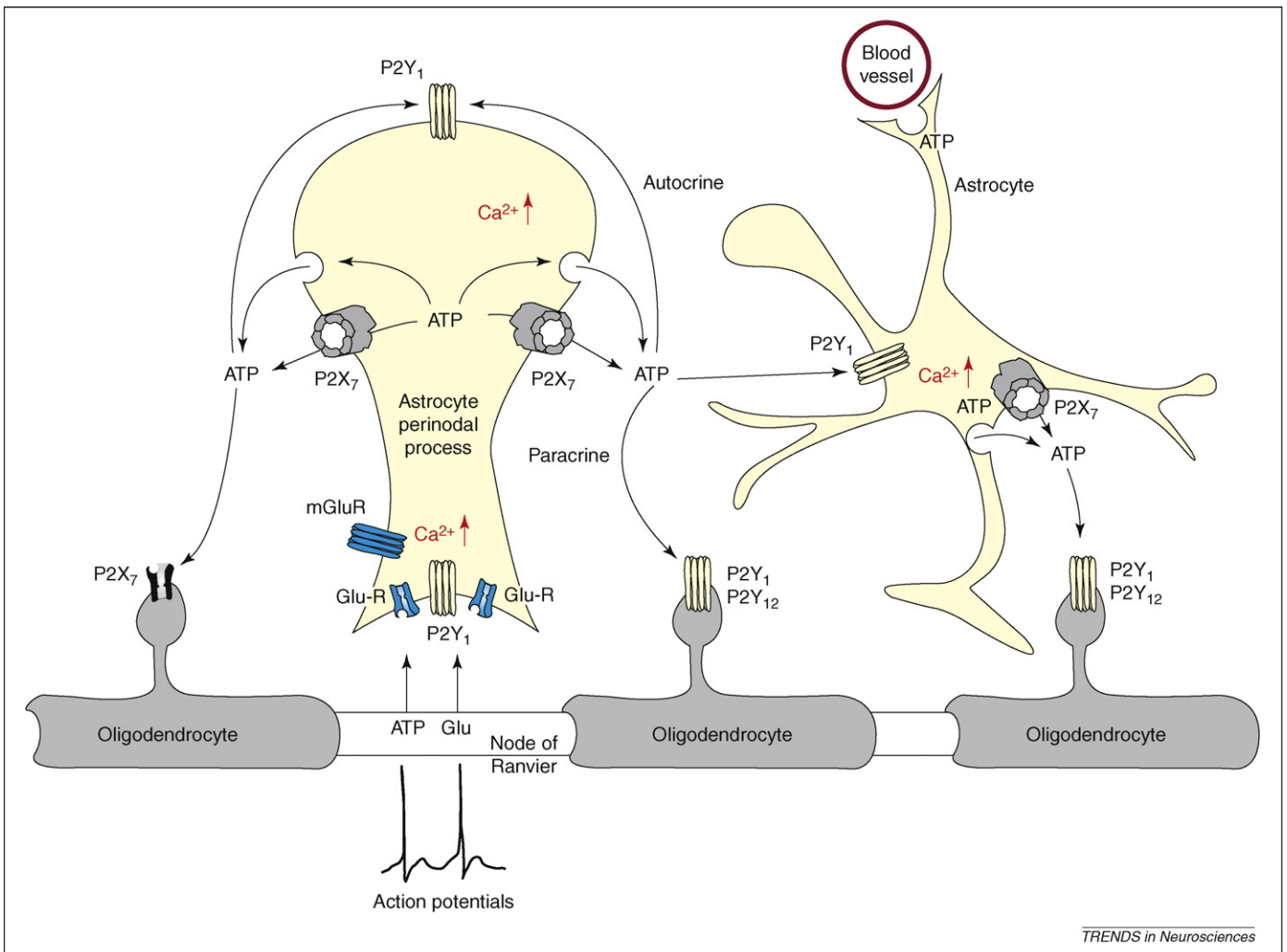


Figure 3. Nucleotide-mediated transmission in the axon-glia network. ATP and glutamate (Glu), released during axon-potential generation in axons, stimulate glutamate and P2 receptors localized in astroglial perinodal processes. Activation of these receptors, in turn, is coupled with ATP release from astrocytes, which might occur either via exocytosis or through membrane channels (e.g. connexins, panexins or P2X₇ receptors). ATP released from astrocytes acts both in an autocrine manner by further potentiating the response of a given astrocyte or in a paracrine manner by activating P2Y and P2X receptors localized in neighbouring astrocytes and oligodendrocytes. In addition, ATP is an important signalling substance at the gliovascular interface, including the control of microvascular blood flow. Modified from Ref. [68]. Abbreviations: Glu-R, glutamate receptor; mGluR, metabotropic glutamate receptor.

