Abstract  ATP is a co-transmitter in the central and peripheral nervous system. Extracellular ATP exerts its effects via ionotropic (P2X), as well as metabotropic receptors (P2Y). P2X receptors are involved in fast excitatory synaptic signalling by ATP, whereas the role of P2Y receptors in synaptic transmission is unclear. Seven different mammalian P2X receptor subunits (P2X$_{1-7}$) have been cloned to date. This article gives an overview about the distribution of these P2X receptor subunits in the nervous system. A comparison is made between the pharmacological properties of recombinant receptors and natively occurring neuronal P2X receptors by means of electrophysiological methods. The subcellular distribution of, developmental influences on, and interspecies differences between P2X receptors are also considered. It is concluded that the properties of native P2X receptors are best explained by a heteromeric assembly of different P2X receptor subunits.

Key words  Development · Heteromeric P2X receptor · Homomeric P2X receptor · Neuronal P2X receptor · Presynaptic P2X receptor · Somatodendritic P2X receptor · Interspecies differences

Introduction

Adenosine-5'-triphosphate (ATP) is a transmitter or co-transmitter of neuro-neuronal synaptic transmission in the central (CNS) and peripheral nervous system (PNS; Burnstock 1986; von Kügelgen and Starke 1991; Zimmermann 1994). Thus, ATP mediates fast synaptic potentials or currents at synapses formed by sympathetic neurones dissociated from guinea-pig coeliac ganglia (Evans et al. 1992; Silinsky et al. 1992), or at synapses in the intact coeliac ganglion (Silinsky and Gerzanich 1993), as well as in enteric ganglia (Galligan and Bertrand 1994; LePard et al. 1997). Similar fast postsynaptic ATP-mediated signals were also identified at neuro-neuronal synapses in the rat CNS including structures such as the medial habenula (Edwards et al. 1992), the pontine nucleus locus coeruleus (Nieber et al. 1997), the CA1 region of the hippocampus (Pankratov et al. 1998) and, eventually, the dorsal horn of the spinal cord (Bardoni et al. 1997).

ATP acts via two distinct families of ATP (P2) receptors which can be separated on the basis of structural differences, as well as their mechanisms of signal transduction after nucleotide binding: P2Y and P2X receptors (Abbracchio and Burnstock 1994; Fredholm et al. 1997). The members of the P2Y family, bearing seven transmembrane α-helices, are classical G protein-coupled receptors, whereas the members of the P2X family, bearing two transmembrane regions, are oligomeric ATP-gated cationic channels (reviewed in North and Barnard 1997 and Ralevic and Burnstock 1998). It is unclear whether P2Y receptors are involved in ATP-mediated synaptic transmission, because slow ATP-induced synaptic currents or synaptic potentials have not yet been demonstrated. Hence, this article concentrates on neuronal P2X receptors which mediate fast ATP signalling, as well as on their localisation and their functional characteristics (for further information pertaining to P2Y receptors see e.g. von Kügelgen, this issue). At the present time, seven mammalian P2X receptors (P2X$_{1-7}$) have been cloned and functionally characterised (North and Barnard 1997; Ralevic and Burnstock 1998). One putative new member of the P2X family, the P2XM clone (Urano et al. 1997), has not yet been identified unequivocally as a ligand-gated ion channel and a second putative new member of the P2X-family, P2X$_8$, has so far only been identified in chick skeletal muscle (Bo et al. 2000). Hence, P2XM and P2X$_8$ are not considered further in this review.
Localisation and properties of neuronal P2X receptors

Distribution of P2X receptor mRNA and P2X proteins

Activation of ATP-gated P2X receptor channels supplies a depolarising excitatory input to the neurones bearing them. The understanding of such possible excitatory functions of the co-transmitter ATP under physiological conditions requires the mapping of P2X receptors in the nervous system. In the last 5 years, the use of in situ hybridisation techniques by subtype-specific riboprobes, as well as immunolabelling with antisera containing subtype-specific antibodies, yielded a wealth of data about the distribution of mRNAs and receptor proteins for the seven cloned P2X receptor subunits.

In the rat nervous system, P2X mRNA or P2X proteins or both have been detected throughout the neuraxis (Table 1). Thus, the epithalamic region of the medial habenula, the locus coeruleus, the hippocampus, the dorsal horn of the spinal cord, as well as sympathetic coeliac ganglia and the myenteric plexus of the enteric nervous system have in common that they all express receptor proteins for at least one subclass of P2X subunit (P2X2; Table 1). In all these regions a co-transmitter role of ATP is strongly suggested by electrophysiological evidence (see Introduction). Inspection of Table 1 also indicates that the co-localisation of P2X proteins or P2X mRNAs from several P2X subclasses seems to be the rule rather than an exception. Such exceptions are e.g. the rostral ventrolateral medulla, the intermediolateral region of the spinal cord and the myenteric plexus, regions where to date only P2X2 has been identified (Table 1). This finding, however, may reflect the limited set of data available for these structures rather than a real break in the rule. The observed overlap in tissue distribution for the different P2X mRNAs and receptor proteins strongly suggests that heteromeric P2X receptors also occur. The fact that different P2X subunits indeed assemble into functional channels was first documented for P2X2 and P2X3 heterologously expressed in HEK293 cells (Lewis et al. 1995). ATP-gated currents, closely resembling those seen with the P2X2,3 heteromers, were also found in sensory neurones cultured from rat nodose ganglia (Khakh et al. 1995; Lewis et al. 1995) and dorsal root ganglia (Grubb and Evans 1999; Ueno et al. 1999); both structures express proteins for the P2X2 and P2X3 subunits (Table 1).

The tissue distribution of the seven P2X receptors ranges from abundant to discrete. Thus, P2X2, P2X4, and P2X6 mRNAs or proteins have been detected throughout the brain and spinal cord, as well as in sensory and autonomic ganglia (Table 1). P2X7 receptors were initially believed to be absent in the brain (Valera et al. 1994). However, later on it turned out that P2X7 mRNA is localised in the cerebral cortex, striatum, hippocampus and cerebellum, where P2X7 subunit proteins have also been detected (Table 1). Outside the brain, P2X7 was found in the dorsal and ventral horns of the spinal cord, sensory ganglia (trigeminal, nodose, dorsal root ganglia), sensory systems (spiral ganglion in the cochlea of the inner ear) and autonomic sympathetic ganglia (superior cervical and coeliac ganglia; Table 1).

The P2X3 receptor is believed to mostly occur in capsaicin-sensitive sensory neurones involved in nociception (Chen et al. 1995; Lewis et al. 1995; Vulchanova et al. 1998). Accordingly, P2X3 protein or its mRNA was found in cell bodies of sensory neurones, and in their central termination fields (dorsal horn of the spinal cord, nucleus of the solitary tract), as well as in their peripheral endings, e.g. in taste buds of the tongue and the spiral ganglion of the inner ear (Table 1). However, exceptions exist. In the adult rat nervous system, P2X3 was also found in the hypothalamic supraoptic nucleus, as well as in autonomic sympathetic ganglia (Table 1).

