Available online at www.sciencedirect.com
SCIENCE DIRECT®
European Journal of Pharmacology 483 (2004) 5–17
www.elsevier.com/locate/ejphar

Review

Molecular physiology of P2 receptors in the central nervous system

Peter Illes[a,*], J. Alexandre Ribeiro[b]

[a] Rudolf-Boehm-Institute of Pharmacology, University of Leipzig, Haertelstrasse 16-18, 04107 Leipzig, Germany
[b] Laboratory of Neurosciences, Faculty of Medicine, University of Lisbon, Av. Professor Egas Moniz, 1649-028 Lisbon, Portugal

Received 7 August 2003; received in revised form 16 October 2003; accepted 24 October 2003

Abstract

Neurons of the central nervous system (CNS) are endowed with ATP-sensitive receptors belonging to the P2X (ligand-gated cationic channels) and P2Y (G protein-coupled receptors) types. Whereas a number of P2X receptors mediate fast synaptic responses to the transmitter ATP, P2Y receptors mediate either slow changes of the membrane potential in response to non-synaptically released ATP or the interaction with receptors for other transmitters. To date seven P2X and seven P2Y receptors of human origin have been molecularly identified and functionally characterized. P2X subunits may occur as homooligomers or as heterooligomeric assemblies of more than one subunit. $P2X_7$ subunits do not form heterooligomeric assemblies and are uniqe in mediating apoptosis and necrosis of glial cells and possibly also of neurons. The $P2X_2$, $P2X_4$, $P2X_4/P2X_6$ and $P2Y_1$ receptors appear to be the predominant neuronal types. The localisation of these receptors may be at the somato-dendritic region (postsynaptic) or at the nerve terminals (presynaptic). Postsynaptic P2 receptors appear to be mostly excitatory, while presynaptic P2 receptors may be either excitatory (P2X) or inhibitory (P2Y). Since in the CNS the stimulation of a single neuron may activate multiple networks, a concomitant stimulation of facilitatory and inhibitory circuits as a result of ATP release is also possible. Finally, the enzymatic degradation of ATP may lead to the local generation of adenosine which can modulate via A_1 or A_{2A} receptor-activation the ATP effect.

Keywords: Presynaptic; Postsynaptic; P2X receptor; P2Y receptor; ATP

1. Introduction

Extracellular nucleotide receptors belong to the P2X (ligand-gated cationic channels) or P2Y type (G protein-coupled receptors) (Abbracchio and Burnstock, 1994; Ralevic and Burnstock, 1998; North, 2002). Both receptor-types are widely distributed in the central nervous system (CNS) and exhibit various effects both at neuronal and glial cells. Astrocytes are endowed with a minor population of P2X and a major population of P2Y receptors, whose activation leads initially to an increase of intracellular Ca^{2+} and subsequently to long-term changes including proliferation and apoptosis (Neary et al., 1996; Fields and Stevens, 2000; Ciccarelli et al., 2001). Neurons also possess both classes of P2 receptors; P2X mediates fast synaptic responses to the transmitter ATP, whereas P2Y mediates either slow changes of the membrane potential in response to non-synaptically released ATP or the interaction with receptors for other transmitters (Illes et al., 1996; Nörenberg and Illes, 2000; Khakh, 2001; Masino and Dunwiddie, 2001; Robertson et al., 2001; Burnstock, 2003). In addition to these changes occurring within short time periods, also long-term, trophic effects were observed in response to ATP, such as neuronal maturation, neurite outgrowth (Huang and Kao, 1996; Swanson et al., 1998) and the expression of transmitter receptors at target cells (Choi et al., 2003). The present review will concentrate on the most important recent developments in the field of neuronal P2 receptors in the CNS, with an emphasis on functional aspects.

2. Recombinant P2 receptors

2.1. P2X receptors

P2X receptors form a family of at least seven subunits containing 379 ($P2X_6$) to 595 ($P2X_7$) amino acids (Khakh, 2001). The subunits have two transmembrane domains, a large extracellular loop containing the ATP binding site, as

* Corresponding author. Tel.: +49-341-9724600; fax: +49-341-9724609.
E-mail address: illp@medizin.uni-leipzig.de (P. Illes).

0014-2999/$ - see front matter © 2003 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejphar.2003.10.030

well as intracellular N and C terminal tails (Khakh et al., 2001a). Biochemical evidence indicates that the receptors occur as stable trimers or hexamers of three or six subunits (Nicke et al., 1998; Stoop et al., 1999). Moreover, heteromultimeric assemblies of $P2X_2/P2X_3$ (Radford et al., 1997), $P2X_4/P2X_6$ (Le et al., 1998a) and $P2X_1/P2X_5$ (Torres et al., 1998b) have been described, while $P2X_7$ does not coassemble with any other subunit (Torres et al., 1999).

Neuronal P2X receptors in the CNS appear to belong mostly to the $P2X_2$, $P2X_4$ or $P2X_4/P2X_6$ types. The ATP analogue α,β-methylene ATP (α,β-meATP) is an agonist at $P2X_1$ and $P2X_3$ subunits, but not at the residual homomeric subunits (Ralevic and Burnstock, 1998). However, the $P2X_4/P2X_6$ heteromeric receptors acquire a sensitivity to this agonist (Le et al., 1998a). Furthermore, the $P2X_2$ receptor-antagonistic pyridoxalphosphate-6-azophenyl-2′,4′-disulfonic acid (PPADS) does not block rat recombinant $P2X_4$ receptors (Buell et al., 1996), although the human homolog of this receptor is sensitive to the antagonist (Garcia-Guzman et al., 1997).

There is a wealth of data available about recombinant $P2X_2$ receptors. Alanin-scanning mutagenesis identified a region proximal to the first transmembrane domain which contained two lysine residues that were critical for the effect of ATP (Jiang et al., 2000). This region was supposed to contribute to the ATP-binding site of the receptor. In order to identify residues necessary for proton and Zn^{2+} modulation (Wildman et al., 1998), alanines were singly substituted for each of the nine histidines in the extracellular domain of the rat $P2X_2$ receptor (Dylan Clyne et al., 2002a). Different mutations selectively eliminated pH or Zn^{2+} potentiation implicating that these are two independent sites of action, even though the mechanisms of pH and Zn^{2+} potentiation appeared similar. *N*-glycosylation sites that have an amide group of asparagines were only found in the extracellular domain (182–185, 239–242, 298–301). The amide groups bind covalently carbohydrates. Such Asp residues contribute probably to solubility, cell-surface expression, and ATP potency (Torres et al., 1998a; Hu et al., 2002). Deletion of any two out of the three asparagine residues (182N, 239N and 298N) that are *N*-glycosylated led to non-functional channels (Hu et al., 2002).

The $P2X_1$ (Ennion and Evans, 2002) and $P2X_2$ (Dylan Clyne et al., 2002b) subunits contain ten conserved cysteines in the extracellular loop which are involved in the formation of disulfide bonds. None of these bonds are individually essential for channel function. However, trafficking of the $P2X_1$ receptor to the cell membrane was severely reduced by disruption of the C261–C270 disulfide bound or disruption of C117–C165 together with another bond (Ennion and Evans, 2002). $P2X_4$ receptors heterologously expressed in cultured olfactory bulb neurons underwent rapid constitutive internalisation and subsequent reinsertion into the plasma membrane (Bobanovic et al., 2002). The presence of the $P2X_4$ subunit in a $P2X_4/P2X_6$ heteromer governed the trafficking properties of the receptor. Finally, it has been shown that channel opening is necessary for P2X receptor internalisation (Jensik and Cox, 2002).

The second transmembrane region (TM2; amino acids in positions 330–353) was shown to line the ion pore of $P2X_2$ (Rassendren et al., 1997; Egan et al., 1998). The substituted cysteine accessibility method identified a functional domain of the channel pore (L338 and D349) by mapping residues on either side of the channel gate. Domains near either end of the first transmembrane region (TM1; amino acids in positions 30–50) also influenced the ion conduction through the pore of the $P2X_2$ subunit (Jiang et al., 2001; Haines et al., 2001).

P2X receptors can be classified into rapidly ($P2X_1$, $P2X_3$) and slowly desensitizing types ($P2X_2$, $P2X_5$, $P2X_6$, $P2X_7$) (Ralevic and Burnstock, 1998). Two $P2X_2$ isoforms differing only in their C terminal regions desensitize at different rates (Brändle et al., 1997). The $P2X_{2(b)}$ isoform is missing a sixty-nine amino acid segment that is present in $P2X_{2(a)}$ and it desensitizes about fivefold faster than the longer isoform. It was concluded from mutagenesis studies that the rate of desensitization of the $P2X_2$ receptor is controlled by distinct amino acids located in the C-terminus (Smith et al., 1999; Koshimizu et al., 1999). Moreover, a protein kinase C phosphorylation site in the N-terminus is necessary for the full expression of slowly desensitizing ATP-gated channels (Surprenant et al., 1995; Ennion et al., 2000; Bouet-Grabot et al., 2000).

$P2X_7$ channels were reported to change their ion selectivity during prolonged exposure to ATP (Surprenant et al., 1996). However, recently neuronal $P2X_2$, $P2X_{2/3}$ and $P2X_4$ receptor-channels were also found to progressively diate during prolonged ATP activation (Khakh et al., 1999; Virginio et al., 1999). The binary properties of channels such as whether or not certain ions permeate in particular cellular settings, can have profound effects on the encoding properties of the synapses.

A cross-inhibition of $P2X_2$ and α_3,β_4 nicotinic acetylcholine receptors (Khakh et al., 2000) as well as of $5\text{-}HT_3$ receptors (Boue-Grabot et al., 2003) has been shown to occur during co-activation. The inhibitory cross-talk between $P2X_2$ and $5\text{-}HT_3$ receptors was disrupted when the intracellular C-terminal domain of the $P2X_2$ subunit was deleted (Boue-Grabot et al., 2003).

2.2. P2Y receptors

So far the P2Y family comprises of ten cloned and functionally defined subtypes (Von Kügelgen and Wetter, 2000; Barnard and Simon, 2001; Communi et al., 2001). Eight of them ($P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$, $P2Y_{11}$, $P2Y_{12}$, $P2Y_{13}$, $P2Y_{14}$) are present in human tissues of which $P2Y_1$, $P2Y_6$, $P2Y_{11}$, $P2Y_{12}$, $P2Y_{13}$, and $P2Y_{14}$ occur in the CNS (Moore et al., 2001; Abbracchio et al., 2003). The receptor proteins contain the typical features of G protein-coupled receptors including seven predicted hydrophobic transmem-

brane regions. $P2Y_1$, $P2Y_{12}$ and $P2Y_{13}$ receptors react to adenine nucleotides only, whereas $P2Y_6$ is a uridine nucleotide-sensitive receptor (Ralevic and Burnstock, 1998; Communi et al., 2001; Hollopeter et al., 2001). In addition, ADP and especially its structural analogues i.e. 2-methyltio ADP (2-MeSADP) activate $P2Y_1$ and $P2Y_{12}$ receptors with a much higher potency than ATP itself (Barnard and Simon, 2001). Several antagonists clerarly differentiate between the individual receptor types, such as N^6methyl-modified 2′-deoxyadenosine 3′,5′-bisphosphate (MRS 2179) which is selective for $P2Y_1$ receptors (Camaioni et al., 1998) and 2-propythio-D-β,γ-difluoromethylene ATP (PFL 66096) which is selective for $P2Y_{12}$ receptors (Humphries et al., 1994). Recently, an UDP-glucose receptor (Chambers et al., 2000) has been described to exhibit a considerable sequence homology with P2Y receptors (Abbracchio et al., 2003). Based on this fact as well as on (1) its chromosomal co-localization with other P2Y receptors, (2) the similarity of its cognate ligand to the natural ligand for P2Y receptors and (3) the identification of UDP-glucose in the extracellular space, it was renamed the $P2Y_{14}$ receptor (Abbracchio et al., 2003; Lazarowski et al., 2003).