receptor phenotypes is determined by assembly of individual subunits; hitherto six homomeric (P2X₁–P2X₅ and P2X₇; P2X₆ subunits apparently do not oligomerise [29]) and six heteromeric (P2X_{1/2}, P2X_{1/4}, P2X_{1/5}, P2X_{2/3}, P2X_{2/6} and P2X_{4/6}) channels have been identified, and P2X_{4/7} channels were also claimed [30,32]. Homomeric P2X₇ receptors are activated at 100–1000 μ M ATP concentrations and the rest of receptors have an EC₅₀ of \sim 1–10 μ M. All P2X subunits are expressed in neural cells; this expression is heterogeneous in different brain regions and cell types. In many central neurons, expression is mosaic (and far from being fully characterised), which determines exceptional variability of ATP-induced current responses [33]. Peripheral neurons (both sensory and autonomic) predominantly express P2X₃ and P2X_{2/3} receptors, which are implicated in pain and temperature perception. The homomeric P2X₃ channels have a unique temperature sensitivity [34]; their rate of desensitisation is temperature insensitive, whereas the recovery from desensitization shows exceptional temperature dependence (temperature coefficient, Q₁₀ \sim 10). There is very little evidence for the

presence of functional P2X receptors *in situ* or in freshly isolated glia; so far, heteromeric P2X_{1/5} receptors were identified in cortical astrocytes [35], P2X₁ and P2X₅ subunits are possibly expressed in Schwann cells [36] and P2X₄ receptors in spinal cord microglia [37].

All P2X receptors are permeable to Ca²⁺; the ratio of Ca²⁺ to monovalent cations permeability varies between 1 and $>$ 5–10 for various P2X subunit combinations [38,39]. Native ATP-induced currents in several types of central neurons, such as neurons from medial habenula and somatosensory cortex, have an exceptionally high Ca²⁺ permeability ($P_{Ca}:P_{monovalent} = \sim$ 10–12 [40,41]), which is comparable to the Ca²⁺ permeability of *N*-methyl-D-aspartate (NMDA) receptors. As a result, P2X receptors might provide the most important route for Ca²⁺ influx at the postsynaptic density at resting membrane potentials when NMDA receptors are unavailable owing to Mg²⁺ block [33]. Indeed, stimulation of P2X receptors triggers cytosolic Ca²⁺ signals in many CNS neurons [39], and P2X-mediated presynaptic Ca²⁺ signals can regulate neurotransmitter release [42]. Finally, the involvement of the P2X₇ pore in

apoptosis induction via sustained entry of Ca^{2+} into cells has been repeatedly suggested [43].

Postsynaptic P2X receptors interact with several ionotropic receptors including nicotinic acetylcholine receptors, GABA_A receptors and NMDA receptors; for all these a reciprocal inhibition was demonstrated. The mechanisms for these interactions might be mediated by intracellular Ca^{2+} , by Ca^{2+} -activated kinases phosphorylating the receptors, or by direct interactions between receptors molecules (for reviews, see Refs [39,44]).

P2Y receptors

All P2Y receptors (Figure 1; Table 2) share the seven-transmembrane-domain topology of G-protein-coupled receptors [45]. Based on phylogenetic similarity, presence of amino acids important for ligand binding and selectivity of G-protein coupling, two distinct P2Y subgroups with a high level of sequence divergence are recognized: the P2Y₁, P2Y₂, P2Y₄, P2Y₆, and P2Y₁₁ subgroup and the P2Y₁₂, P2Y₁₃, and P2Y₁₄ subgroup [45]. Receptors of the first subgroup principally use G_q/G₁₁ to activate the phospholipase C/inositol triphosphate (InsP₃) endoplasmic reticulum Ca^{2+} -release pathway [46], whereas receptors of the second subgroup almost exclusively couple to G_{i/o}, which inhibits adenylyl cyclase and modulate ion channels [45]. Coupling of the same P2Y receptor to different G proteins is also possible, indicating agonist-specific signalling involving distinct active receptor conformations. For instance, activation of P2Y₁₁ receptors by ATP increases cAMP, InsP₃ and cytosolic Ca^{2+} , whereas activation by UTP produces Ca^{2+} mobilization without InsP₃ or cAMP increase [47]. Such agonist-specific signalling might differentially guide the neuronal or glial response under conditions in which different types of nucleotides are concomitantly released. Under some circumstances, P2Y receptors (e.g. P2Y₂) can interact directly with other proteins (e.g. integrins) without G-protein mediation [48].