P2X5 also showed a restricted localisation. In the CNS, P2X5 mRNA was found only in the mesencephalic nucleus of the trigeminal nerve at which proprioceptive primary afferents from muscles of mastication terminate, and in motoneurones of the spinal cord ventral horn (Table 1). In the peripheral nervous system, P2X5 mRNA, P2X5 protein, or both were identified in sensory ganglia and the spiral ganglion of the inner ear (Table 1), whereas sympathetic ganglia such as superior cervical and coeliac ganglia did not express P2X5 proteins (Xiang et al. 1998b).

It is generally believed that P2X7 is not expressed in neuronal cells although it was originally cloned from superior cervical ganglia and brain tissue (Surprenant et al. 1996). Since P2X7 receptors form large diameter cytolytic pores upon prolonged contact with ATP (Surprenant et al. 1996), it was argued that “it is not obvious why neurones would exhibit such responses” (Collo et al. 1997). However, native P2X receptors in nodose ganglion neurones, as well as heterologously expressed P2X2 and P2X4 subunits (showing widespread distribution in the nervous system; Table 1) were also shown to develop large pores during prolonged application of ATP; these pores were comparable in diameter to those generated by the cytolytic P2X7 receptor (Virginio et al. 1999). Indeed, P2X7 seems to be present also in neuronal tissues, such as in the rat spiral ganglion of the inner ear and in rat retinal ganglion cells (Table 1) as demonstrated by RT-PCR on single cells (Brändle et al. 1999) and P2X7 immunostaining, respectively (Brändle et al. 1998a).

Functional studies

P2X receptors, when activated, open within a few milliseconds and allow the non-selective passage of cations (Na+, K+, Ca2+) through their channel pore (Surprenant 1996). Hence, measurement of rapidly activating cationic inward currents, evoked either by ATP or some of its structural analogues in neurones voltage-clamped at a negative membrane potential, provides the most direct way to functionally characterise native somatodendritic P2X receptors (Krishtal et al. 1983). This can be done in neurones kept either in cell cultures or contained in nervous tissue slices. Although the latter preparations repre-
sent a more physiological situation, possible indirect actions of ATP via nearby neurones may be complicating factors. In this setting, therefore, it is preferable to exclude the involvement of other transmitters such as glutamate by suitable antagonists. This minor disadvantage, however, is by far outweighed by the possibility to study ATP-mediated neuro-neuronal synaptic transmission as shown for the first time in the medial habenula slice. In this preparation, electrical stimulation evoked excitatory postsynaptic currents (EPSC) in voltage-clamped habenula neurones. These EPSCs showed a fast rise time, indicating the involvement of ligand-gated ion channels, and were inhibited by the P2 receptor antagonist suramin (Edwards et al. 1992). Hence, electrophysiological methods allow the detection of P2X receptor activation both by endogenously released as well as exogenously applied ATP (e.g. Jahr and Jessel 1983).

More indirectly, P2X receptors can also be detected by virtue of their appreciable Ca^{2+} permeability (Rogers and Dani 1995; Evans et al. 1996), provided some precautions

<table>
<thead>
<tr>
<th>Brain</th>
<th>P2X1</th>
<th>P2X2</th>
<th>P2X3</th>
<th>P2X4</th>
<th>P2X5</th>
<th>P2X6</th>
<th>P2X7</th>
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<td>PPADS</td>
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<td>Inward current</td>
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<td>Suramin, PPADS</td>
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<td>Block of EPSC</td>
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<td>Firing↑</td>
<td>2MeSATP = α,βme-ATP</td>
<td>Suramin, PPADS</td>
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<td>Suramin, PPADS</td>
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<td>Inward current</td>
<td>ATP = α,βme-ATP</td>
<td>Suramin</td>
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<td>Firing↑</td>
<td>2MeSATP = α,βme-ATP</td>
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<td>Slic, rat</td>
<td>WPC</td>
<td>Inward current</td>
<td>ATP, α,βme-ATP inactive</td>
<td>Suramin</td>
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<tr>
<td>Dorsal motor nucleus of vagus</td>
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<td>WPC</td>
<td>Inward current</td>
<td>ATP &gt; 2MeSATP, α,βme-ATP inactive</td>
<td>Suramin, PPADS</td>
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<td>Slice, mouse</td>
<td>WPC</td>
<td>Inward current</td>
<td>ATP</td>
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<td>Inward current</td>
<td>2MeSATP &gt;&gt; α,βme-ATP</td>
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<td>Firing↑</td>
<td>ATP &gt; ATP, 2MeSATP</td>
<td>Suramin</td>
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<td>Culture, rat</td>
<td>MFM</td>
<td>ATP &gt; 2MeSATP; α,βme-ATP inactive</td>
<td>Suramin, PPADS</td>
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<td>Cerebellar granule cells</td>
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<td>MFM</td>
<td>No effect</td>
<td>ATP, α,βme-ATP</td>
<td>Suramin</td>
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<tr>
<td>Spinal cord</td>
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<td>Inward current</td>
<td>ATP &gt; α,βme-ATP</td>
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</tr>
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<td></td>
<td>Culture, guinea pig</td>
<td>WPC</td>
<td>Inward current</td>
<td>2MeSATP ≥ ATP ≥ α,βme-ATP</td>
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Table 2 (continued)

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<th>Agonists</th>
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<td>Inward current</td>
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<td>WPC</td>
<td>Inward current</td>
<td>ATP, α,βme-ATP, Suramin</td>
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<tr>
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<td>ECR</td>
<td>Firing</td>
<td>ATP, α,βme-ATP, Suramin, PPADS</td>
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</tbody>
</table>

Autonomic, sympathetic ganglia

| Superior cervical ganglion | Culture, rat | WPC | Inward current | ATP = 2MeSATP, α,βme-ATP inactive, Suramin, PPADS |
| Thoracolumbar ganglia | Culture, rat | MFM | [Ca²⁺],↑ | ATP |
| Coeliac ganglion | Culture, guinea pig | WPC | Inward current | 2MeSATP ≥ ATP ≥ α,β-me-ATP, Suramin, PPADS |
| Culture, rat | WPC | Inward current | ATP, α,β-me-ATP inactive, Suramin, PPADS |
| Culture, mouse | WPC | Inward current | ATP, α,β-me-ATP inactive, Suramin, PPADS |

Cardiac ganglia

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<td>ATP ≥ 2MeSATP &gt;&gt; α,β-me-ATP, PPADS</td>
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</tbody>
</table>