Mutation of the transmembrane (TM) helical domains indicated the critical function of residues on the exofacial side of TM3 and TM7 in the binding of ATP (Jacobson et al., 1999). In addition, two essential disulfide bridges in the extracellular domains and several charged residues in the extracellular loops (EL) 2 and 3 have been found to be critical for receptor activation (Hoffmann et al., 1999). Recently $P2Y_1$ and adenosine A_1 receptors have been found to form constitutive hetero-oligomers in human embryonic kidney (HEK) 293 cells and this process was promoted by the simultaneous activation of both receptors by their respective agonists (Yoshioka et al., 2002). It is interesting to note that the modulation of an endogenous voltage-gated ion channel in *Xenopus* oocytes by $P2Y_1$ receptors has been shown to critically depend on the C-terminal region of the receptor (Lee et al., 2003).

3. Distribution of P2 receptors

3.1. P2X receptors

Both $P2X_2$ receptor mRNA and $P2X_2$ receptor protein shows a large scale and matching distribution in the rat CNS as proved by riboprobe-based in situ hybridization and specific antisera (Kanjhan et al., 1999). An extensive expression pattern was observed in the cerebral cortex, hippocampus, habenula, substantia nigra pars compacta, ventromedial and arcuate hypothalamic nuclei (Xiang et al., 1998), supraoptic and paraventricular nuclei (Loesch et al., 1999), mesencephalic trigeminal nucleus, ventrolateral medulla, dorsal vagal complex (Atkinson et al., 2000) and nucleus of the solitary tract (Kanjhan et al., 1999). In the rat spinal cord, $P2X_2$ receptor expression was highest in the dorsal horn, with significant neuronal labeling in the ventral horn and intermediolateral cell column.

$P2X_3$ receptor mRNA and protein appears to be selective for a subpopulation of small-diameter neurons of dorsal root ganglia (Chen et al., 1995; Lewis et al., 1995). The confinement of $P2X_3$ receptor immunoreactivity to brain structures involved in pain transmission (nucleus tractus solitarius, solitary tract, spinal trigeminal nucleus; Vulchanova et al., 1996, 1997) appears to be stringent in the adult rat brain in contrast to a more widespread distribution observed in the embryonic and neonatal rat brain (Kidd et al., 1998).

In situ hybridization for $P2X_4$ mRNA (Bo et al., 1995; Buell et al., 1996) or antibodies directed against $P2X_4$ receptors (Le et al., 1998b) indicated strong expression in the rat cerebellum and spinal cord. $P2X_4$ immunoreactivity was also evident in areas including the cerebral cortex, hippocampus, thalamus and brainstem.

Both light microscopic and postembedding immunocytochemistry at the ultrastructural level indicated a localization of $P2X_2$ and $P2X_4$ receptors at postsynaptic specializations of parallel fibre synapses in the cerebellum and of Schaffer collateral synapses in the rat hippocampus (Rubio and Soto, 2001). Analyis of their distribution at the synapse showed that P2X receptors are localized at peripheral portions of the postsynaptic specialization, where ionotropic glutamate receptor density decreases.

Investigations at the light and electron microscopic levels have revealed that in the nervous system the $P2X_{1-6}$ receptor subtypes are localized predominantly at neurons, while the $P2X_7$ receptor-subtype appears to be limited to e.g. activated microglia, lymphocytes, macrophages or astroglia, agreeing with its role in brain repair following inflammation, infarction or immune insult (Di Virgilio et al., 1999; Gu et al., 2000). More recently, both $P2X_7$ receptor mRNA and immunoreactivity was found to be targeted to presynaptic excitatory but not inhibitory terminals in the spinal cord and brain (Deuchars et al., 2001; Sperlagh et al., 2002). In contrast, the $P2X_7$ receptor protein was also present in the nuclear envelop of a subgroup of inhibitory neurons in the hippocampus (Atkinson et al., 2002). It is suggested that activation of this intracellular receptor leads to a permeability increase towards Ca^{2+}which may via changes in the nuclear Ca^{2+} concentration alter the transcription of several genes implicated in neuronal plasticity (Atkinson et al., 2002).

3.2. P2Y receptors

A quantitative reverse transcriptase-polymerase chain reaction (RT-PCR) study demonstrated the presence of large quantities of $P2Y_1$ and $P2Y_{11}$ mRNA in the human brain when compared with other tissues (Moore et al., 2001). $P2Y_1$ mRNA was most highly expressed in regions of the basal ganglia including the striatum, nucleus accumbens, putamen and caudate nucleus and was detected in the globus

pallidus, hippocampus, cerebellum and many regions of the cerebral cortex. A polyclonal $P2Y_1$ receptor antibody indicated a striking localisation to neuronal structures of the human cerebral cortex, cerebellar cortex, hippocampus, caudate nucleus, putamen, globus pallidus and midbrain (Moore et al., 2000). A basically similar distribution of the $P2Y_1$ receptor immunoreactivity was demonstrated in the rat brain (Moran-Jimenez and Matute, 2000).

Cellular resolution of the expression of $P2Y_{12}$ mRNA was obtained by in situ hybridization histochemistry of brain sections; the observations are consistent with a glial expression pattern (Hollopeter et al., 2001). $P2Y_{13}$ mRNA was detected in different brain regions (thalamus, caudate nucleus, substantia nigra, hippocampus and cerebellum) (Communi et al., 2001). Autoradiography of ADP stimulated [^{35}S]GTP-γ-S binding in brain tissue, supposed to reflect the distribution of $G_{i/o}$-linked $P2Y_{12}$ or $P2Y_{13}$ receptors, showed high levels in the cerebellum, cerebral cortex, hippocampus, stria terminalis and ventrolateral medulla (Laitinen et al., 2001). It is interesting to note that in contrast to $P2Y_1$ receptor-protein distribution the white matter in the cerebellum exhibited homogenous labeling with scattered intensive small spots.

Widespread distribution of $P2Y_{14}$ mRNA has been observed in humans, with highest expression in placenta, adipose tissue, stomach and intestine, with moderate levels in the brain, spleen, lung and heart (Chambers et al., 2000). Although there is no indication for the neuronal localization of this receptor hitherto, it has been detected in rat primary astrocytes (Charlton et al., 1997) which responded to UDP-glucose with increases of their intracellular Ca^{2+} concentration (Fumagalli et al., 2003).

4. Effects at postsynaptic P2 receptors situated at the somato-dendritic region

4.1. P2X receptors

Various neurons of the CNS are endowed with functional P2X receptors. In slices of the medial habenula electrical stimulation evokes ATPergic synaptic potentials (Edwards et al., 1992). Exogenous ATP itself failed to initiate a whole-cell current response, whereas the structural analogue α,β-methylene ATP (α,β-meATP) caused small inward currents as well as in a minority of the outside-out patches tested single channel openings. This indicates that the overall receptor density is low, with receptors probably concentrated at the synapse. In addition, it has been shown that neurones of the medial habenula lack functional NMDA receptors and possess AMPA receptors that have low permeability to Ca^{2+} (Robertson et al., 1999). It appears that P2X receptor-mediated synaptic currents are the only calcium-permeable fast-transmitter gated currents in these neurones which may be important for their physiological function.

In cultured dorsal horn neurons of the rat spinal cord, fast synaptic currents were detected which were due to the co-release of ATP and γ-aminobutyric acid (GABA) (Jo and Schlichter, 1999). Similarly, in the lateral hypothalamus of mice and chick, both electrically evoked and spontaneous ATP and GABA co-release has been observed (Jo and Role, 2002a). The miniature postsynaptic currents recorded at ATP-GABA co-synapses were only partially inhibited by the $GABA_A$ receptor antagonist bicuculline. The remaining miniature postsynaptic currents were blocked by the P2 receptor antagonist suramin. Neurons of the lateral hypothalamus obtain cholinergic input from the pedunculopontine and laterodorsal tegmental nuclei as well as from cholinergic interneurons within the lateral hypothalamus. GABAergic transmission was modulated in an opposite way by facilitatory nicotinic and inhibitory muscarinic acetylcholine receptors (Jo and Role, 2002b).

The cell bodies of central noradrenergic neurons of the rat nucleus locus coeruleus contain hyperpolarizing α_2-adrenoceptors (Aghajanian and Wang, 1986) as well as depolarizing P2X and P2Y receptors (Harms et al., 1992; Shen and North, 1993). It is noteworthy that these receptor-types are consecutively activated by the two transmitters noradrenaline and ATP co-released on electrical stimulation from recurrent axon collaterals (Poelchen et al., 2001). Whereas locus coeruleus neurons from older rats (18–23 days of age) responded to α,β-meATP with inward current, the same neurons from younger rats (10–14 days of age) were insensitive to this agonist (Wirkner et al., 1998). At the same time noradrenaline evoked α_2-adrenoceptor-mediated outward currents in all tissues investigated, suggesting that probably no functional P2 receptors are present at locus coeruleus neurons after birth and maturity of these receptors is reached only in animals older than 18 days.

The proprioceptive sensory neurons of the rat trigeminal mesencephalic nucleus are situated in the immediate neighbourhood of the locus coeruleus. These neurons respond to ATP or its analogues (adenosine 5′-[γ-thio]triphosphate, ATP-γ-S; α,β-meATP) with a rapidly desensitizing inward current; all agonists cross-desensitized with each other (Khakh et al., 1997). The $P2X_1$-, $P2X_3$-selective antagonist 2′-3′-*O*-(2,4-trinitrophenyl)-ATP (TNP-ATP; Thomas et al., 1998) reduced, but did not abolish the current evoked by ATP-γ-S (Patel et al., 2001). However, an agonist for these receptor-types, α,β-meATP failed to evoke an inward current. Hence, the TNP-ATP-sensitive component of the current did not match with any of the known cloned homomeric or heteromeric P2X receptors.

In rat brain slices, ATP caused inward current in both CA1 (Pankratov et al., 1998) and CA3 (Mori et al., 2001) hippocampal neurons. ATP, co-released with glutamate, induced synaptic responses in these neurons that were observed mainly under conditions of synchronous discharge from pultiple presynaptic inputs. The ATP-gated receptor involved appears to belong to the $P2X_2$ type. When green fluorescent protein (GFP)-tagged $P2X_2$ receptors were engi-

neered and expressed in embryonic hippocampal neurons, ATP application to dendrites caused receptor redistribution (Khakh et al., 2001b). This led to the formation of varicose hot spots of higher $P2X_2$-GFP receptor density accompanied by an activation-dependent enhancement of the ATP-evoked current. It is interesting to note that presynaptic facilitatory $P2X_2$ receptors are also involved in synaptic transmission onto stratum radiatum interneurons but not CA1 pyramidal neurons (Khakh et al., 2003). Finally, rat pyramidal neurons of the sensory cortex possess P2X receptors as well, since electrically evoked excitatory postsynaptic currents could be blocked by a combination of NMDA, non-NMDA and P2X receptor antagonists only (Pankratov et al., 2002a). In addition, application of ATP or α,β-meATP caused in these neurons inward currents, supporting the role of ATP as a fast synaptic transmitter.