P2Y receptors are expressed very early in the embryonic CNS and are broadly distributed on both neurons and glia. Currently, eight P2Y receptors are identified, which exhibit sensitivity to adenine nucleotides, uracil nucleotides, sugar nucleotides, or both adenine and uracil nucleotides (i.e. P2Y₂). An additional P2Y-like uracil-nucleotide-responsive receptor also activated by cysteinyl-leukotrienes (cysLTs) has been reported [49,50]. P2Y₁₂ is also promiscuously activated by ADP and cysLTE4 [51], indicating that dual responsiveness to nucleotides and cysLTs might be a feature common to several P2Y or P2Y-like receptors.

P2Y receptors might generate homodimers or heterodimers with other P2Y receptors [52] or with other transmitter receptors (e.g. A₁ adenosine receptors [53]). Interactive molecular pathways include: nucleotide-induced transactivation of tyrosine kinase receptors (such as epidermal growth factor receptor, platelet-derived growth factor receptor and vascular endothelial growth factor receptor-2); activation of soluble tyrosine kinases (such as Src, or of ectometalloproteases [54]); and interaction with integrins or the nerve growth factor (NGF) receptor and their signalling pathways [55–58].

P2X-receptor-mediated synaptic currents in the CNS

Synaptic currents mediated through activation of P2X receptors were found in many CNS regions, including the medial habenula, hippocampus, cortex and spinal cord [3,4]. With the exception of the medial habenula, in which activation of ATP-containing terminals evokes excitatory postsynaptic currents (EPSCs) solely mediated by P2X receptors [59], the ATP-mediated synaptic responses represent a component of total EPSC largely mediated by glutamate. The P2X-mediated synaptic currents account for only 5–15% of total EPSCs, thus their modulatory action is probably associated with their high Ca^{2+} permeability at hyperpolarised membrane potentials [4,39].

Activation of P2X receptors has been implicated in regulation of synaptic plasticity in several brain circuits. The role of P2X receptors in controlling both long-term potentiation (LTP) and long-term depression remains controversial; different groups have reported positive effects, negative effects or no effects at all (for review, see Ref. [39]). For example, in the hippocampus there is evidence showing that activation of P2X receptors inhibits LTP [60] in addition to opposite data showing that activation of P2X₄ receptors facilitates LTP [61,62]. It is likely that these discrepancies reflect the complex nature of LTP induction and/or maintenance, which can be differentially regulated by cytosolic Ca^{2+} .

Signalling in neuronal–glial networks

Information processing in the brain results from continuous interaction of two cellular circuits: neuronal and glial. Neuronal networks, integrated through electrically excitable processes and synaptic contacts, are embedded into a glial syncytium, which uses intercellular diffusion of ions, second messengers and metabolites as a long-range communication route [63]. Functional and cognitive importance of these alternative signalling pathways, operating within glial circuits, remains largely unknown, yet it becomes increasingly clear that coordination between neuronal and glial webs are of ultimate importance for normal operation of the brain. Furthermore, glial cells are particularly important in shaping brain pathological reactions, which determine the outcome of a multitude of neurological diseases [64].

ATP has a special role in signalling in neuronal–glial networks (Figures 2 and 3), being involved in all varieties of neuronal–glial and glial–glial signalling [65]. Indeed, all types of glia, peripheral (Schwann cells) and central (astrocytes, oligodendrocytes and microglia), express functional purinergic receptors [36,66–69]. Almost invariably, glial cells express P2Y receptors linked to the InsP₃-mediated Ca^{2+} -signalling cascade [70]; expression of P2X receptors is much more segregated. In astroglia, functional P2X_{1/5} receptors were hitherto found in the cortex but not in other brain regions; oligodendrocytes in the optic nerve express P2X₇ receptors, which, upon sustained activation, might cause cell death and can be implicated in the pathogenesis of multiple sclerosis [71]; microglial cells in the spinal cord express P2X₄ receptors, which were shown to mediate chronic neuropathic pain [37].