Enteric system

| Myenteric plexus | Culture, guinea pig | WPC | Inward current | ATP ≥ 2MeSATP >> α,β-me-ATP, PPADS |


are taken. Hence, Ca²⁺ entry through voltage-gated Ca²⁺ channels, as well as depletion of intracellular Ca²⁺ stores, should be avoided. Under these conditions, P2X receptors would increase the intracellular free Ca²⁺ concentration (measured by Ca²⁺-sensitive fluorescent dyes such as fura-2/AM) in a manner absolutely dependent on extracellular Ca²⁺. Eventually, the measurement of biological responses to the ATP analogue α,β-meATP (α,β-methylene ATP), which is known to be inactive at P2Y receptors (Ralevic and Burnstock 1998), strongly suggests but does not exclude (see below) the involvement of P2X receptors. These responses include an increase in the action potential frequency of rat locus coeruleus neurones (Tschöp et al. 1992; Fröhlich et al. 1996; Sansum et al. 1998) or a decrease in blood pressure after microinjection of α,β-meATP into the nucleus of the solitary tract (Ergene et al. 1994). However, one should be aware that α,β-meATP is only a weak agonist (EC₅₀ ≥ 100 μM) or even inactive at recombinant P2X₂ and P2X₄ homomeric receptors (Table 3) and, therefore, negative results should be treated with caution. P2X receptor-mediated effects have been detected throughout the neuraxis (Table 2). Again, the largest amount of data relates to the rat nervous system. It appears that functional P2X receptor identification was successful in regions where P2X mRNA or P2X proteins have also been observed (see Table 1). Exceptions were e.g. the caudate/putamen ( striatum), the ventral tegmental area, and cerebellar granule cells. In the striatum, ATP was superfused to medium spiny neurones contained in a brain slice preparation (Nörenberg et al. 1997). Hence, ATP may have been degraded by ecto-ATPases (Zimmermann 1999) before it reached its site of action. Alternately, medium spiny neurones could have been the wrong cell type investigated for ATP actions, because at least four additional neuronal cell populations, which can be differentiated by morphological, immunohistochemical and electrophysiological criteria, are also present in the striatal complex (Kawaguchi et al. 1995). The discrepancy between P2X receptor localisation in the ventral tegmental area as well as cerebellar granule cells (Table 1) and the lack of evidence for the presence of functional
Table 3: Properties of recombinant P2X receptor subunits from rat or human (P2X<sub>1</sub>) tissues functionally expressed as homomeric (P2X<sub>1-7</sub>) or heteromeric receptors (P2X<sub>2,3</sub>, P2X<sub>4,5</sub>, P2X<sub>6,8</sub>). P2X cDNAs were microinjected into Xenopus oocytes (Brake et al. 1994; Valera et al. 1994; Poelchen et al. 1998; Chen et al. 1995; Evans et al. 1995; Séguela et al. 1996; Soto et al. 1996; Wang et al. 1996; Simon et al. 1997; Lé et al. 1998a; Lé et al. 1999) or transfected to HEK293 cells (Evans et al. 1995; Lewis et al. 1995; Collo et al. 1996; Evans and Surprenant 1996; Garcia-Guzman et al. 1996; Surprenant 1996; Surprenant et al. 1996; Torres et al. 1998; Haines et al. 1999; Jones et al. 2000) and ATP-induced inward currents were measured by two-electrode voltage-clamp and the whole-cell patch clamp technique in Xenopus oocytes and HEK293 cells, respectively.

<table>
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<tr>
<th>Agonists&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Antagonists&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Desensitization&lt;sup&gt;b&lt;/sup&gt;</th>
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<tr>
<td>P2X&lt;sub&gt;1&lt;/sub&gt; &lt;sup&gt;1-4&lt;/sup&gt;</td>
<td>ATP (0.9 µM) ≥ 2MeSATP (1 µM) &gt; α,β-meATP (2.2 µM)</td>
<td>PPADS (1 µM) = suramin (1 µM)</td>
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<tr>
<td>P2X&lt;sub&gt;2&lt;/sub&gt; &lt;sup&gt;5-7&lt;/sup&gt;</td>
<td>2MeSATP (3 µM) &gt; ATP (8 µM); α,β-meATP inactive&lt;sup&gt;c&lt;/sup&gt;</td>
<td>PPADS (1 µM) &gt; suramin (8 µM)</td>
</tr>
<tr>
<td>P2X&lt;sub&gt;3&lt;/sub&gt; &lt;sup&gt;7, 8&lt;/sup&gt;</td>
<td>ATP (0.3 µM) &gt; ATP (0.6 µM) ≥ α,β-meATP (0.7 µM)</td>
<td>PPADS (1.5 µM) &gt; suramin (3 µM)</td>
</tr>
<tr>
<td>P2X&lt;sub&gt;4&lt;/sub&gt; &lt;sup&gt;9-13&lt;/sup&gt;</td>
<td>ATP (5.5 µM) ≥ 2MeSATP&lt;sup&gt;d&lt;/sup&gt; (12.7 µM) &gt; α,β-meATP</td>
<td>PPADS (100 µM) &gt; suramin (&gt;100 µM)</td>
</tr>
<tr>
<td>P2X&lt;sub&gt;5&lt;/sub&gt; &lt;sup&gt;14, 15&lt;/sup&gt;</td>
<td>ATP (15.4 µM) ≥ 2MeATP (20 µM); α,β-meATP inactive&lt;sup&gt;c&lt;/sup&gt;</td>
<td>PPADS (2.6 µM) &gt; suramin (4 µM)</td>
</tr>
<tr>
<td>P2X&lt;sub&gt;6&lt;/sub&gt; &lt;sup&gt;16&lt;/sup&gt;</td>
<td>2MeSATP (9 µM) &gt; ATP (12 µM); α,β-meATP inactive&lt;sup&gt;c&lt;/sup&gt;</td>
<td>PPADS (45 µM) &gt; suramin (&gt;300 µM)</td>
</tr>
<tr>
<td>P2X&lt;sub&gt;7&lt;/sub&gt; &lt;sup&gt;17, 18&lt;/sup&gt;</td>
<td>ATP (115 µM) ≥ 2MeSATP&lt;sup&gt;e&lt;/sup&gt;; α,β-meATP inactive&lt;sup&gt;c&lt;/sup&gt;</td>
<td>80% inhibition by 10 µM PPADS</td>
</tr>
<tr>
<td>P2X&lt;sub&gt;8&lt;/sub&gt; &lt;sup&gt;19, 20&lt;/sup&gt;</td>
<td>2MeSATP (1 µM) &gt; ATP (2 µM) ≥ α,β-meATP (3 µM)</td>
<td>PPADS (0.6 µM) &gt; suramin (1.6 µM)</td>
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</table>

<sup>a</sup>Numbers in parentheses are EC<sub>50</sub> and IC<sub>50</sub> values of agonists and antagonists, respectively, where available

<sup>b</sup>Desensitisation is used descriptively for the decay in current amplitude during continuous agonist application (EC<sub>50</sub> to EC<sub>100</sub>) measured either by determination of time constants or fraction of current remaining at the end of the application period. Desensitisation is considered to be slow for time constants (time for an e-fold decrease in amplitude) > 10 s or remaining current amplitudes > 50% of peak currents at the end of agonist application (≥2 s). The P2X<sub>1,5</sub> heterooligomer displayed a composite phenotype consisting of a transient peak current followed by a sustained plateau.

<sup>c</sup>No effect at 100 µM

P2X receptors in both preparations (Mateo et al. 1998; Poelchen et al. 1998) is harder to cope with, since the P2X-selective ATP analogue α,βmeATP, which is resistant to enzymatic degradation (Kennedy and Leff 1995), was inactive. By contrast, the P2 agonist 2-methylthio ATP (2MeSATP) released dopamine from the endings of ventral tegmental area neurones in their termination field in the nucleus accumbens (Krügel et al. 1999). Hence, a possible explanation may be that in ventral tegmental area neurones, P2X receptors are preferentially transported to the axon endings and function as presynaptic release-modulating receptors. The same may hold true for cerebellar granule cells because at least P2X<sub>1</sub> immunoreactivity was detected presynaptically in their parallel fibre varicosities (Loesch and Burnstock 1998).