Although Ca^{2+}-permeable P2X receptors mediate a minor component of the excitatory synaptic current in hippocampal CA1 pyramidal neurons, they may be involved in the Ca^{2+}-dependent inactivation of the NMDA component of the excitatory postsynaptic current (Pankratov et al., 2002b). During high frequency stimulation of synaptic inputs P2X receptors desensitise and thereby the NMDA component of the excitatory postsynaptic current may increase, leading to long-term potentiation. In agreement with this suggestion, inhibition of P2X receptors by PPADS or their desensitisation by α,β-meATP dramatically facilitated the induction of long-term potentiation which is believed to be a cellular model of learning and memory. In partial contradiction to these findings, in mouse (Wieraszko and Seyfried, 1989; Chen et al., 1996) and guinea-pig (Fujii et al., 2002) hippocampal CA1 neurons, ATP was reported to induce long-term potentiation by phosphorylating the extracellular domain of the NMDA receptor-channel and thereby increasing its permeability. A possible explanation for this discrepancy is that in the rat and mouse/guinea-pig hippocampus different purinergic mechanisms regulate long-term potentiation.

In the rat dorsal motor nucleus of the vagus, ATP causes inward current (Ueno et al., 2001). Low concentrations of Zn^{2+} potentiated these currents in agreement with results obtained on recombinant (Wildman et al., 1998) and native (Li et al., 1993) $P2X_2$ receptors. Moreover, rat cerebellar Purkinje cells responded to ATP with a rapid increase in intracellular Ca^{2+} (Mateo et al., 1998; Garcia-Lecea et al., 1999). The effect of ATP was mimicked by 2-methylthio ATP (2-MeSATP) but not by α,β-meATP and was potentiated by Zn^{2+} and H^+ ions indicating the involvement of $P2X_2$ subunits. Patch-clamp recordings and single-cell RT-PCR indicated the existence of $P2X_2$ receptors in histaminergic neurons of the rat tuberomamillary nucleus (Vorobjev et al., 2003). Finally, ATP increased the cytosolic Ca^{2+} concentration in neurons of the ventral tegmental area acutely dissociated from the rat brain (Sorimachi et al., 2002). This effect was due to the activation of Ca^{2+}-permeable P2X receptors.

4.2. P2Y receptors

In various primary cultures of the rat brain (striatum, medulla oblongata, hippocampus and superior colliculus), ATP or ADP evoked an outwardly rectifying K^+ current (Ikeuchi and Nishizaki, 1995; Ikeuchi et al., 1995a,b, 1996). It has been concluded that ATP stimulates an outwardly rectifying K^+ channel which is regulated by protein kinase C activation through a P2Y receptor, linked to a pertussis toxin-insensitive G protein. These results are in partial agreement with recent data demonstrating the coupling of the $P2Y_2$ receptor-type to recombinant inwardly rectifying K^+ channels of the Kir3.0 subfamily (Mosbacher et al., 1998).

ATP, ATP-γ-S (Dave and Mogul, 1996) and diadenosine pentaphosphate (Panchenko et al., 1996) all potentiated the high-voltage activated current through Ca^{2+} channels in freshly dissociated hippocampal neurons. Pyramidal neurons of the sensorimotor cortex (Lalo and Kostyuk, 1998) and neocortical neurons (Lalo et al., 1998) exhibited an increase of the cytoplasmic free calcium concentration ($[Ca^{2+}]_i$ transient) in response to ATP. This effect may involve the $G_{q,11}$/phospholipase C/inositoltrisphosphate/Ca^{2+} pathway, which is a typical signal transduction mechanism for neuronal $P2Y_1$ receptors (King et al., 1998).

In addition to these effects, P2Y receptors were also able to potentiate the conductance of ionotropic glutamate receptors of the *N*-methyl-D-aspartate (NMDA)-, but not of the α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA)-type in layer V pyramidal neurons of the rat prefrontal cortex (Wirkner et al., 2002). This interaction takes place via G protein-activation and can be observed only in a subgroup of the pyramidal neurons. Since ligand-gated cationic channels, such as NMDA receptors have either been shown to be regulated by protein phosphorylation or to contain consensus sequences for phosphorylation by protein kinases (Moss and Smart, 1996), protein kinase C or calmodulin kinase II are possible candidates for the transduction mechanism of P2Y receptors. It was suggested that $P2Y_2$ receptors are involved in this process, since ATP and UTP, but not the $P2Y_1$ receptor-selective ADP-β-S facilitated the NMDA current (Wirkner et al., 2002).

In fact, ADP-β-S inhibited the NMDA-induced current in layer V pyramidal neurons of the rat prefrontal cortex (Luthardt et al., 2003). The blockade of this effect by the $P2Y_1$ receptor antagonists PPADS and 2′ -deoxy-N^6-methyladenosine-3′,5′-diphosphate (MRS 2179) supported the involvement of a $P2Y_1$ receptor. Since the intracellular application of the stable GDP analogue GDP-β-S which is known to interfere with G protein-mediated reactions did not alter the inhibitory effect of ADP-β-S, the two receptors were suggested to interact via direct protein–protein apposition (Khakh et al., 2000; Liu et al., 2000). A non-receptor mediated blockade by ATP of NMDA currents was also reported to occur in cultured rat hippocampal cells and in *Xenopus* oocytes transfected with recombinant NR1a/NR2B

receptors (Ortinau et al., 2003). ATP failed to alter other subunit combinations such as those of NR1a/NR2A, NR1a/NR2C and NR1a/NR2D. GTP and ITP but not UTP and CTP mimicked the inhibitory action of ATP. It was suggested that binding of ATP occurs to a glycine-rich consensus nucleotide-binding motif in the N-terminal part of the NR2B subunit.

5. Effects at presynaptic P2 receptors situated at nerve terminals

5.1. P1 receptors of the A_1-type

Besides the above described postsynaptic effects, ATP also acts presynaptically. However a difficulty to interpret these effects is caused by the fact that ATP is degraded enzymatically to adenosine (see Cunha and Ribeiro, 2000). Adenosine A_1 receptor activation causes a profound inhibition of synaptic transmission and of evoked neurotransmitter release through inhibition of calcium influx and/or inhibition of the exocytotic machinery (Ribeiro, 1995). The ecto-nucleotidase pathway subserving the breakdown of ATP is very efficient; adenosine A_1 receptor activation can occur within milliseconds after adding ATP to the preparations (Dunwiddie et al., 1997). A channelling process involving ecto-nucleotidases and adenosine A_1 receptors, i.e. the ability of ATP-derived adenosine to activate adenosine A_1 receptors without equilibrating with the biophase, probably accounts for some of the proposed direct actions of adenine nucleotides (Cunha et al., 1998; Sebastiao et al., 1999). Furthermore, the excitatory actions of ATP are difficult to distinguish from the facilitatory effects of adenosine A_{2A} receptors (Sebastiao and Ribeiro, 1996). The use of P2 receptor antagonists does not entirely disriminate P2 from P1 receptor responses, since most P2 receptor antagonists are effective inhibitors of extracellular ATP catabolism (Ralevic and Burnstock, 1998).

Recently the hypothesis has been advanced that there may be a nucleotide receptor that inhibits neurotransmitter release, is activated directly by ATP and ATP analogues, and is insensitive to most P2 receptor antagonists, but is sensitive to purportedly selective adenosine A_1 receptor antagonists such as 8-cyclopentyl-theophylline (Smith et al., 1997; Mendoza-Fernandez et al., 2000). However, in mice lacking the adenosine A_1 receptor (Johansson et al., 2001), the inhibitory effects of both adenosine, ATP and ATP-γ-S on synaptic transmission onto hippocampal CA1 pyramidal cells were completely absent, suggesting that all these effects are mediated *via* conventional adenosine A_1 receptors (Masino et al., 2002).

5.2. P2 receptors of the P2X- and P2Y-types

ATP analogues inhibit evoked acetylcholine release from the rat cerebral cortical synaptosomes more potently than adenosine itself, and the effect of ATP is not prevented either upon blocking the extracellular formation of ATP-derived adenosine or upon removal of extracellular adenosine (Cunha et al., 1994). However, the type of receptor(s) involved in the effect of ATP on acetylcholine release from rat cerebral cortical synaptosomes remains to be defined (Cunha et al., 1994).

ATP changes in a biphasic manner the release of noradrenaline in the CNS. Thus, it has been reported that P2Y-like receptor-activation inhibits noradrenaline release from cerebral cortical (Von Kügelgen et al., 1994) and hippocampal slices (Von Kügelgen et al., 1999) of rats. Using a microdialysis approach in the dorsal and ventral striatum (nucleus accumbens), it was reported that ATP induces dopamine release (Zhang et al., 1995; Krügel et al., 1999). This effect was suggested to consist of two components. The first component involves a direct stimulation of dopaminergic nerve terminals in the nucleus accumbens, while the second inhibitory component involves a sequential activation of glutamate and finally GABA neurons projecting to the dopaminergic cell bodies in the ventral tegmentum (Krügel et al., 2001a,b). An inhibitory effect of ATP was observed by fast cyclic voltammetry in rat neostriatal slices on dopamine release (Trendelenburg and Bültmann, 2000). In addition, P2 receptors were shown to increase the dendritic release of dopamine in the ventral tegmental area (Krügel et al., 2001a,b).

Activation of P2 receptors inhibits serotonin release from rat brain cortical slices (Von Kügelgen et al., 1997). This effect of ATP may be again biphasic, since a presynaptic P2X receptor-mediated facilitation of serotonin release was observed in the rat hippocampus (Okada et al., 1999). However, the use of slices makes it difficult to distinguish between direct presynaptic P2 receptor-involvement and indirect modulatory actions.

In rat neurohypophysial terminals, the ATP-mediated modulation of vasopressin release was investigated and contradicting conclusions were reached. Whereas, in one of the studies the authors described a P2 receptor-mediated facilitation (Troadec et al., 1998), in another study a P2 receptor-mediated inhibition was observed (Sperlagh et al., 1999).

Investigations of the effects of ATP on glutamatergic synaptic transmission have been limited in their interpretation by the rapid metabolism of ATP into adenosine, since adenosine causes an intense inhibition of excitatory synaptic transmission in different CNS areas (Ribeiro, 1995). This led to the conclusion that if ATP has any effect on glutamatergic synaptic transmission, it is mediated by adenosine A_1 receptors and not necessarily by P2 receptor-activation. However, more recent studies have convincingly demonstrated an increase of glutamate release mediated by a P2X-like receptor, measured as an increase in the frequency of miniature excitatory postsynaptic currents in primary sensory afferents in the spinal cord (Nakatsuka and Gu, 2001), and in various brainstem nuclei (Khakh and Henderson, 1998; Kato and Shigetomi, 2001). Additional electro-

physiological studies in cultured rat hippocampal neurons suggest that ATP depresses glutamate release (Inoue et al., 1999), a conclusion further supported by the ability of ATP to inhibit the evoked release of glutamate in rat hippocampal neurons (Koizumi and Inoue, 1997; Mendoza-Fernandez et al., 2000) and in cortical slices (Bennett and Boarder, 2000). It appears that ATP may biphasically modulate (inhibit or facilitate) glutamate release in the CNS.