Furthermore, ATP acts as a widespread gliotransmitter, and regulated release of ATP from astrocytes, either

via exocytosis or through release via membrane channels (e.g. connexins, volume-regulated Cl^- channels or P2X_7 receptors) has been observed in various glial preparations (for example, see Refs [4,72]). ATP released from astroglia is specifically important for generation and maintenance of propagating Ca^{2+} waves within the glial syncytium in many (but not in all) brain regions [72,73]. Finally, ATP controls and regulates many pathological reactions of glia, for example reactive gliosis or microglial motility and activation; the latter is specifically controlled by P2Y_6 and P2Y_{12} receptors [74,75].

Plasticity, development and regeneration

The ubiquity of purinergic signalling predestines nucleotides for exerting multiple functional roles in the development, plastic remodelling and regeneration of the nervous system [5,76]. Nucleotides elicit multiple trophic effects on cultured neural cells. For example, NGF- and P2Y -receptor co-activation preferentially results in neuronal survival and neurite outgrowth [77]. One of the exciting recent developments is the role of nucleotides in the control of neurogenesis [5]. The ATP hydrolyzing ectonucleotidase NTPDase2 represents a hallmark of the adult neurogenic niches. It is selectively expressed by the stem cells in the adult subventricular zone of the lateral ventricles and in the dentate gyrus of the hippocampus and also with retinal Müller cells. Activation of the metabotropic P2Y_1 and P2Y_2 receptors in cultured adult neural stem cells augmented cell proliferation in the presence of mitogenic growth factors, inferring nucleotide-receptor- and growth-factor-receptor-mediated synergism in progenitor cell proliferation. Cultured neurospheres constitutively released bursts of ATP to activate endogenous P2 receptors [78,79]. Although little is known concerning the role of nucleotides in hippocampal neurogenesis, the presence of functional P2X receptors on hippocampal progenitors has been demonstrated [80]. There is also ample evidence for a functional role of purinergic signalling pathways in mammalian embryonic neurogenesis [5,81], including in the retina [82]. In *Xenopus laevis*, ADP is an important agonist for the induction of the eye-field transcription factor Pax6 and eye development. Abrogating this pathway deteriorates or abolishes eye formation [83].

In vitro and *in vivo* experiments underpin the important role of nucleotides in the reactive responses of astrocytes to brain injury. These include stellation, proliferation and increased glial fibrillary acidic protein synthesis in addition to chemotactic and chemokinetic cell migration [84,85]. Via P2Y_4 receptors, ATP and UTP induce astrocyte expression and release of thrombospondin-1, a large multi-domain matrix glycoprotein participating in cell-cell and cell-matrix interactions including synapse formation [86]. In addition, ATP stimulates N-cadherin expression, a Ca^{2+} -dependent cell-adhesion molecule involved in glia-glia and axon-glia interactions [87].

Microglia, the immune effector cells of the CNS implicated in the pathogenesis of almost every CNS disease, express several P2X and P2Y receptors [69] in addition to adenosine receptors, which affect multiple cellular responses including microglial proliferation, process moti-

lity and migration *in vitro* and *in vivo* [74,88]. The microglial P2X_7 receptor has received special attention because it stimulates the release of plasminogen, interleukin (IL)- 1β or tumour necrosis factor [88,89]. Nucleotide-activated microglia, thus, have an important role in the control of both eliminating damaged cells and in creating a tissue environment favouring repair and neuroregeneration [90].

Nucleotides and nucleosides exert multiple effects on cultured oligodendrocyte precursors including increased motility, proliferation and differentiation [91,92]. After electrical axonal activity, adenosine seems to be a primary activity-dependent signal promoting the differentiation of premyelinating progenitor cells into myelinating oligodendrocytes, whereas ATP is of primary importance for regulating early development and myelination of Schwann cells [65]. A crosstalk between electrical-impulse activity in axons, astrocytes and myelination mediated by leukaemia inhibitory factor (LIF) has been demonstrated. In particular, LIF is released by astrocytes in response to ATP liberated from axons firing action potentials, and LIF promotes myelination by mature oligodendrocytes (for review, see Ref. [75]).