Comparison between native and recombinant P2X receptors

Where available, Table 2 gives also relative rank orders of agonist potencies for the commonly used P2X receptor agonists ATP, 2MeSATP and α,β-meATP and, moreover, indicates whether P2X receptors mediated responses were sensitive to the P2 antagonists pyridoxalphosphate-6-azophenyl-2',4'-disulfonic acid (PPADS) and suramin. In the rat CNS, two functional P2X receptor phenotypes appear to be present. In most regions of the brain, P2X receptors were activated by α,β-meATP (≤100 µM) and markedly antagonised by suramin and/or PPADS (≤100 µM; see Table 1 and references therein). In addition, these P2X receptors showed only little desensitisation (decay of the response duration during continuous agonist application) or rundown (decay of the response upon repeated agonist application) as e.g. demonstrated in neurones from the cerebral cortex (Lallo et al. 1998), the hippocampus (Pankratov et al. 1998), the supraoptic nucleus (Shibuya et al. 1999), the mesencephalic trigeminal nucleus (Khakh et al. 1997), the locus coeruleus (Tschöp et al. 1992) and the nucleus of the solitary tract (Ueno et al. 1992). Apparent exceptions to this major P2X phenotype have been described in neurones from the dorsal motor nucleus of the vagus, in cerebellar Purkinje cells and in a small subset (<5%) of neurones from the dorsal horn of the spinal cord. These receptors were not activated by α,β-meATP but were also blocked by low concentrations of PPADS and/or suramin (Table 2) and did only marginally desensitise or rundown (Nabekura et al. 1995; Bardoni et al. 1997; García-Lecea et al. 1999). Of the recombinant homomeric receptors, P2X<sub>2</sub>, P2X<sub>3</sub>, P2X<sub>5</sub> and P2X<sub>7</sub> were insensitive to α,β-meATP but only P2X<sub>2</sub> and P2X<sub>5</sub> were blocked by suramin and PPADS at low micromolar concentrations (Table 3). Since P2X<sub>2</sub> subunits have not yet been detected in the dorsal motor nucleus of the vagus, or in cerebellar Purkinje
cells and the dorsal horn of the spinal cord (Table 1), at least in rats, P2X2 may be the predominant subunit in these CNS regions.

Nevertheless, the pharmacological properties of the first and greater proportion of CNS P2X receptors do not correlate well with those of recombinant P2X subunits: functionally expressed, α,β-meATP-sensitive homomeric receptors showed a prominent desensitisation (P2X1, P2X3) or were rather insensitive to suramin and PPADS (P2X2; cf. Table 3). Hence, most rat brain P2X receptors may belong to a hitherto unknown subclass. However, another possibility is the formation of heteromeric channel assemblies. Native P2X receptor channels are oligomers of currently unknown stoichiometry although it seems that a trimeric assembly constitutes the structural basis of the P2X pore as recently suggested for heterologously expressed P2X1, P2X2 and P2X3 subunits (Nicke et al. 1998; Stoop et al. 1999). Whatever the exact number of P2X subunits may be, functional ATP-gated channels can arise from heteropolymerisation of different P2X subunits.

It has been shown recently that rat P2X4 and P2X6 subunits can co-assemble into functional ATP-gated channels (Lê et al. 1998a). P2X6, when expressed alone, did either fail to produce ATP-gated channels (Xenopus oocytes; Lê et al. 1998a) or did so only in a small fraction of cells (HEK293 cells; Collo et al. 1996). P2X4 alone produced functional P2X receptors in all cells (Lê et al. 1998a) which, however, were only weakly activated by α,β-meATP and rather insensitive to PPADS and suramin (Table 3). P2X4/P2X6 heteromeric receptors were activated by intermediate concentrations of α,β-me-ATP, were blocked by low concentrations of PPADS or suramin, and desensitised slowly (Table 3).

Provided that also native subunits heteropolymerise, brain P2X receptors may be P2X4/6, in good agreement with the abundant occurrence and co-localisation of P2X4/P2X6 mRNA at least in the rat brain (Table 1). Interestingly, the presence of α,β-meATP-sensitive brain P2X receptors has also been demonstrated in the supraoptic nucleus, the nucleus of the solitary tract and the ventrolateral medulla of rats, regions thought to be involved in the central regulation of cardiovascular and respiratory control (Table 2). Moreover, in anaesthetised rabbits, microinjection of α,β-meATP into the ventrolateral medulla elicited either an increase or a decrease of blood pressure dependent on whether sympatheic or sympahtoinhibitory neurones were activated (Horiuchi et al. 1999). These effects were antagonised by suramin (Table 2). Hence, α,β-meATP-sensitive, P2X4/6-like brain receptors may occur in different species.

In the periphery, P2X receptors have been functionally characterised in the sensory, the autonomic sympathetic and parasympathetic, as well as the enteric divisions of the PNS. These peripheral P2X receptors, just as their central counterparts, were sensitive to the P2 receptor antagonists PPADS and suramin at concentrations ≤100 μM (Table 2 and references therein). Hence, it seems unlikely that these neurones contained homomeric P2X4, P2X6 or P2X7 receptors (Table 3), although P2X4, P2X6 and P2X7 subunits have been detected in rat sensory ganglia, and P2X4 as well as P2X6 subunits occurred in sympathetic neurones (Table 1).

P2X receptors in sensory systems, including neurones of nodose, dorsal root and retinal ganglia, were sensitive to α,β-meATP, irrespective of whether they were cultured from rat or guinea-pig ganglia (Table 2). However, there were also differences between these neurones. Cells of nodose ganglia responded to P2X receptor agonists with currents which showed only little desensitisation or rundown (Khakh et al. 1995). In contrast, dorsal root ganglion neurones showed either rapidly desensitising (85% of cells), slowly desensitising (5%) or biphasic responses (10%), i.e. currents composed of an initial fast decaying component followed by a sustained plateau (Burgard et al. 1999; Grubb and Evans 1999). Similarly, in retinal ganglion cells, fast-decaying as well as slowly decaying ATP-evoked responses were found, indicating a heterogeneity of the P2X receptor population.

The slowly desensitising P2X receptor phenotype found in sensory ganglia, which was highly sensitive to α,β-meATP, suramin and PPADS, does not fit to the profile of any known recombinant homomeric receptor (Table 3) and, hence, these native P2X receptors may be heteromers. Rat nodose and dorsal root ganglia synthesise proteins for P2X1–P2X6 (Table 1). Up to date, in rat retinal ganglion cells only P2X3 subunit proteins have been demonstrated unequivocally (Table 1) but RT-PCR experiments showed the additional presence of P2X5 mRNA in the retina (Brändle et al. 1998b). Hence, within certain limitations, a great number of different subunit compositions are possible. However, not all known recombinant subunits can form heteromeric receptors, as suggested by observations from a co-immunoprecipitation study where protein-protein interactions for P2X1/P2X4, P2X4/P2X6, P2X3/P2X4, P2X6/P2X5, or for any combination containing P2X7 subunits could not be detected (Torres et al. 1999).