The divergent effects of ATP reported in neurochemical or electrophysiological studies performed in slices or neuronal cultures could be due to opposite effects of ATP on glutamatergic and GABAergic systems. In rat cultured hippocampal neurons, ATP and ATP analogues enhance GABAergic transmission, but not the effects of iontophoretically applied GABA, in a PPADS-sensitive manner (Inoue et al., 1999). However, the observation that ATP and ATP analogues failed to modify GABA release from superfused hippocampal synaptosomes (Hugel and Schlichter, 2000), raises the question of whether the ATP-induced increase in GABAergic transmission reported in hippocampal neurons might be secondary to an increase of glutamate release. In cultured spinal cord neurons, the release of GABA, assessed by miniature inhibitory postsynaptic currents or evoked inhibitory postsynaptic currents, is facilitated by ATP in 22% of the synapses, an effect proposed to be mediated by $P2X_2$ receptors (Hugel and Schlichter, 2000).

Pharmacological activation of $P2X_7$ receptors in spinal cord slices resulted in glutamate-mediated excitation of the recorded neurons. More recent studies suggested the involvement of the $P2X_7$ receptor-type in the release of glutamate evoking a subsequent release of GABA in the rat hippocampus (Sperlagh et al., 2002). Furthermore, extracellular and patch-clamp recordings in CA3 pyramidal cells of the hippocampus revealed that the potent $P2X_7$ receptor agonist dibenzoylATP caused a long-lasting inhibition of glutamatergic neurotransmission at mossy fiber-CA3 synapses (Armstrong et al., 2002).

ATP has been described to facilitate the spontaneous glycinergic inhibitory postsynaptic current frequency at dissociated rat dorsal horn interneuron synapses (Rhee et al., 2000). The P2X receptor responding to α,β-meATP was functionally expressed at neurons obtained from 28 to 30, but not from 10- to 12-day-old rats (Jang et al., 2001). Glycine release was also facilitated by the activation of presynaptic P2X receptors in dissociated trigeminal nucleus pars caudalis neurons (Wang et al., 2001). Although substance P itself had no effect on miniature inhibitory postsynaptic current frequency or amplitude distribution, the peptide abolished the facilitation by ATP. This blockade was due to a Ca^{2+}/calmodulin-dependent protein kinase II-mediated modulation of P2X receptors.

Since glutamatergic and GABAergic terminals account for nearly 90% of CNS nerve terminals, the monitoring of the effects of ATP on calcium influx in CNS synaptosomes should mainly reveal effects of ATP on GABAergic and/or glutamatergic nerve terminals. Numerous studies have documented that ATP increases intracellular free calcium concentration, an effect apparently mediated by a P2X-like receptor (possibly $P2X_3$; Pintor et al., 1999; Gomez-Villafuertes et al., 2000, 2001). These receptors may be co-localized with $GABA_B$ receptors, since almost 90% of the terminals responded with an increase of the intracellular Ca^{2+} concentration both to ATP and baclofen (Gomez-Villafuertes et al., 2003).

6. Release of ATP, glutamate and GABA from astrocytes

ATP is a co-transmitter of noradrenaline (Sperlagh et al., 1998; Poelchen et al., 2001) and GABA (Jo and Schlichter, 1999; Jo and Role, 2002a). In addition to the neuronal secretion of ATP, stimulation of astrocytes by pharmacological means as well as by mechanical or osmotic stimulation may release ATP by four alternative mechanisms. Firstly, by an exocytotic vesicular release (Coco et al., 2003); secondly, by ATP-binding cassette (ABC) proteins such as the multidrug resistance-associated protein (Ballerini et al., 2002); thirdly, by connexin hemichannels providing the substrate for gap junction formation (Cotrina et al., 2000); and finally, by osmolytic transporters linked to anion channels (Darby et al., 2003). Calcium waves in astrocytes were shown to be initiated and supported by the release of ATP (Cotrina et al., 2000) leading to the stimulation of astrocytic $P2Y_1$ receptors (Fam et al., 2003). These waves are defined as oscillations of intracellular free Ca^{2+} that propagate between neighboring astrocytes and are presently considered to constitute an information-processing system operating in parallel with neuronal circuits.

In addition to ATP, astrocytes were shown to release also excitatory (glutamate, aspartate) and inhibitory (GABA) amino acid transmitters (Bezzi and Volterra, 2001; Nedergaard et al., 2002). The release of glutamate appears to utilize mechanisms similar to that of ATP; both astrocytic vesicles (Araque et al., 2000; Jeremic et al., 2001) and connexin hemichannels (Ye et al., 2003) may be involved. Moreover, the stimulation of $P2X_7$ receptors on astrocytes mediates glutamate release providing a link between the secretion of ATP and glutamate (Duan et al., 2003). Hence, astrocytic ATP and glutamate appear to modulate both synaptic transmission in neuronal systems and Ca^{2+} wave propagation in astrocytic networks (Innocenti et al., 2000; Koizumi et al., 2003; Newman, 2001, 2003).

Finally, the ATP-induced stimulation of $P2X_7$ receptors has been shown to release not only glutamate (see above) but also GABA from astrocytes of the brain or Müller cells of the retina (Pannicke et al., 2000; Wang et al., 2002) and, thereafter, e.g. potentiate inhibitory synaptic transmission via $GABA_B$ receptor-activation (for hippocampal slices, see Kang et al., 1998). Hence, glia should be considered an active partner at the synapse, dynamically regulating synaptic transmission (Newman, 2003).

7. Complexity of purinergic signaling in consequence to enzymatic degradation of ATP to adenosine

ATP has biphasic presynaptic neuromodulatory effects: an inhibitory effect through the activation of P2Y receptors and a facilitatory effect via the activation of P2X receptors. Potential contamination with adenosine modulation, needs to be discarded, i.e., it is necessary to exclude if adenosine (A_1 and A_{2A}) receptors are involved. It is also important to use biochemical methods to support a presynaptic localisation of P2 receptors observed by using radioligands. Furthermore, immunological approaches, namely the electron microscopy immunocytochemistry appear to be very helpful in this respect (Loesch and Burnstock, 1998, 2001; Cunha and Ribeiro, 2000). Finally, it will be important to test whether the synaptic role of ATP is, indeed, mediated by P2 receptor activation, or whether ATP is directly acting as a phosphate donor for ecto-protein kinase modification of presynaptic proteins, which are involved in the control of neurotransmitter release (Ehrlich and Kornecki, 1999).

The existence of an ATP-mediated auto-modulatory system allows ATP to join the signal-pattern of most neurotransmitters, such as glutamate, GABA, acetylcholine, noradrenaline and serotonin. These neurotransmitters possess both post-synaptic and pre-synaptic receptors (Starke et al., 1989). It is interesting that purines display a dual presynaptic neuromodulatory pattern: one mediated by ATP and another by adenosine. ATP is stored in vesicles and can be released by exocytosis, whereas adenosine is neither stored in vesicles nor released as a classical neurotransmitter, i.e. via exocytosis. It, therefore, appears that adenosine behaves mainly as a neuromodulator, whereas ATP appears to be a neurotransmitter, in conformity with the initial idea of Burnstock (1972). One may consider that these two different purinergic systems work in different time scales and are eventually not functionally interconnected. Therefore, the high efficiency of extracellular ATP catabolism would rapidly shut down ATP signaling, generating, upon further time-delayed catabolism, another signaling molecule, adenosine. An alternative hypothesis is that the activity of the two purinergic systems may be highly inter-dependent, and the ecto-nucleotidase cascade would assume a key role in balancing the action of these two neuromodulatory systems. This possibility has previously been addressed (Cunha and Ribeiro, 2000) and is further supported by the marked extracellular gradient of adenosine concentration at the synaptic level (Lopes et al., 1999) and by the intense control of presynaptic ATP receptors by adenosine receptor activation. The critical role of ecto-nucleotidases in determining the relative importance of the two purinergic neuromodulatory systems is supported by the observation that different enzymatic activities are found in different preparations, with conversions of ATP into adenosine ranging from milliseconds to minutes (Dunwiddie et al., 1997) and that ecto-nucleotidases are released upon stimulation of preparations (e.g. Todorov et al., 1997). Another possible reason for the existence of two different purinergic neuromodulatory systems may reside in their different roles, ATP being an auto-modulatory system (i.e. acting on the nerve terminal from where it is released), whereas adenosine would mostly fulfil a hetero-modulatory role (i.e. acting also in neighboring neurons from where it is generated). It is hoped that as more information is available on the presynaptic role of ATP and on the strict need to distinguish ATP from adenosine-mediated presynaptic effects, it will be possible to discover the relative roles and the functional relation of these two presynaptic purinergic neuromodulatory systems.

8. Conclusions and outlook

Numerous P2X/P2Y receptors were reported to pre- or postsynaptically modify CNS neuronal functions. These receptors are targets of ATP released by exocytotic mechanisms or by cellular damage. Recent work indicates that ATP may in addition to elucidating fast or slow synaptic responses may also initiate the neuronal/astrocytic cross-talk. In response to ATP-induced stimulation, astrocytes exocytotically release glutamate and/or GABA which both may act at the respective neuronal receptors, thereby contributing to the complexity of the neuronal networks. Although much is known about the cellular mechanisms of the ATP(ADP)/UTP(UDP) effects, there is limited information on the function of P2 receptors at the gross behavioural level. There is a great need for analyses on the involvement of purinergic mechanisms in the regulation of locomotion and food intake as well as in the expression of sensitization, reward and anxiety (Kittner et al., 2000a,b, 2001, 2003). Clinical studies in humans have to follow in order to elucidate purinergic disregulation as a possible cause of certain illnesses such as schizophrenia or drug addiction. In consequence, P2X and P2Y receptors are possible targets of therapeutic manipulations in CNS illnesses.

Acknowledgements

This work was supported by grants from the DFG, BMBF and EU (P.I.) and from the FCT and EU (J.A.R.). One of the authors (P.I.) expresses his gratitude to Dr. T.V. Dunwiddie for being his host during a sabbatical stay in Denver in 2000.

References

Abbracchio, M.P., Burnstock, G., 1994. Purinoceptors: are there families of P2X and P2Y purinoceptors? Pharmacol. Ther. 64, 445–475.

Abbracchio, M.P., Boeynaems, J.-M., Barnard, E.A., Boyer, J.L., Kennedy, C., Miras-Portugal, M.T., King, B.F., Gachet, C., Jacobson, K.A., Weisman, G.A., Burnstock, G., 2003. Characterization of the

UDP-glucose receptor (re-named here the $P2Y_{14}$ receptor) adds diversity to the P2Y receptor family. Trends Pharmacol. Sci. 24, 52–55.

Aghajanian, G.K., Wang, Y.-Y., 1986. Pertussis toxin blocks the outward currents evoked by opiate and α_2-agonists in locus coeruleus neurons. Brain Res. 371, 390–394.

Araque, A., Li, N., Doyle, R.T., Haydon, P.G., 2000. SNARE protein-dependent glutamate release from astrocytes. J. Neurosci. 20, 666–673.

Armstrong, J.N., Brust, T.B., Lewis, R.G., MacVicar, B.A., 2002. Activation of presynaptic $P2X_7$-like receptors depresses mossy fiber-CA3 synaptic transmission through p38 mitogen-activated protein kinase. J. Neurosci. 22, 5938–5945.