Nucleotide receptors in peripheral neurotransmission and neuromodulation

ATP receptors of both modalities, the P2X and P2Y , are abundantly expressed in all elements of the peripheral nervous system, in neuronal bodies and terminals, in satellite glia and in Schwann cells [36]. Although mRNA transcripts and immunocytochemistry revealed expression of almost all P2X subunits, the predominant type of receptors in the peripheral sensory nerve cells are P2X_2 , P2X_3 and heteromeric $\text{P2X}_{2/3}$ receptors [3]. In sympathetic and parasympathetic ganglia, P2X -receptor subtypes are expressed in both presynaptic and postsynaptic compartments; their role in fast transmission is modulatory mostly through regulation of neurotransmitter release.

The ATP sensitivity is not necessarily restricted to the terminals; increased axonal excitability to ATP and/or adenosine of unmyelinated fibres in rat vagus, sural and dorsal root nerves in addition to the human sural nerve has been described [3]. P2X and P2Y receptors are present on nociceptive sensory fibres and these are involved in modulation of pain transmission [93]. The unifying purinergic hypothesis for the initiation of pain was promulgated by Burnstock in 1996 [94]; this hypothesis postulated that purinergic mechanosensory transduction occurs in visceral tubes and sacs, including the ureter, bladder and gut, in which ATP released from epithelial cells during distension acts on P2X_3 homomeric and $\text{P2X}_{2/3}$ heteromeric receptors on subepithelial sensory nerves and initiates impulses in sensory pathways to pain centres in the CNS. Supporting evidence for this hypothesis has been recently provided [3]. These findings have opened up the possibility of reducing pain sensation with P2X antagonists; recent developments are represented by compound A-317491 (synthesized by Abbott Laboratories) and compound RO3 (synthesized by Roche Palo Alto), which are both effective P2X_3 and $\text{P2X}_{2/3}$ antagonists; the latter one is orally bioavailable and stable *in vivo* and currently in clinical trials.

Special senses

Purinergic mechanisms play an important part in specialized sensory pathways. In the eye, almost all cellular elements are influenced by ATP released from nerves and by paracrine or autocrine release from retinal cells, in particular from pigment epithelial cells, retinal astrocytes and inner retinal amacrine-like neurons [3]. In the inner ear, ATP acts as a co-transmitter with glutamate generating $[Ca^{2+}]_i$ signals in cochlea inner hair cells. Various P2X- and P2Y-receptor subtypes were shown to be expressed in other cell types in the cochlea, including outer hair cells, nonsensory epithelial cells, Henson cells, supporting cells, Deiters' cells in the organ of Corti, mucociliary cells in the inner ear, epithelial cells of the endolymphatic sac, strial marginal cells and vestibular dark cell epithelium [95,96]. In the olfactory system, P2X₅ and P2X₇ receptors are expressed in squamous, respiratory and olfactory epithelial cells, whereas P2Y₁ receptors are present in respiratory epithelium submucosal glandular tissue and P2Y₂ and P2Y₁₁ receptors are localized to the mucous-secreting cells within the vomeronasal organ [97]. Damaged cells release ATP, which activates purinergic receptors on neighbouring sustentacular cells, olfactory receptor neurons and basal cells, thereby initiating a stress-signalling cascade involving heat-shock proteins for neuroprotection. Taste-bud cells and associated sensory-nerve fibres express P2X₂- and P2X₃-receptor subunits [98] and P2Y₁, P2Y₂ and P2Y₄ receptors [99]. ATP is the key transmitter acting via P2X₂ and P2X₃ receptors on taste-receptor cells detecting chemicals in the oral cavity [15]. Elimination of P2X₂ and P2X₃ receptors abolished responses of the taste nerves, although the nerves remained responsive to touching, temperature and menthol.

Behaviour

There are only a few studies of the involvement of purinergic signalling in behavioural pathways even though purinergic neurotransmission and neuromodulation is now well established in the brain. Purinergic pathways have been described in relation to feeding behaviour [100,101], locomotor co-ordination [102,103], sleep and arousal [104,105] and mood and motivation [106,107]. A detailed review of behavioural studies involving purinergic signalling in healthy and pathological conditions is available [3].