With regard to the hitherto functionally expressed recombinant heteromeric receptors, either P2X2/3 or P2X4/6 may account for the slowly desensitising P2X receptors in sensory ganglia (Table 3). However, two observations strongly argue against P2X4/6. The first observation is that α,β-meATP was a full agonist in rat nodose neurones (Khakh et al. 1995), as well as dorsal root neurones, displaying slowly desensitising currents (Ueno et al. 1999), and was roughly equipotent with ATP in retinal ganglion cells (Taschenberger et al. 1999). In contrast, at recombinant heteromeric P2X4/6 receptors α,β-meATP was a partial agonist (Lê et al. 1998a; Table 3). The second observation relates to the differential modulation of P2X receptor-mediated responses by extracellular protons. Acidification of the extracellular milieu potentiated ATP-evoked currents in nodose (Li et al. 1996), dorsal root (Burgard et al. 1999) and retinal ganglion neurones (Taschenberger et al. 1999) but decreased the responses mediated by recombinant P2X4/6 receptors (Lê et al. 1998a).

Hence, P2X4/6 heteromeric channels may represent one type, but not the exclusive type of native P2X receptors.
encountered in the somatosensory nervous system. In line with this latter assumption is the finding in rat nodose ganglion that P2X2 and P2X3 immunoreactivity was co-localised in the majority of neurones although cells that were positive for only one antibody also occurred (Vulchanova et al. 1997). Since the preferential P2X2/3 antagonist trinitrophenyl-ATP (Virgino et al. 1998) produced a biphasic inhibition curve of the non-desensitising ATP-gated currents, indicating more than one binding site, it was suggested that individual nodose ganglion neurones express homomeric P2X2 and heteromeric P2X2/3 but not fast desensitising homomeric P2X3 receptors (Thomas et al. 1998). Interestingly, mesenteric vagal afferents, whose cell bodies are located in the nodose and dorsal root ganglia, were excited by α,β-meATP in a suramin- and PPADS-sensitive manner (Table 2); this response did not desensitise (Kirkup et al. 1999). Hence, sensory neurones may express P2X2/3 heteromeric receptors not only in their somatodentritic region but also at their peripheral endings.

Eventually, the question arises which types of α,β-meATP-sensitive P2X subunits might be responsible for the fast-desensitising currents observed in the majority of dorsal root neurones, as well as in a proportion of retinal ganglion cells. Of recombinant homomeric receptors, P2X1, as well as P2X3, fulfil the criteria required for the fast-desensitising response (Table 3). However, the receptor in dorsal root ganglia appears to be P2X3, since β,γ-me-ATP (β,γ-methylene-ATP), which is a potent agonist at recombinant P2X1 receptors (Evans et al. 1995), as well as at prototypic native P2X1 receptors in smooth muscle (Trezise et al. 1995), had no effect in dorsal root neurones (Rae et al. 1998). The fact that the rate of receptor desensitisation in dorsal root neurones increased with the agonist concentration (Robertson et al. 1996; Grubb and Evans 1999), a behaviour typical for recombinant P2X1 (Chen et al. 1995; Lewis et al. 1995) but not P2X3 receptors (Evans and Surprenant 1996), also supports this conclusion. Accordingly, the receptor in retinal ganglion neurones may also belong to the P2X1 subtype, since ocular tissue lacked P2X1 mRNA (Brändle et al. 1998b). As a reason of the biphasic response seen in some dorsal root neurones, it was proposed that P2X3 homomeric (fast-desensitising) together with P2X2/3 heteromeric receptors (slowly desensitising) (Burgard et al. 1999; Grubb and Evans 1999; Ueno et al. 1999) co-exist. However, an alternative explanation is also possible. Sensory ganglia express P2X1 and P2X2 (Table 1), and these subunits can co-assemble into functional heteromeric channels which are sensitive to α,β-meATP, PPADS and suramin. Moreover, currents through P2X2/3 heteromeric receptor channels showed a biphasic decay in the presence of agonists (Table 3), similar to the currents observed with native receptors in a proportion of dorsal root ganglion neurones.

In the autonomic division of the PNS, P2X receptors have been functionally characterised in the sympathetic as well as in the parasympathetic branch of the autonomic nervous system (Table 2). All these receptors had in common that they showed slowly desensitising responses in the presence of agonists (Fieber and Adams 1991; Silinsky and Gerzanich 1993; Khakh et al. 1995; Boehm 1999; Nörenberg et al. 1999; Zhong et al. 2000a) and that they were potently antagonised by low micromolar concentrations of suramin and PPADS (Table 2). Furthermore, these “autonomic” P2X receptors were either insensitive to α,β-meATP in the concentration range of up to 100 µM (rat superior cervical ganglion; Khakh et al. 1995; rat and mouse coeliac ganglion; Zhong et al. 2000a) or were only marginally activated at concentrations ≥100 µM (thoracolumbar sympathetic ganglia; Nörenberg et al. 1999; cardiac parasympathetic ganglia; Fieber and Adams 1991). Of the seven cloned P2X receptors all but P2X5 and P2X7 have been detected in sympathetic ganglia (Table 1). Hence, P2X2 may be the predominant subunit in sympathetic ganglia, because other homomeric or heteromeric receptors can be excluded by virtue of their high sensitivities to α,β-meATP, low sensitivities to antagonists and desensitising behaviour (Table 3).

However, Table 2 suggests one exception from this pattern: guinea-pig “autonomic” P2X receptors (i.e. in guinea-pig coeliac ganglia) differed from rat and mouse “autonomic” P2X receptors in that they were activated by low concentrations of α,β-meATP (EC50 13 μM; Khakh et al. 1995), an observation which favours interspecies differences. Since α,β-meATP behaved as a full agonist and since ATP-evoked currents desensitised slowly but not in a biphasic manner (Khakh et al. 1995), it is tempting to speculate that P2X receptors in guinea-pig coeliac ganglia are P2X2/3 (cf. Table 3). In line with this assumption, the likely existence of functional heteromeric P2X2/3 receptors has been demonstrated in neurones from guinea-pig superior cervical ganglia, where they may co-exist with homomeric P2X2 receptors (Zhong et al. 2000b).

Eventually, P2X receptors have been functionally characterised in neurones isolated from the guinea-pig myenteric plexus belonging to the enteric division of the PNS (Table 2). Two types of cells have been identified. The major proportion of neurones (92%) responded to ATP with slowly desensitising inward currents, which were markedly antagonised by PPADS. In these cells, α,β-meATP was a rather poor agonist (Table 2). In a minority of neurones (8%), however, ATP and α,β-meATP activated with a similar potency rapidly desensitising inward currents, indicating that heterogeneous P2X receptor populations may exist. Based on these observations, it was proposed that the smaller proportion of myenteric neurones possess P2X receptors which may be P2X2, or P2X3 (cf. Table 3), whereas the greater proportion of neurones may bear P2X3-like receptors (Zhou and Galligan 1996), in agreement with the detection of P2X2 protein, at least in the myenteric plexus of rats (Table 1).