Atkinson, L., Batten, T.F.C., Deuchars, J., 2000. $P2X_2$ receptor immunoreactivity in the dorsal vagal complex and area postrema of the rat. Neuroscience 99, 683–696.

Atkinson, L., Milligan, C.J., Buckley, N.J., Deuchars, J., 2002. An ATP-gated ion channel at the cell nucleus. Nature 420, 42.

Ballerini, P., Di Iorio, P., Ciccarelli, R., Nargi, E., D'Alimonte, I., Traversa, U., Rathbone, M.P., Caciagli, F., 2002. Glial cells express multiple ATP binding cassette proteins which are involved in ATP release. NeuroReport 13, 1789–1792.

Barnard, E.A., Simon, J., 2001. An elusive receptor is finally caught: $P2Y_{12}$, an important drug target in platelets. Trends Pharmacol. Sci. 22, 388–391.

Bennett, G.C., Boarder, M.R., 2000. The effect of nucleotides and adenosine on stimulus-evoked glutamate release from rat brain cortical slices. Br. J. Pharmacol. 131, 617–623.

Bezzi, P., Volterra, A., 2001. A neuron-glia signalling network in the active brain. Curr. Opin. Neurobiol. 11, 387–394.

Bo, X., Zhang, Y., Nassar, M., Burnstock, G., Schoepfer, R., 1995. A P2X purinoceptor cDNA conferring a novel pharmacological profile. FEBS Lett. 375, 129–133.

Bobanovic, L.K., Royle, S.J., Murrell-Lagnado, R.D., 2002. P2X receptor trafficking in neurons is subunit specific. J. Neurosci. 22, 4814–4824.

Boue-Grabot, E., Barajas-Lopez, C., Chakfe, Y., Blais, D., Belanger, D., Emerit, M.B., Seguela, P., 2003. Intracellular cross talk and physical interaction between two classes of neurotransmitter-gated channels. J. Neurosci. 23, 1246–1253.

Bouet-Grabot, E., Archambault, V., Seguela, P., 2000. A protein kinase C site highly conserved in P2X subunits controls the desensitization kinetics of $P2X_2$ ATP-gated channels. J. Biol. Chem. 275, 10190–10195.

Brändle, U., Spielmann, P., Osteroth, R., Sim, J., Surprenant, A., Buell, G., Ruppersberg, J.P., Plinkert, P.K., Zenner, H.P., Glowatzki, E., 1997. Desensitization of the $P2X_2$ receptor controlled by alternative splicing. FEBS Lett. 404, 294–298.

Buell, G., Lewis, C., Collo, G., North, R.A., Surprenant, A., 1996. An antagonist-insensitive P_{2X} receptor expressed in epithelia and brain. EMBO J. 15, 55–62.

Burnstock, G., 1972. Purinergic nerves. Pharmacol. Rev. 24, 509–581.

Burnstock, G., 2003. Purinergic receptors in the nervous system. Curr. Top. Membr. 54, 307–368.

Camaioni, E., Boyer, J.L., Mohanram, A., Harden, T.K., Jacobson, K.A., 1998. Deoxyadenosine bisphosphate derivatives as potent antagonists at $P2Y_1$ receptors. J. Med. Chem. 41, 183–190.

Chambers, J.K., Macdonald, L.E., Sarau, H.M., Ames, R.S., Freeman, K., Foley, J.J., Zhu, Y., McLaughlin, M.M., Murdock, P., et al., 2000. A G protein-coupled receptor for UDP-glucose. J. Biol. Chem. 275, 10767–10771.

Charlton, M.E., Williams, A.S., Fogliano, M., Sweetnam, P.M., Duman, R.S., 1997. The isolation and characterization of a novel G protein-coupled receptor regulated by immunologic challenge. Brain Res. 764, 141–148.

Chen, C.-C., Akopian, A.N., Sivilotti, L., Colquhoun, D., Burnstock, G., Wood, J.N., 1995. A P2X purinoceptor expressed by a subset of sensory neurons. Nature 377, 428–431.

Chen, W., Wieraszko, A., Hogan, M.V., Yang, H.-A., Kornecki, E., Ehrlich, Y.H., 1996. Surface protein phosphorylation by ecto-protein kinase is required for the maintenance of hippocampal long-term potentiation. Proc. Natl. Acad. Sci. U. S. A. 93, 8688–8693.

Choi, R.C.Y., Siow, N.L., Cheng, A.W.M., Ling, K.K.Y., Tung, E.K., Simon, J., Barnard, E.A., Tsim, K.W.K., 2003. ATP acts via $P2Y_1$ receptors to stimulate acetylcholinesterase and acetylcholine receptor expression: transduction and transcription control. J. Neurosci. 23, 4445–4456.

Ciccarelli, R., Ballerini, P., Sabatino, G., Rathbone, M.P., D'Onofrio, M., Caciagli, F., Di Iorio, P., 2001. Involvement of astrocytes in purine-mediated reparative processes in the brain. Int. J. Dev. Neurosci. 19, 395–414.

Coco, S., Calegari, F., Pravettoni, E., Pozzi, D., Taverna, E., Rosa, P., Matteoli, M., Verderio, C., 2003. Storage and release of ATP from astrocytes in culture. J. Biol. Chem. 278, 1354–1362.

Communi, D., Suarez Gonzalez, N., Detheux, M., Brezillon, S., Lannoy, V., Parmentier, M., Boeynaems, J.-M., 2001. Identification of a novel human ADP receptor coupled to G_i. J. Biol. Chem. 276, 41479–41485.

Cotrina, M.L., Lin, J.H.-C., Lopez-Garcia, J.C., Naus, C.C.G., Nedergaard, M., 2000. ATP-mediated glia signaling. J. Neurosci. 20, 2835–2844.

Cunha, R.A., Ribeiro, J.A., 2000. ATP as a presynaptic modulator. Life Sci. 68, 119–137.

Cunha, R.A., Ribeiro, J.A., Sebastiao, A.M., 1994. Purinergic modulation of the evoked release of [^{3}H]acetylcholine from the hippocampus and cerebral cortex of the rat: role of the ectonucleotidases. Eur. J. Neurosci. 6, 33–42.

Cunha, R.A., Sebastiao, A.M., Ribeiro, J.A., 1998. Inhibition by ATP of hippocampal synaptic transmission requires localized extracellular catabolism by ecto-nucleotidases into adenosine and channeling to adenosine A_1 receptors. J. Neurosci. 18, 1987–1995.

Darby, M., Kuzmiski, J.B., Panenka, W., Feighan, D., MacVicar, B.A., 2003. ATP released from astrocytes during swelling activates chloride channels. J. Neurophysiol. 89, 1870–1877.

Dave, S., Mogul, D.J., 1996. ATP receptor activation potentiates a voltage-dependent Ca channel in hippocampal neurons. Brain Res. 715, 208–216.

Deuchars, S.A., Atkinson, L., Brooke, R.E., Musa, H., Milligan, C.J., Batten, T.F.C., Buckley, N.J., Parson, S.H., Deuchars, J., 2001. Neuronal $P2X_7$ receptors are targeted to presynaptic terminals in the central and peripheral nervous systems. J. Neurosci. 21, 7143–7152.

Di Virgilio, F., Sanz, J.M., Chiozzi, P., Falzoni, S., 1999. The $P2Z/P2X_7$ receptor of microglial cells: a novel immunomodulatory receptor. Prog. Brain Res. 120, 355–368.

Duan, S., Anderson, C.M., Keung, E.C., Chen, Y., Swanson, R.A., 2003. $P2X_7$ receptor-mediated release of excitatory amino acids from astrocytes. J. Neurosci. 23, 1320–1328.

Dunwiddie, T.D., Diao, L., Proctor, W.R., 1997. Adenine nucleotides undergo rapid, quantitative conversion to adenosine in the extracellular space in rat hippocampus. J. Neurosci. 17, 7673–7682.

Dylan Clyne, J., LaPointe, L.D., Hume, R.I., 2002a. The role of histidine residues in modulation of the rat $P2X_2$ purinoceptor by zinc and pH. J. Physiol. 539, 347–359.

Dylan Clyne, J., Wang, L.-F., Hume, R.I., 2002b. Mutational analysis of the conserved cysteines of the rat $P2X_2$ purinoceptor. J. Neurosci. 22, 3873–3880.

Edwards, F.A., Gibb, A.J., Colquhoun, D., 1992. ATP receptor-mediated synaptic currents in the central nervous system. Nature 359, 144–147.

Egan, T.M., Haines, W.R., Voigt, M.M., 1998. A domain contributing to the ion channel of ATP-gated $P2X_2$ receptors identified by the substituted cysteine accessibility method. J. Neurosci. 18, 2350–2359.

Ehrlich, Y.H., Kornecki, E., 1999. Ecto-protein kinases as mediators for the action of secreted ATP in the brain. Prog. Brain Res. 120, 411–426.

Ennion, S.J., Evans, R.J., 2002. Conserved cysteine residues in the extracellular loop of the human $P2X_1$ receptor form disulfide bonds and are involved in receptor trafficking to the cell surface. Mol. Pharmacol. 61, 303–311.

Ennion, S., Hagen, S., Evans, R.J., 2000. The role of positively charged amino acids in ATP recognition by human $P2X_1$ receptors. J. Biol. Chem. 275, 29361–29367.

Fam, S.R., Gallagher, C.J., Kalia, L.V., Salter, M.W., 2003. Differential frequency dependence of $P2Y_1$- and $P2Y_2$- mediated Ca^{2+} signaling in astrocytes. J. Neurosci. 23, 4437–4444.

Fields, R.D., Stevens, B., 2000. ATP: an extracellular signaling molecule between neurons and glia. Trends Neurosci. 23, 625–633.

Fujii, S., Kato, H., Kuroda, Y., 2002. Cooperativity between extracellular adenosine 5′-triphosphate and activation of *N*-methyl-D-aspartate receptors in long-term potentiation induction in hippocampal CA1 neurons. Neuroscience 113, 617–628.

Fumagalli, M., Brambilla, R., D'Ambrosi, N., Volonte, C., Matteoli, M., Verderio, C., Abbracchio, M.P., 2003. Nucleotide-mediated calcium signaling in rat cortical astrocytes: role of P2X and P2Y receptors. Glia 43, 218–230.

Garcia-Guzman, M., Soto, F., Gomez-Hernandez, J.M., Lund, P.-E., Stühmer, W., 1997. Characterization of recombinant human $P2X_4$ receptor reveals pharmacological differences to the rat homologue. Mol. Pharmacol. 51, 109–118.

Garcia-Lecea, M., Delicado, E.G., Miras-Portugal, M.T., Castro, E., 1999. $P2X_2$ characteristics of the ATP receptor coupled to $[Ca^{2+}]_i$ increases in cultured Purkinje neurons from neonatal rat cerebellum. Neuropharmacology 38, 699–706.

Gomez-Villafuertes, R., Gualix, J., Miras-Portugal, M.T., Pintor, J., 2000. Adenosine 5′-tetraphosphate (Ap_4), a new agonist on rat midbrain synaptic terminal P2 receptors. Neuropharmacology 39, 2381–2390.