Box 1 summarizes some of the important physiological roles of ATP in the nervous system.

Pathologies of the CNS

Several studies document pathological insults to the CNS accompanied by high concentrations of extracellular nucleotides. Examples are hypoxia, ischemia, traumatic insults (stab wound or spinal cord injury [SCI]) and high-frequency neuronal activation during epilepsy-associated seizures (for reviews, see Refs [75,84]). Two recent *in vivo* imaging studies shed light on the mechanisms and importance of nucleotides (mainly ATP) released at the site of injury. In a model of SCI, ATP release was very low in the injured region, probably as a result of disturbed cellular synthesis and severe cellular damage [108]. Instead, in the peri-traumatic region, ATP concentration was markedly

Box 1. Important physiological roles of ATP in the nervous system

- In the peripheral nervous system ATP acts as an excitatory or inhibitory neurotransmitter to smooth muscle.
- In the central nervous system ATP mediates excitatory synaptic currents, usually contributing a fraction of the excitatory postsynaptic current.
- ATP functions as a co-transmitter with various neurotransmitters and as an important prejunctional and postjunctional modulator, often after breakdown to adenosine.
- ATP is an important gliotransmitter and mediator of Ca^{2+} waves between astrocytes. In addition, it is involved in reciprocal neuron–glia signalling.
- ATP acts as a danger signal alerting the nervous tissue to aversive influences. It has an important role in inducing microglial proliferation, migration and secretion of inflammatory mediators and in immunomodulation and immunosuppression. It induces astrogliosis, with stellation, migration and proliferation of astrocytes and the release of molecules supporting tissue remodelling.
- ATP and adenosine participate in neuroprotection.
- ATP has an important role in sensory pain perception and in neuropathic pain conditions involving microglial activation. Purinergic mechanosensory transduction is a mechanism underlying visceral pain.
- ATP and probably other nucleotides play a part in neuroregeneration, including proliferation and differentiation of neural stem cells.
- ATP is a transmitter in sensory reception in the tongue and plays an important part in the function of additional sensory organs such as the eye, inner ear and olfactory system.

increased, not only owing to lack of efficient metabolism by degrading enzymes [109] but also owing to active release from surviving cells. Immediately after ischemic or traumatic damage to the brain, astrocytes released ATP resulting in rapid activation of microglia that formed a barrier between the healthy and injured tissue [110]. These findings indicate that, in a similar way to the immune system [111], early during acute CNS injury actively released ATP (and maybe other nucleotides) might act as diffusible 'danger signals' to alert responses to damage and start repair.

In the periphery, danger signals are essential defense mechanisms in the initial phase of inflammation and have been shown to become detrimental if inappropriately sustained. Similarly, in the CNS, when released in excess and/or for prolonged periods of time, nucleotides become toxic and contribute to neurodegeneration. Under these circumstances, P2 receptors function as an amplification device to spread the ATP wave and inflammation via activation of P2 receptors on neurons or, indirectly, via release of proinflammatory cytokines from macrophages and/or microglia (for review, see Refs [75,84]). Both P2X and P2Y receptors are involved in these events. P2X₂, P2X₄ and P2X₇ receptors are upregulated in neurons and glia in both *in vitro* and *in vivo* ischemia models [112,113].

The P2X₇ receptors might regulate the cleavage and release of IL-1 from macrophages and/or microglia, thus contributing to neuroinflammation [114,115]. Moreover, the role of P2X₇ in cell death is clearly established [43,71]. An increased expression of P2Y₁ receptors in the peri-infarct area after focal ischemia in rats has also been documented [116]. Moreover, the *in vivo* knock-down of the

new putative P2Y receptor GPR17 by pharmacological agents or anti-sense oligonucleotides markedly attenuated ischemic damage [50].

Dysregulation of P2 receptors might also contribute to chronic neuroinflammatory diseases. ATP increases neuronal vulnerability to β -amyloid peptide-induced toxicity, and P2X₇ receptors are specifically upregulated around β -amyloid plaques in a mouse model of Alzheimer's disease (AD) [117]. In neurofibrillary tangles and neuritic plaques in the brain tissue of AD patients, changes in P2Y₁ receptors have been described [118]. Increased expression of P2X₇ receptors was also observed in reactive astrocytes in multiple sclerosis lesions [119] and a causal link between activation of P2X₇ receptors and oligodendrocytes death was established [71]. In line with all these findings, P2-receptor antagonists seem to be beneficial and have been used to reduce neuronal damage in several models of acute and chronic neurodegeneration [84].