However, the slowly desensitising P2X receptors in myenteric neurones were unusual in that ATP-induced inward currents were not antagonised but actually potentiated by the P2 antagonist suramin, an effect which was not due to the known ecto-ATPase-inhibiting activity of this compound (Barajas-López et al. 1993, 1996). There are data to indicate that recombinant P2X2 subunits may
display a similar behaviour. Whereas some studies report that this subunit is rather insensitive to antagonism by suramin (Soto et al. 1996; Wang et al. 1996; Jones et al. 2000), other studies showed that rat P2X4-mediated responses were potentiated by suramin or by two other P2 agonists, cibacron blue and reactive blue-2, probably at allosteric modulatory sites (see Fig. 6 in Bo et al. 1995; Lê et al. 1998a; Miller et al. 1998). Hence, it is tempting to speculate that the majority of myenteric neurones may possess heteromeric P2X receptors, where P2X4 may be the constituent responsible for the potentiation by suramin, whereas other (unknown) subunits confer the sensitivity to the antagonism by PPADS. Of the recombinant receptors, P2X2 and P2X3 showed a high sensitivity to PPADS as well as a slow desensitisation during agonist application (Table 3). Since P2X4 co-assembled with P2X3 but not with P2X2 (Torres et al. 1999), it would be interesting to investigate whether possible recombinant P2X4/3 heteromeric receptors could mimic the unusual behaviour of native P2X receptors found in guinea-pig myenteric neurones.

So far, neuronal P2X receptors, which were most likely somatodendritic receptors at least when studied in isolated cells, displayed properties in some regions of the brain, as well as in the sensory and enteric nervous system, which can be explained by a heteromeric assembly of different P2X subunits. In most neurones from dorsal root ganglia and the autonomic nervous system, however, native receptors had properties compatible with a homomeric P2X3- and P2X2-assembly, respectively. However, there are some caveats. Of course, the existence of hitherto unknown P2X subunits cannot be excluded or a single subunit may predominate the phenotype of a heteromeric complex. Such a predominance has been shown e.g. for the AMPA type of glutamate-gated ion channels, where the GluR-B subunit governs Ca2+ permeability, as well as rectification properties of the AMPA receptor heteromeric complex (Verdoorn et al. 1991; Geiger et al. 1995). The properties of the P2X3/4 heteromeric channel, where the slowly desensitising characteristics of P2X3 prevailed (Lewis et al. 1995), had already suggested that the situation may be similar for P2X receptors. A further complication may arise from alternative splicing. Several splice variants e.g. of the rat P2X2 subunit have been detected which P2X2b (alternative name P2X2b) formed functional homomeric ATP-gated channels, which differed from prototypic P2X2 (P2X2a) in that P2X2b-mediated currents desensitised faster and were less sensitive to the antagonists suramin and PPADS (Simon et al. 1997). Taken together the previous evidence, the assembly of P2X receptors from different subunits, the coexistence of such heteromeric receptors together with homomeric receptors in the same cell, the predominance of single subunits in heteromeric receptors and eventually the contribution of splice variants, all may determine the pharmacological/biophysical properties of native P2X receptors and, hence, shape the response of a given neurone to extracellular (co-transmitter) ATP.

Finally, although beyond the scope of the present paper, a last complicating factor should be also shortly mentioned, i.e. the likely coexistence of ionotrophic P2X receptors together with metabotropic P2Y and P1 (adenosine) receptors which may also influence the neuronal response to ATP (e.g. locus coeruleus; Tschöp et al. 1992; myenteric plexus; Barajas-López et al. 1994; spinal cord dorsal horn; Jo and Schlichter 1999). For further information pertaining to nucleotide/nucleoside receptor (P receptor) co-expression, the reader is referred to the detailed review of Ralevic and Burnstock (1998).

Presynaptic P2X receptors

Subcellular distribution of P2X receptor proteins

Determination of the subcellular localisation of P2X receptors is critical for a closer understanding of their possible roles in co-transmitter ATP-mediated synaptic transmission. Thus, the release of ATP from the axon terminals of a presynaptic neurone may activate P2X receptors in the receptive somatodendritic region of a second, postsynaptic neurone and by this way contribute to fast excitatory neurotransmission. However, released ATP may also act at presynaptic P2X receptors, located either at the axon terminal where it was released from (autoreceptors) or at nearby endings (heteroreceptors). With regard to the appreciable Ca2+ permeability of the known recombinant P2X subunits (Rogers and Dani 1995; Evans et al. 1996), activation of such presynaptic P2X receptors may lead to facilitation of ongoing action potential-dependent exocytotic transmitter release. Two different experimental approaches using P2X subtype-specific antibodies either in combination with electron-microscopy or surgical removal of sensory ganglia prior to the determination of P2X immunostaining in their central termination fields, indeed provided evidence for a presynaptic localisation of P2X1, P2X3, P2X1 and P2X4 subunits.

By means of the first approach, P2X1 immunoreactivity was localised at the electron-microscopic level in the rat cerebellum in parallel fibre varicosities arising from granule cells, as well as in dendritic spines of Purkinje cells (Loesch and Burnstock 1998). Since immunopositive varicosities formed synapses with immunonegative dendritic spines and immunonegative granule cell varicosities synapsed on stained Purkinje cells, it was suggested that ATP may modulate transmitter release at some granule cell/Purkinje cell synapses and may serve as a co-transmitter at others (Loesch and Burnstock 1998). At the electron-microscopic level, P2X3 was also localised at nerve endings in the dorsal horn of the spinal cord and in the nucleus of the solitary tract (Llewellyn-Smith and Burnstock 1998), P2X4 immunoreactivity was evident within axon terminals of the olfactory bulb and the dorsal horn of spinal cord (Lê et al. 1998b).

By means of the second approach, it was shown that nodose gangliectomy decreased the intensity of P2X2 immunoreactivity in the nucleus of the solitary tract whereas ipsilateral removal of the dorsal root ganglia decreased the intensity of P2X2 and P2X3 immunoreactivities in the
dorsal horn of the spinal cord. Hence, these P2X proteins were not only present at the level of cell bodies contained in both ganglia (Table 1) but also presynaptically at the endings of the primary sensory afferents in their respective central termination fields (Vulchanova et al. 1996, 1997, 1998). Taken together, these observations provide strong evidence that P2X receptors are present at some nerve terminals and thus may be involved in the modulation of transmitter release.

Functional studies

Additional indications for the presence of presynaptic P2X receptors have been obtained in functional studies at synaptic terminals in the neuraxis. Thus, ATP evoked an increase in the concentration of intracellular free Ca2+ in synaptic terminals prepared from the human cortex, which was absolutely dependent on transmembrane Ca2+ entry and was antagonised by PPADS, but only partially affected by the blockade of voltage-operated Ca2+ channels. Hence, terminals in the cerebral cortex possess (presynaptic) P2X receptors (Pintor et al. 1999), although their actual role in transmitter release needs further clarification.