Gomez-Villafuertes, R., Gualix, J., Miras-Portugal, M.T., 2001. Single GABAergic synaptic terminals from rat midbrain exhibit functional P2X and dinucleotide receptors, able to induce GABA secretion. J. Neurochem. 77, 84–93.

Gomez-Villafuertes, R., Pintor, J., Gualix, J., Miras-Portugal, M.T., 2003. $GABA_B$ receptor-mediated presynaptic potentiation of ATP ionotropic receptors in rat midbrain synaptosomes. Neuropharmacology 44, 311–323.

Gu, B.J., Zhangh, W.Y., Bendall, L.J., Chessell, I.P., Buell, G.N., Wiley, J.S., 2000. Expression of $P2X_7$ purinoceptors on human lymphocytes and monocytes: evidence for non-functional $P2X_7$ receptors. Am. J. Physiol., Cell Physiol. 279, C1189–C1197.

Haines, W.R., Voigt, M.M., Migita, K., Torres, G.E., Egan, T.M., 2001. On the contribution of the first transmembrane domain to whole-cell current through an ATP-gated ionotropic P2X receptor. J. Neurosci. 21, 5885–5892.

Harms, L., Finta, E.P., Tschöpl, M., Illes, P., 1992. Depolarization of rat locus coeruleus neurons by adenosine 5′-triphosphate. Neuroscience 48, 941–952.

Hoffmann, C., Moro, S., Nicholas, R.A., Harden, T.K., Jacobson, K.A., 1999. The role of amino acids of the extracellular loops of the human $P2Y_1$ receptor in surface expression and activation processes. J. Biol. Chem. 274, 14639–14647.

Hollopeter, G., Jantzen, H.-M., Vincent, D., Li, G., England, L., Ramakrishnan, V., Yang, R.-B., Nurden, P., Nurden, A., Julius, D., Conley, P.B., 2001. Identification of the platelet ADP receptor targeted by antithrombotic drugs. Nature 409, 202–207.

Hu, B., Senkler, C., Yang, A., Soto, F., Liang, B.T., 2002. $P2X_4$ receptor is a glycosylated cardiac receptor mediating a positive inotropic response to ATP. J. Biol. Chem. 277, 15752–15757.

Huang, C.M., Kao, L.S., 1996. Nerve growth factor, epidermial growth factor, and insulin differentially potentiate ATP-induced $[Ca^{2+}]_i$ rise and dopamine secretion in PC12 cells. J. Neurochem. 66, 124–130.

Hugel, S., Schlichter, R., 2000. Presynaptic P2X receptors facilitate inhibitory GABAergic transmission between cultured rat spinal cord dorsal horn neurons. J. Neurosci. 20, 2121–2130.

Humphries, R.G., Tomlinson, W., Ingall, A.H., Cage, P.A., Leff, P., 1994. FPL 66096: a novel, highly potent and selective antagonist at human platelet P2T-purinoceptors. Br. J. Pharmacol. 113, 1057–1063.

Ikeuchi, Y., Nishizaki, T., 1995. ATP-evoked potassium currents in rat striatal neurons are mediated by a P_2 purinergic receptor. Neurosci. Lett. 90, 89–92.

Ikeuchi, Y., Nishizaki, T., Mori, M., Okada, Y., 1995a. Adenosine activates the potassium channel via a P_2 purinoceptor but not via an adenosine receptor in cultured rat superior colliculus neurons. Neurosci. Lett. 198, 205–208.

Ikeuchi, Y., Nishizaki, T., Okada, Y., 1995b. A P_2 purinoceptor activated by ADP in rat medullary neurons. Neurosci. Lett. 198, 71–74.

Ikeuchi, Y., Nishizaki, T., Okada, Y., 1996. Repetitive applications of ATP potentiate potassium current by Ca^{2+}/calmodulin kinase in cultured rat hippocampal neurons. Neurosci. Lett. 203, 115–118.

Illes, P., Nieber, K., Nörenberg, W., 1996. Electrophysiological effects of ATP on brain neurones. J. Auton. Pharmacol. 16, 407–411.

Innocenti, B., Parpura, V., Haydon, P.G., 2000. Imaging extracellular waves of glutamate during calcium signaling in cultured astrocytes. J. Neurosci. 20, 1800–1808.

Inoue, K., Koizumi, S., Ueno, S., Kita, A., Tsuda, M., 1999. The functions of ATP receptors in the synaptic transmission in the hippocampus. Prog. Brain Res. 120, 193–206.

Jacobson, K.J., Hoffmann, C., Kim, Y.-C., Camaioni, E., Nandanan, E., Jang, S.Y., et al., 1999. Molecular recognition in P2 receptors: ligand development aided by molecular modeling and mutagenesis. Prog. Brain Res. 120, 119–132.

Jang, I.-S., Rhee, J.S., Kubota, H., Akaike, N., Akaike, N., 2001. Developmental changes in P2X purinoceptors on glycinergic presynaptic nerve terminals projecting to rat substantia gelatinosa neurons. J. Physiol. 536, 505–519.

Jensik, P., Cox, T., 2002. ATP-induced internalization of amphibian epithelial P2X receptors is linked to channel opening. Pflugers Arch. 444, 795–800.

Jeremic, A., Jeftinija, K., Stevanovic, J., Glavaski, A., Jeftinija, S., 2001. ATP stimulates calcium-dependent glutamate release from cultured astrocytes. J. Neurochem. 77, 664–675.

Jiang, L.-H., Rassendren, F., Surprenant, A., North, R.A., 2000. Identification of amino acid residues contributing to the ATP-binding site of a purinergic P2X receptor. J. Biol. Chem. 275, 34190–34196.

Jiang, L.-H., Rassendren, F., Spelta, V., Surprenant, A., North, R.A., 2001. Amino acid residues involved in gating identified in the first membrane-spanning domain of the rat $P2X_2$ receptor. J. Biol. Chem. 276, 14902–14908.

Jo, Y.-W., Role, L.W., 2002a. Coordinate release of ATP and GABA at in vitro synapses of lateral hypothalamic neurons. J. Neurosci. 22, 4794–4804.

Jo, Y.-H., Role, L.W., 2002b. Cholinergic modulation of purinergic and GABAergic co-transmission at in vitro hypothalamic synapses. J. Neurophysiol. 88, 2501–2508.

Jo, Y.-W., Schlichter, R., 1999. Synaptic corelease of ATP and GABA in cultured spinal neurons. Nat. Neurosci. 2, 241–245.

Johansson, B., Halldner, L., Dunwiddie, T.V., Masino, S.A., Poelchen, W., Gimenez-Llort, L., Escorihuela, R.M., Fernandez-Teruel, A., Wiesenfeld-Hallin, Z., Xu, X.J., et al., 2001. Hyperalgesia, anxiety and decreased hypoxic neuroprotection in mice lacking the adenosine A_1 receptor. Proc. Natl. Acad. Sci. U. S. A. 98, 9407–9412.

Kang, J., Jiang, L., Goldman, S.A., Nedergaard, M., 1998. Astrocyte-mediated potentiation of inhibitory synaptic transmission. Nat. Neurosci. 8, 683–692.

Kanjhan, R., Housley, G.D., Burton, L.D., Christie, D.L., Kippenberger, A., Thorne, P.R., Luo, L., Ryan, A.F., 1999. Distribution of the $P2X_2$ receptor subunit of the ATP-gated ion channels in the rat central nervous system. J. Comp. Neurol. 407, 11–32.

Kato, F., Shigetomi, E., 2001. Distinct modulation of evoked and spontaneous EPSCs by purinoceptors in the nucleus tractus solitarii of the rat. J. Physiol. 530, 469–486.

Khakh, B., 2001. Molecular physiology of P2X receptors and ATP signalling at synapses. Nat. Rev. 2, 165–174.