Activation of P2 receptors after neurodegenerative insults also displays neuroprotective effects, including the release of neurotrophic molecules and beneficial cytokines, activation of neuritogenesis and other reparative processes. According to a recent hypothesis [75], nucleotide-induced effects originate as a time- and site-specific defence mechanism to protect neurons and facilitate post-injury recovery. Dysregulation of these mechanisms upon chronic inflammation might turn this reaction into a destructive and uncontrolled chain of events resulting in brain damage and functional loss.

Finally, as revealed by analysis of receptor polymorphisms, nucleotides are also involved in psychiatric disease. The P2X₇ receptor gene, *P2RX7*, was found to be associated with major depressive disorder [120].

Peripheral pathologies

Purinergic pathways are also implicated in numerous pathologies associated with malfunction of the peripheral nervous system. For example, ATP-mediated effects are implicated in enhanced sympathetic-nerve activity, which causes cardiac dysfunction, arrhythmias, heart failure and sudden cardiac death in myocardial ischemia and in sympathetic nerves supplying hypertensive blood vessels [121]. Incidentally, ATP is a rapidly acting hypotensive agent that compares favourably with sodium nitropruside, and ATP-MgCl₂ is a safe, effective and preferential pulmonary vasodilator in children with pulmonary hypertension. The purinergic component of peripheral co-transmission substantially (up to 40%) increases in urogenital pathologies such as interstitial cystitis, outflow obstruction, idiopathic detrusor instability and some types of neurogenic bladder [122–125]. Purinergic transmission might have a role in male infertility; the knockout mice lacking P2X₁ receptors seem to be normal but fail to breed, and this is associated with loss of the purinergic component of sympathetic co-transmission in the vas deferens. Vagal afferent purinergic signalling might be involved in the hyperactivity associated with asthma and chronic obstructive pulmonary diseases. It was recognized early that the nervous system might contribute to the pathophysiology of rheumatoid arthritis, and a role for purinergic signalling in rheumatic diseases has been considered. Quinacrine

(Atabrine), a drug that binds strongly to ATP, has been used for the treatment of rheumatoid arthritis patients for many years. Spinal P1-receptor activation has been claimed to inhibit inflammation and joint destruction in rat adjuvant-induced arthritis, supporting the view that therapeutic strategies that target the CNS might be useful in arthritis [126].

Future directions

Although important molecular, cellular, physiological and pathophysiological functions of ATP and other nucleotides have been uncovered, much needs to be learned about their function in the nervous system. Recent exciting data indicate that ATP and its receptors might be targeted to obtain new and potent agents of potential use in pain, neuroinflammation, neurodegenerative diseases such as stroke and Alzheimer's, and neuropsychiatric conditions such as depression and drug addiction [127]. More needs to be learned regarding the role of nucleotides in the control of behaviour and mental processes, in mechanosensory transduction, in the development of neural disease and in regenerative processes, and their potential for novel therapeutic strategies.

The development of novel biosensors is expected to enable the local real-time measurement of ATP from individual cells and defined neural circuitries under physiological and pathological conditions. The release mechanisms for nucleotides turned out to be multi-faceted and their relative importance for individual nucleotides, cell types and type of physiological stimulus needs to be deciphered. Many CNS cells carry multiple nucleotide receptors, the individual functional impact and integrative role of which need to be further elaborated. Microglia, with its P2Y₆, P2Y₁₂, P2X₄ and P2X₇ receptors, serve as an example.

An additional challenge is the integration of the nucleotide signalling pathway with the adenosine-receptor system (which are often co-localized) and the interaction with other transmitter systems, such as glutamate and dopamine for behaviour, cytokines for inflammation and growth factors for neuro-repair and development. The functional role of nucleotides in the embryonic and postnatal development of the central and peripheral nervous system is just evolving. Perhaps the main limiting factor for such studies is the absence of selective and stable purinoceptor-subtype agonists and antagonists that can cross the blood–brain barrier.

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