In the rat motor trigeminal nucleus, ATP failed to elicit inward currents typical for a postsynaptic response. In contrast, ATP as well as α,β-meATP increased the frequency of spontaneous glutamatergic postsynaptic currents, in a suramin- and PPADS-sensitive manner, even when action potential propagation was suppressed by tetrodotoxin, a blocker of voltage-dependent Na+ channels. Hence, mesencephalic sensory trigeminal neurones may not only possess postsynaptic P2X receptors at their cell somata (Tables 1, 2) but also presynaptic P2X receptors at their terminals projecting to the motor trigeminal nucleus (Khakh and Henderson 1998). The activation of these presynaptic receptors increased spontaneous glutamate release.

In synaptic terminals isolated from the rat pituitary, ATP released vasopressin and evoked an increase in intracellular Ca2+, which was sensitive to suramin and PPADS, was abolished by the withdrawal of extracellular Ca2+ and was not affected by the blockade of voltage-operated Ca2+ channels (Troadeck et al. 1998). Hence, magnocellular neurosecretory neurones may possess P2X receptors at their cell bodies contained i.a. in the hypothalamic supraoptic nucleus (Tables 1, 2), as well as at their neurohypophyseal terminals where they may be involved in neuropeptide secretion.

In a rat spinal cord slice preparation, electrical stimulation of terminals from dorsal root ganglia evoked excitatory postsynaptic currents in dorsal horn neurones. These EPSCs were abolished by a mixture of antagonists at the AMPA/kainate- (6-cyano-7-nitroquinazoline-2,3-dione, CNQX) and NMDA-type glutamate receptors (2-amino-5-phosphonopentanoic acid, AP-5) but were also diminished by PPADS (Li et al. 1998). This can be taken as an indication that glutamate is the main transmitter between sensory afferent terminals and dorsal horn neurones, in agreement with the finding that only a small proportion of these latter cells (<5%) was sensitive to exogenously applied ATP (Bardoni et al. 1997). Nevertheless, this finding also suggests that co-released ATP might have modulated glutamate release via presynaptic P2 receptors situated at the sensory nerve terminals. These presynaptic receptors are most likely P2X receptors as shown in a co-culture preparation, where spinal cord dorsal horn neurones formed synaptic contacts with neurones from dorsal root ganglia. In this preparation, ATP, as well as α,β-meATP, increased the frequency of spontaneous glutamatergic EPSCs recorded from spinal cord dorsal horn neurones. The effects of the two P2 receptor agonists were prevented by withdrawal of extracellular Ca2+ or co-application of PPADS but were partially resistant to the blockade of voltage-operated Ca2+ channels (Gu and MacDermott 1997). Hence, sensory neurones possess P2X receptors at their cell bodies, e.g. in dorsal root ganglia (Tables 1, 2), at their central terminals in the dorsal horn of the spinal cord (P2X2, P2X3 and P2X4 proteins were described at this location; see preceding section) and most likely also at their peripheral endings (P2X1 immunoreactivity was also observed in nerve fibres terminating in the epidermis; Vulchanova et al. 1998).

The frequency of spontaneous glycinergic inhibitory postsynaptic currents (IPSCs) in rat substantia gelatmosa neurones of the spinal cord was increased by ATP and 2-MeSATP but not α,β-meATP (Rhee et al. 2000). This effect was antagonized by suramin and PPADS; it was also eliminated in a Ca2+-free extracellular medium. Hence, presynaptic P2X receptors of glycinergic terminals ending at substantia gelatmosa neurones appear to depress the excess excitability and thereby decrease overactive pain signaling. The frequency of spontaneous gabaergic IPSCs in neurones cultured from laminae I–III of the neonatal rat dorsal horn was also increased by ATP (Hugel and Schlichter 2000). Again, this effect was antagonized by suramin and PPADS; it was eliminated in a Ca2+-free extracellular medium, but not by depletion of intracellular Ca2+ stores. Hence, presynaptic P2X receptors of gabaergic interneurones in the dorsal horn of the spinal cord may also be involved in the processing of nociceptive information.

In neurones, either cultured from rat superior cervical or thoracolumbar sympathetic ganglia, ATP evoked the release of previously incorporated [3H]noradrenaline (Boehm 1994; von Kügelgen et al. 1997). This effect depended on the presence of extracellular Ca2+, and was only partially inhibited, when action potential propagation or voltage-operated Ca2+ channels were blocked (Boehm 1999; von Kügelgen et al. 1999a). Moreover, ATP released noradrenaline even in “axonal” cultures, where cell bodies had been removed (Boehm 1999; von Kügelgen et al. 1999b). Hence, postganglionic sympathetic neurones appear to possess P2X receptors at their varicosities, which may modulate noradrenaline release in addition to the P2X receptors situated in the somatodendritic region (Tables 1, 2).

Extracellular ATP potentiated the frequency of spontaneous synaptic currents recorded in Xenopus nerve-mus-
cle cultures from postsynaptic muscle cells; this effect was mediated by the influx of Ca$^{2+}$ through the plasma membrane (Fu and Poo 1991). Moreover, ATP, as well as $\alpha,\beta$-meATP, evoked rapid cationic inward currents, sensitive to suramin, in terminals of calyx-type giant synapses from the chicken ciliary ganglion (Sun and Stanley 1996). Taken together, these observations indicate that postsynaptic P2X receptors also occur at cholinergic synapses, where they may modulate acetylcholine release.

In conclusion, biochemical and functional studies indicate the likely existence of release-facilitating presynaptic P2X receptors, which are potential targets of co-transmitter ATP. Eventually the question arises which P2X subunit(s) may contribute to the assembly of presynaptic P2X receptors. From the available data, no definite conclusion can be drawn. However, receptors at nerve terminals in the motor trigeminal nucleus, neuropituitary and dorsal horn of the spinal cord were sensitive to both $\alpha,\beta$-meATP and PPADS ($\leq 100\, \mu$M), and only marginally desensitised (Gu and McDermott 1997; Khakh and Henderson 1998; Troudec et al. 1998); these properties do not match those of recombinant homomeric P2X receptors (Table 3). Hence, presynaptic P2X receptors, at least at some nerve terminals, may be heteromeric receptors (i.e. P2X$_{4/6}$ or P2X$_{1/5}$).

#### Developmental influences

**P2X receptor mRNA expression**

Morphological studies describe either an age-dependent increase or decrease of P2X receptor expression in the rat nervous system. Thus, P2X$_1$ as well as P2X$_2$ mRNA was found at significantly lower levels in brains as well as nucleus and superior cervical ganglia of adult rats in comparison with those taken from 5-day-old animals (Kidd et al. 1995). Similarly, rat brain P2X$_3$ receptor immunoreactivity declined during early postnatal development (Kidd et al. 1998). In contrast to these findings, P2X$_2$ mRNA in the rat spinal ganglion was not detected before postnatal day 8 (Brändle et al. 1999).

**Functional studies**

A limited set of functional data indicates that neuronal P2X receptor expression may be influenced by developmental factors. Thus, 2MeSATP-evoked P2X receptor-mediated inward currents of locus coeruleus neurons contained in brain slices were much larger in 18- to 23-day-old than in 8- to 14-day-old rats. In contrast, $\alpha_2$-adrenoeceptor-mediated K$^+$ currents were similar in both groups of animals (Wirkner et al. 1998). Hence, in the locus coeruleus, the expression of P2X, but not of $\alpha_2$-adrenoeceptors, seems to be up-regulated during postnatal development.