Khakh, B.S., Henderson, G., 1998. ATP receptor-mediated enhancement of

fast excitatory neurotransmitter release in the brain. Mol. Pharmacol. 54, 372–378.
Khakh, B.S., Humphrey, P.P.A., Henderson, G., 1997. ATP-gated cation channels (P2X purinoceptors) in trigeminal mesencephalic nucleus neurones of the rat. J. Physiol. 498, 709–715.
Khakh, B.S., Bao, X.R., Labarca, C., Lester, H.A., 1999. Neuronal P2X transmitter-gated cation channels change their ion selectivity in seconds. Nat. Neurosci. 2, 322–330.
Khakh, B., Zhou, X., Sydes, J., Galligan, J.J., Lester, H.A., 2000. State-dependent cross-inhibition between transmitter-gated cation channels. Nature 406, 405–410.
Khakh, B.S., Burnstock, G., Kennedy, C., King, B.F., North, R.A., Seguela, P., Voigt, M., Humphrey, P.P.A., 2001a. International union of pharmacology: XXIV. Current status of the nomenclature and properties of P2X receptors and their subunits. Pharmacol. Rev. 53, 107–118.
Khakh, B.S., Smith, W.B., Chiu, C.-S., Ju, D., Davidson, N., Lester, H.A., 2001b. Activation-dependent changes in receptor distribution and dendritic morphology in hippocampal neurons expressing $P2X_2$-green fluorescent protein receptor. Proc. Natl. Acad. Sci. U. S. A. 98, 5288–5293.
Khakh, B.S., Gittermann, D., Cockayne, D.A., Jones, A., 2003. ATP modulation of excitatory synapses onto interneurons. J. Neurosci. 23, 7426–7437.
Kidd, E.J., Miller, K.J., Sansum, A.J., Humphrey, P.P., 1998. Evidence for $P2X_3$ receptors in the developing rat brain. Neuroscience 87, 533–539.
King, B.F., Townsend-Nicholson, A., Burnstock, G., 1998. Metabotropic receptors for ATP and UTP: exploring the correspondence between native and recombinant nucleotide receptors. Trends Pharmacol. Sci. 19, 506–514.
Kittner, H., Krügel, U., El-Ashmawy, I., Illes, P., 2000a. Suppression of feeding-evoked dopamine release in the rat nucleus accumbens by the blockade of P2 receptors. Eur. J. Pharmacol. 406, R13–R14.
Kittner, H., Krügel, U., Hoffmann, E., Illes, P., 2000b. Effects of intra-accumbens injection of 2-methylthio ATP: a combined open field and electroencephalographic study in rats. Psychopharmacology 150, 123–131.
Kittner, H., Krügel, U., Illes, P., 2001. The P2 receptor antagonist PPADS prevents both the acute locomotor effects of amphetamine and the behavioural sensitization caused by repeated amphetamine injections in rats. Neuroscience 102, 241–243.
Kittner, H., Franke, H., Fischer, W., Schultheis, N., Krügel, U., Illes, P., 2003. Stimulation of $P2Y_1$ receptors causes anxiolytic-like effects in the rat elevated plus-maze: implications for the involvement of $P2Y_1$ receptor-mediated nitric oxide production. Neuropsychopharmacology 28, 435–444.
Koizumi, S., Inoue, K., 1997. Inhibition by ATP of calcium oscillations in rat cultured hippocampal neurones. Br. J. Pharmacol. 122, 51–58.
Koizumi, S., Fujishita, K., Tsuda, M., Shigemoto-Mogami, Y., Inoue, K., 2003. Dynamic inhibition of excitatory synaptic transmission by astrocyte-derived ATP in hippocampal cultures. Proc. Natl. Acad. Sci. U. S. A. 100, 11023–11028.
Koshimizu, T., Koshimizu, M., Stojilkovic, S.S., 1999. Contributions of the C-terminal domain to the control of P2X receptor desensitization. J. Biol. Chem. 274, 37651–37657.
Krügel, U., Kittner, H., Illes, P., 1999. Adenosine 5′-triphosphate induced dopamine release in the rat nucleus accumbens in vivo. Neurosci. Lett. 265, 49–52.
Krügel, U., Kittner, H., Franke, H., Illes, P., 2001a. Stimulation of P2 receptors in the ventral tegmental area enhances dopaminergic mechanisms in vivo. Neuropharmacology 40, 1084–1093.
Krügel, U., Kittner, H., Illes, P., 2001b. Mechanisms of adenosine 5′-triphosphate-induced dopamine release in the rat nucleus accumbens in vivo. Synapse 39, 222–232.
Laitinen, J.T., Uri, A., Raidaru, G., Miettinen, R., 2001. [^{35}S]GTPγS autoradiography reveals a wide distribution of $G_{i/o}$-linked ADP receptors in the nervous system; close similarities with the platelet $P2Y_{ADP}$ receptor. J. Neurochem. 77, 505–518.
Lalo, U., Kostyuk, P., 1998. Developmental changes in purinergic calcium signalling in rat neocortical neurones. Dev. Brain Res. 111, 43–50.
Lalo, U., Voitenko, N., Kostyuk, P., 1998. Iono- and metabotropically induced purinergic signalling in rat neocortical neurons. Brain Res. 799, 285–291.
Lazarowski, E.R., Shea, D.A., Boucher, R.C., Harden, T.K., 2003. Release of cellular UDP-glucose as a potential extracellular signaling molecule. Mol. Pharmacol. 63, 1190–1197.
Le, K.T., Babinski, K., Seguela, P., 1998a. Central $P2X_4$ and $P2X_6$ channel subunits coassemble into a novel heteromeric ATP receptor. J. Neurosci. 18, 7152–7159.
Le, K.-T., Villeneuve, P., Ramjaun, A.R., McPherson, P.S., Beaudet, A., Seguela, P., 1998b. Sensory presynaptic and widespread somatodendritic immunolocalization of central ionotropic P2X ATP receptors. Neuroscience 83, 177–190.
Lee, S.Y., Wolff, S.C., Nicholas, R.A., O'Grady, S.M., 2003. P2Y receptors modulate ion channel function through interactions involving the C-terminal domain. Mol. Pharmacol. 63, 878–885.
Lewis, C.J., Neidhart, S., Holy, C., North, R.A., Buell, G., Surprenant, A., 1995. Coexpression of $P2X_2$ and $P2X_3$ receptor subunits can account for ATP-gated currents in sensory neurons. Nature 377, 432–435.
Li, C., Peoples, R.W., Weight, F.F., 1993. Zn^{2+} potentiates excitatory action of ATP on mammalian neurons. Proc. Natl. Acad. Sci. U. S. A. 90, 8264–8267.
Liu, F., Wan, Q., Pristupa, Z.B., Yu, X.-M., Wang, Y.T., Niznik, H.B., 2000. Direct protein–protein coupling enables cross-talk between dopamine D5 and γ-aminobutyric acid A receptors. Nature 403, 274–280.
Loesch, A., Burnstock, G., 1998. Electron-immunocytochemical localization of the $P2X_1$ receptors in the rat cerebellum. Cell Tissue Res. 294, 253–260.
Loesch, A., Burnstock, G., 2001. Immunoreactivity to $P2X_6$ receptors in the rat hypothalamo-neurohypophysial system: an ultrastructural study with ExtrAvidin and colloidal gold–silver immunolabelling. Neuroscience 106, 621–631.
Loesch, A., Miah, S., Burnstock, G., 1999. Ultrastructural localisation of ATP-gated $P2X_2$ receptor immunoreactivity in the rat hypothalamo-neurohypophysial system. J. Neurocytol. 28, 495–504.
Lopes, L.V., Cunha, R.A., Ribeiro, J.A., 1999. Interaction between adenosine A_1 and A_{2A} receptors in the control of neurotransmitter release-Pandalai, S.I. (Ed.), Recent Research Developments in Neurochemistry, vol. 2, part II. Research Signpost, Trivandrum, pp. 463–472.
Luthardt, J., Borvendeg, S.J., Sperlagh, B., Poelchen, W., Wirkner, K., Illes, P., 2003. $P2Y_1$ receptor activation inhibits NMDA receptor-channels in layer V pyramidal neurons of the rat prefrontal and parietal cortex. Neurochem. Int. 42, 161–472.
Masino, S.A., Dunwiddie, T.V., 2001. Role of purines and pyrimidines in the central nervous system. In: Abbracchio, M.P., Williams, M. (Eds.), Handbook of Experimental Pharmacology. Purinergic and Pyrimidinergic Signalling I-Molecular, Nervous and Urogenitary System Function, vol. 151/1. Springer-Verlag, Berlin, pp. 251–288.
Masino, S.A., Diao, L., Illes, P., Zahniser, N.R., Larson, G.A., Johansson, B.B., Fredholm, B.B., Dunwiddie, T.V., 2002. Modulation of hippocampal glutamatergic transmission by ATP is dependent on adenosine A_1 receptors. J. Pharmacol. Exp. Ther. 303, 356–363.
Mateo, J., Garcia-Lecea, M., Miras-Portugal, M.T., Castro, E., 1998. Ca^{2+} signals mediated by P2X-type purinoceptors in cultured cerebellar Purkinje cells. J. Neurosci. 18, 1704–1712.
Mendoza-Fernandez, V., Andrew, R.D., Barajas-Lopez, C., 2000. ATP inhibits glutamate synaptic release by acting at P2Y receptors in pyramidal neurons of hippocampal slices. J. Pharmacol. Exp. Ther. 293, 172–179.
Moore, D., Chambers, J., Waldvogel, H., Faull, R., Emson, P., 2000. Regional and cellular distribution of the $P2Y_1$ purinergic receptor in the human brain: striking neuronal localisation. J. Comp. Neurol. 421, 374–384.
Moore, D.J., Chambers, J.K., Wahlin, J.P., Tan, K.B., Moore, G.B., Jenkins, O., Emson, P.C., Murdock, P.R., 2001. Expression pattern of

human P2Y receptor subtypes: a quantitative reverse transcription-polymerase chain reaction study. Biochim. Biophys. Acta 1521, 107–119.
Moran-Jimenez, M.-J., Matute, C., 2000. Immunohistochemical localization of the $P2Y_1$ purinergic receptor in neurons and glial cels of the central nervous system. Mol. Brain Res. 78, 50–58.
Mori, M., Heuss, C., Gähwiler, B.H., Gerber, U., 2001. Fast synaptic transmission mediated by P2X receptors in CA3 pyramidal cells of rat hippocampal slice cultures. J. Physiol. 535, 115–123.
Mosbacher, J., Maier, R., Fakler, B., Glatz, A., Crespo, J., Bilbe, G., 1998. P2Y receptor subtypes differentially couple to inwardly-rectifying potassium channels. FEBS Lett. 436, 104–110.
Moss, S.J., Smart, T.G., 1996. Modulation of amino acid-gated ion channels by protein phosphorylation. Int. Rev. Neurobiol. 39, 1–52.
Nakatsuka, T., Gu, J.G., 2001. ATP P2X receptor-mediated enhancement of glutamate release and evoked EPSCs in dorsal horn neurons of the rat spinal cord. J. Neurosci. 21, 6522–6531.
Neary, J.T., Rathbone, M.P., Cattabeni, F., Abbracchio, M.P., Burnstock, G., 1996. Trophic actions of extracellular nucleotides and nucleosides on glial and neuronal cells. Trends Neurosci. 19, 13–18.
Nedergaard, M., Takano, T., Hansen, A.J., 2002. Beyond the role of glutamate as a neurotransmitter. Nat. Rev. 3, 748–755.
Newman, E.A., 2001. Propagation of intercellular calcium waves in retinal astrocytes and Müller cells. J. Neurosci. 21, 2215–2223.
Newman, E.A., 2003. New roles for astrocytes: regulation of synaptic transmission. Trends Neurosci. 26, 536–542.
Nicke, A., Baumert, H.G., Rettinger, J., Eichele, A., Lambrecht, G., Mutschler, E., Schmalzing, G., 1998. $P2X_1$ and $P2X_3$ receptors form stable trimers: a novel structural motif of ligand-gated ion channels. EMBO J. 17, 3016–3028.
Nörenberg, W., Illes, P., 2000. Neuronal P2X receptors: localisation and functional properties. Naunyn-Schmiedeberg's Arch. Pharmacol. 362, 324–339.
North, R.A., 2002. Molecular physiology of P2X receptors. Physiol. Rev. 82, 1013–1067.
Okada, M., Kawata, Y., Murakarni, T., Wada, K., Mizuno, K., Kaneko, S., 1999. Interaction between purinoceptor subtypes on hippocampal serotonergic transmission using in vivo microdialysis. Neuropharmacology 38, 707–715.
Ortinau, S., Laube, B., Zimmermann, H., 2003. ATP inhibits NMDA receptors after heterologous expression and in cultured hippocampal neurons and attenuates NMDA-mediated neurotoxicity. J. Neurosci. 23, 4996–5003.
Panchenko, V.A., Pintor, J., Tsyndrenko, Y.A., Miras-Portugal, M.T., Krishtal, O.A., 1996. Diadenosine polyphosphates selectively potentiate N-type Ca^{2+} channels in rat central neurons. Neuroscience 70, 353–360.
Pankratov, Y., Castro, E., Miras-Portugal, M.T., Krishtal, O.A., 1998. A purinergic component of the excitatory postsynaptic current mediated by P2X receptors in the CA1 neurons of the rat hippocampus. Eur. J. Neurosci. 10, 3898–3902.
Pankratov, Y., Lalo, U., Krishtal, O., Verkhratsky, A., 2002a. Ionotropic P2X purinoceptors mediate synaptic transmission in rat pyramidal neurones of layer II/III of somato-sensory cortex. J. Physiol. (Lond.) 542, 529–536.
Pankratov, Y.V., Lalo, U.V., Kristhal, O.A., 2002b. Role for P2X receptors in long-term potentiation. J. Neurosci. 22, 8363–8369.
Pannicke, T., Fischer, W., Biedermann, B., Schädlich, H., Grosche, J., Faude, F., Wiedemann, P., Allgaier, C., Illes, P., Burnstock, G., Reichenbach, A., 2000. $P2X_7$ receptors in Müller glial cells from the human retina. J. Neurosci. 20, 5965–5972.
Patel, M.K., Khakh, B.S., Henderson, G., 2001. Properties of native $P2X_3$ receptors in rat trigeminal mesencephalic nucleus neurones: lack of correlation with known, heterologously expressed P2X receptors. Neuropharmacology 40, 96–105.
Pintor, J., Diaz-Hernandez, M., Bustamante, C., Gualix, J., Gomez de Terreros, F.J., Miras Portugal, M.T., 1999. Presence of dinucleotide and ATP receptors in human cerebrocortical synaptic terminals. Eur. J. Pharmacol. 366, 159–165.
Poelchen, W., Sieler, D., Wirkner, K., Illes, P., 2001. Co-transmitter function of ATP in central catecholaminergic neurons of the rat. Neuroscience 102, 493–602.
Radford, K.M., Virginio, C., Surprenant, A., North, R.A., Kawashima, E., 1997. Baculovirus expression provides direct evidence for heteromeric assembly of $P2X_2$ and $P2X_3$ receptors. J. Neurosci. 17, 6529–6533.
Ralevic, V., Burnstock, G., 1998. Receptors for purines and pyrimidines. Pharmacol. Rev. 50, 413–492.
Rassendren, F., Buell, G., Newbolt, A., North, R.A., Surprenant, A., 1997. Identification of amino acid residues contributing to the pore of a P2X receptor. EMBO J. 16, 3446–3454.
Rhee, J.S., Wang, Z.M., Nabekura, J., Inoue, K., Akaike, N., 2000. ATP facilitates spontaneous glycinergic IPSC frequency at dissociated rat dorsal horn interneuron synapses. J. Physiol. 524, 471–483.
Ribeiro, J.A., 1995. Purinergic inhibition of neurotransmitter release in the central nervous system. Pharmacol. Toxicol. 77, 299–305.
Robertson, S.J., Burnashev, N., Edwards, F.A., 1999. Ca^{2+} permeability and kinetics of glutamate receptors in rat medial habenula neurones: implications for purinergic transmission in this nucleus. J. Physiol. 518, 539–549.
Robertson, S.J., Ennion, S.J., Evans, R.J., Edwards, F.A., 2001. Synaptic P2X receptors. Curr. Opin. Neurobiol. 11, 378–386.
Rubio, M.E., Soto, F., 2001. Distinct localization of P2X receptors at excitatory postsynaptic specializations. J. Neurosci. 21, 641–653.
Sebastiao, A.M., Ribeiro, J.A., 1996. Adenosine A_2 receptor-mediated excitatory actions on the nervous system. Prog. Neurobiol. 48, 167–189.
Sebastiao, A.M., Cunha, R.A., Cascalheira, J.F., Ribeiro, J.A., 1999. Adenine nucleotides as inhibitors of synaptic transmission: role of localised ectonucleotidases. Prog. Brain Res. 120, 183–192.
Shen, K.-Z., North, R.A., 1993. Excitation of rat locus coeruleus neurons by adenosine 5′-triphosphate: ionic mechanism and receptor characterization. J. Neurosci. 13, 894–899.
Smith, A.D., Cheek, D.J., Buxton, I.L., Westfall, D.P., 1997. Competition of adenine nucleotides for a 1,3-[^{3}H]-dipropyl-8-cyclopentylxanthine binding site in rat vas deferens. Clin. Exp. Pharmacol. Physiol. 24, 492–497.
Smith, F.M., Humphrey, P.P.A., Murrell-Lagnado, R.D., 1999. Identification of amino acids within the $P2X_2$ receptor C-terminus that regulate desensitization. J. Physiol. 520, 91–99.
Sorimachi, M., Yamagami, K., Wakomori, M., 2002. Activation of ATP receptor increases the cytosolic Ca^{2+} concentration in ventral tegmental area neurons of rat brain. Brain Res. 935, 129–133.
Sperlagh, B., Sershen, H., Lajtha, A., Vizi, E.S., 1998. Co-release of endogenous ATP and [^{3}H]noradrenaline from rat hypothalamic slices: origin and modulation by α_2-adrenoceptors. Neuroscience 82, 511–520.
Sperlagh, B., Mergl, Z., Juranyi, Z., Vizi, E.S., Makara, G.B., 1999. Local regulation of vasopressin and oxytocin secretion by extracellular ATP in the isolated posterior lobe of the rat hypophysis. J. Endocrinol. 160, 343–350.
Sperlagh, B., Köfalvi, A., Deuchars, J., Atkinson, L., Milligan, C.J., Buckley, N.J., Vizi, E.S., 2002. Involvement of $P2X_7$ receptors in the regulation of neurotransmitter release in the rat hippocampus. J. Neurochem. 81, 1196–1211.
Starke, K., Göthert, M., Kilbinger, H., 1989. Modulation of neurotransmitter release by presynaptic autoreceptors. Physiol. Rev. 69, 864–989.
Stoop, R., Thomas, S., Rassendren, F., Kawashima, E., Buell, G., Surprenant, A., North, R.A., 1999. Contribution of individual subunits to the multimeric $P2X_2$ receptor: estimates based on methanethiosulfonate block at T336C. Mol. Pharmacol. 56, 973–981.
Surprenant, A., Buell, G., North, R.A., 1995. P2X receptors bring new structure to ligand-gated ion channels. Trends Neurosci. 18, 224–229.
Surprenant, A., Rassendren, E., Kawashima, E., North, R.A., Buell, G., 1996. The cytolytic P2Z receptor for extracellular ATP identified as a P2X receptor ($P2X_7$). Science 272, 735–738.
Swanson, K.D., Reigh, C., Landreth, G.E., 1998. ATP-stimulated activation

of the mitogen-activated protein kinases through ionotropic $P2X_2$ purinoceptors in PC12 cells. Difference in purinoceptor sensitivity in two PC12 cell lines. J. Biol. Chem. 273, 19965–19971.

Thomas, S., Virginio, C., North, R.A., Surprenant, A., 1998. The antagonist trinitrophenyl-ATP reveals co-existence of distinct P2X receptor channels in rat nodose neurones. J. Physiol. 509, 411–417.

Todorov, L.D., Mihaylova-Todorova, S., Westfall, T.D., Sneddon, P., Kennedy, C., Bjur, R.A., Westfall, D.P., 1997. Neuronal release of soluble nucleotidases and their role in neurotransmitter inactivation. Nature 387, 76–79.

Torres, G.E., Egan, T.M., Voigt, M.M., 1998a. N-linked glycosylation is essential for the functional expression of the recombinant $P2X_2$ receptor. Biochemistry 37, 14845–14851.

Torres, G.E., Haines, W.R., Egan, T.M., Voigt, M.M., 1998b. Co-expression of $P2X_1$ and $P2X_5$ receptor subunits reveals a novel ATP-gated ion channel. Mol. Pharmacol. 54, 989–993.

Torres, G.E., Egan, T.M., Voigt, M.M., 1999. Hetero-oligomeric assembly of P2X receptor subunits. Specificities exist with regard to possible partners. J. Biol. Chem. 274, 6653–6659.

Trendelenburg, A.U., Bültmann, R., 2000. P2 receptor-mediated inhibition of dopamine release in rat neostriatum. Neuroscience 96, 249–252.

Troadec, J.D., Thirion, S., Nicaise, G., Lemos, J.R., Dayanithi, G., 1998. ATP-evoked increases in $[Ca^{2+}]_i$ and peptide release from rat isolated neurohypophysial terminals via a $P2X_2$ purinoceptor. J. Physiol. 511, 89–103.

Ueno, T., Ueno, S., Kakazu, Y., Akaike, N., Nabekura, J., 2001. Bidirectional modulation of P2X receptor-mediated response by divalent cations in rat dorsal motor nucleus of the vagus neurons. J. Neurochem. 78, 1009–1018.

Virginio, C., MacKenzie, A., Rassendren, F.A., North, R.A., Surprenant, A., 1999. Pore dilation of neuronal P2X receptor channels. Nat. Neurosci. 2, 315–321.

Von Kügelgen, I., Wetter, A., 2000. Molecular pharmacology of P2Y-receptors. Naunyn-Schmiedeberg's Arch. Pharmacol. 362, 310–323.

Von Kügelgen, I., Späth, L., Starke, K., 1994. Evidence for P2-purinoceptor-mediated inhibition of noradrenaline release in rat brain cortex. Br. J. Pharmacol. 113, 815–822.

Von Kügelgen, I., Koch, H., Starke, K., 1997. P2-receptor-mediated inhibition of serotonin release in rat brain cortex. Neuropharmacology 36, 1221–1227.

Von Kügelgen, I., Nörenberg, W., Koch, H., Meyer, A., Illes, P., Starke, K., 1999. P2-receptors controlling neurotransmitter release from postganglionic sympathetic neurones. Prog. Brain Res. 120, 173–182.

Vorobjev, V.S., Sharonova, I.N., Haas, H.L., Sergeeva, O.A., 2003. Expression and function of P2X purinoceptors in rat histaminergic neurons. Br. J. Pharmacol. 138, 1013–1019.

Vulchanova, L., Arvidsson, U., Riedl, M., Wang, J., Buell, G., Surprenant, A., North, R.A., Elde, R., 1996. Differential distribution of two ATP-gated channels (P2X receptors) determined by immunocytochemistry. Proc. Natl. Acad. Sci. U. S. A. 93, 8063–8067.

Vulchanova, L., Riedl, M.S., Schuster, S.J., Buell, G., Surprenant, A., North, R.A., Elde, R., 1997. Immunohistochemical study of the $P2X_2$ and $P2X_3$ receptor subunits in rat and monkey sensory neurons and their central terminals. Neuropharmacology 36, 1229–1242.

Wang, Z.-M., Katsubayarashi, S., Rhee, J.-S., Brodwick, M., Akaike, N., 2001. Substance P abolishes the facilitatory effect of ATP on spontaneous glycine release in neurons of the trigeminal nucleus pars caudalis. J. Neurosci. 21, 2983–2991.

Wang, C.-M., Chang, Y.-Y., Kuo, J.-S., Sun, S.H., 2002. Activation of $P2X_7$ receptors induced [^{3}H]GABA release from the RBA-2 type-2 astrocyte cell line through a Cl^-/HCO_3^- dependent mechanism. Glia 37, 8–18.

Wieraszko, A., Seyfried, T.N., 1989. ATP-induced synaptic potentiation in hippocampal slices. Brain Res. 491, 356–359.

Wildman, S.S., King, B.F., Burnstock, G., 1998. Zn^{2+} modulation of ATP-responses at recombinant $P2X_2$ receptors and its dependence on extracellular pH. Br. J. Pharmacol. 1223, 1214–1220.

Wirkner, K., Franke, H., Inoue, K., Illes, P., 1998. Differential age-dependent expression of α_2 adrenoceptor- and P2 purinoceptor-functions in rat locus coeruleus neurons. Naunyn-Schmiedeberg's Arch. Pharmacol. 357, 186–189.

Wirkner, K., Köles, L., Thümmler, S., Luthardt, J., Poelchen, W., Franke, H., Fürst, S., Illes, P., 2002. Interaction between P2Y and NMDA receptors in layer V pyramidal neurons of the rat prefrontal cortex. Neuropharmacology 42, 476–488.

Xiang, Z., Bo, X., Oglesby, I., Ford, A., Burnstock, G., 1998. Localization of ATP-gated $P2X_2$ receptor immunoreactivity in the rat hypothalamus. Brain Res. 813, 390–397.

Ye, Z.-C., Wyeth, M.S., Baltan-Tekkok, S., Ransom, B.R., 2003. Functional hemichannels in astrocytes: a novel mechanism of glutamate release. J. Neurosci. 23, 3588–3596.

Yoshioka, K., Saitoh, O., Nakata, H., 2002. Agonist-promoted heteromeric oligomerization between adenosine A_1 and $P2Y_1$ receptors in living cells. FEBS Lett. 523, 147–151.

Zhang, Y.X., Yamashita, H., Ohshita, T., Sawamoto, N., Nakamura, S., 1995. ATP increases extracellular dopamine level through stimulation of P2y purinoceptors in the rat striatum. Brain Res. 691, 205–212.