A second example is provided by neurones of rat dorsal root ganglia. In cells cultured from neonatal rats (1–6 days old), uridine 5’-triphosphate (UTP) evoked rapid inward currents which cross-desensitised with ATP and were inhibited by suramin and, hence, were mediated by P2X receptors (Robertson and al. 1996; Rae et al. 1998). However, when neurones were prepared from adult rats, ATP remained active, while UTP had no effect (Grubb and Evans 1999). The reason for this development-dependent change in P2X receptor properties is unclear, because all hitherto known recombinant P2X subunits are either insensitive to UTP or are only weakly activated by this agonist (for P2X$_1$ see Ralevic and Burnstock 1998).

Another example relates to glycinergic nerve terminals projecting to substantia gelatinsa neurones of the spinal cord (Jang et al. 2000). In acutely isolated substantia gelatinosa cells with attached glycinergic nerve endings, ATP increased the frequency of spontaneous glycinergic inhibitory currents with a potency which was the same irrespective of the age of the rats (10–30 days postnatally). However, the structural analogue $\alpha,\beta$-meATP was inactive in the younger animals (see also Rhee et al. 2000) and became gradually active in the older animals. Hence, either the properties of the originally present P2X receptors underwent an age-dependent modulation with respect to its $\alpha,\beta$-meATP-sensitivity or a new $\alpha,\beta$-meATP-sensitive subunit or subunit-assembly became expressed in the older rats.

All these observations from biochemical and functional studies indicate that neuronal P2X receptors undergo developmental regulation. However, their expression seems to be regulated differentially in discrete regions of the nervous system.

#### Species differences

**Distribution of P2X receptor proteins**

There are only limited data pertaining to species differences in the tissue distribution of P2X receptor subunits. A study which compared the distribution of P2X$_2$ and P2X$_3$ proteins between rat and monkey (Macaque mutlatta) may support this notion. In contrast to the rat, in the monkey spinal cord dorsal horn P2X$_3$ immunoreactivity could not be detected, and also in contrast to the rat, in the monkey nucleus of the solitary tract, neither P2X$_2$ nor P2X$_3$ could be found. Since the antibodies used were raised against rat but not monkey P2X subunits, the possibility remains that a technical limitation rather than a real interspecies difference in P2X subunit distribution is responsible for the findings (Vulchanova et al. 1997).

Our knowledge on the localisation of P2X receptor subunits in humans is only slowly developing. To date, the human orthologues of P2X$_1$, P2X$_2$, P2X$_3$, P2X$_4$, and P2X$_7$, as well as of a truncated form of P2X$_5$, have been cloned (Valera et al. 1995; Garcia-Guzman et al. 1997a, 1997b; Lê et al. 1997; Rassendren et al. 1997; Lynch et al. 1999; Jones et al. 2000). Judged from RT-PCR experiments, h(uman) P2X$_2$ receptors including hP2X$_2$ splice variants and hP2X$_4$ are found in human brain, as well as...
spinal cord tissues (Garcia-Guzman et al. 1997a, 1997b; Lynch et al. 1999). hP2X_3 was found only in the spinal cord (Garcia-Guzman et al. 1997a), whereas hP2X_5 occurred in a number of nervous tissues including cerebral cortex, caudate/putamen, amygdala, hippocampus, thalamus, substantia nigra, cerebellum and spinal cord (Lê et al. 1997), in a striking difference to the restricted expression of rat P2X_5 (Table 1). hP2X_7 was cloned from monocytes (Rassendren et al. 1997) and until yet, nothing is known about its distribution outside the immune system.

Functional studies

Indications for interspecies differences in P2X receptor properties were found at the level of recombinant receptors and also at the level of native neuronal P2X receptors. Thus, when functionally expressed, the pharmacological properties of the human orthologues hP2X_1, hP2X_2, and hP2X_7, were similar to those found in their rodent counterparts (Valera et al. 1995; Garcia-Guzman et al. 1997a; Lynch et al. 1999). In contrast, hP2X_4 differed from r(at)P2X_4 by its lower sensitivity to the agonist α,β-meATP and its higher sensitivity to the antagonist PPADS (Garcia-Guzman et al. 1997b). hP2X_5 did not assemble into functional ATP-gated channels (Lê et al. 1997) and hP2X_7 showed a lower sensitivity to agonists, as well as lower tendency to form cytolytic pores than rP2X_7 (Rassendren et al. 1997). There are also dissimilarities between P2X receptors from different rodent species. The recombinant mouse P2X_4 receptor, for instance, displayed a higher sensitivity to the agonist α,β-meATP than rP2X_4 (Jones et al. 2000), and recombinant mouse P2X_7 receptors were markedly less sensitive to the agonist 4-benzoyl benzoyl ATP than rP2X_7 (Chessel et al. 1998).

An interspecies difference observed with native neuronal P2X receptors has already been mentioned. P2X receptors in neurons from guinea-pig coeliac ganglia were dissimilar to P2X receptors in neurons from rat and mouse coeliac ganglia by virtue of their high sensitivities to α,β-meATP (Khakh et al. 1995; Zhong et al. 2000a, 2000b; Table 2). This difference may be due to the presence of P2X_2-like and P2X_3-like receptors, respectively. Other examples relate to sensory ganglia. While bullfrog dorsal root neurones responded to ATP always with nondesensitising inward currents (Bean 1990), the greatest proportion of rat DRG neurones showed rapidly desensitising responses (Robertson et al. 1996; Rae et al. 1998; Burgard et al. 1999; Grubb and Evans 1999). Eventually, Zn^{2+} potentiated ATP-gated currents through P2X receptors expressed in rat nodose ganglion neurons (Li et al. 1996), while in bullfrog DRG neurones, ATP-gated currents were depressed by Zn^{2+} (Li et al. 1997).

Taken together, the above data indicate interspecies differences in the tissue distribution of neuronal P2X receptors may exist. Moreover, such differences were also found in functional studies at the level of recombinant, as well as native neuronal P2X receptors. Hence, one should be cautious in extrapolating pharmacological data, based largely on findings in the rat nervous system, to other species including humans.

Conclusions

Both functional and morphological data indicate a widespread distribution of P2X receptors over the neuraxis. These receptors are possible targets of ATP released as a transmitter in its own right or more likely as a co-transmitter of e.g. noradrenaline or acetylcholine. The present review highlights a number of pertinent questions related to neuronal P2X receptors, which belong to the group of ligand-gated ion channels. Firstly, functional differences between the characteristics of native and recombinant P2X receptor channels may be due to the fact that these receptors occur as hetero- rather than homo-oligomers. Further differences may be resolved by the assumption that up to three different subunits may assemble to form a hetero-oligomer. Secondly, the complexity of P2X receptor-mediated synaptic responses may be explained by the existence of these receptors both at somatodendritic and presynaptic sites. Somatodendritic receptors are usually excitatory (both P2X and P2Y), while presynaptic receptors are excitatory (P2X) or inhibitory (P2Y). Furthermore, the same neurone may bear a combination of P2X, P2Y and adenosine-sensitive P1 receptors which can mediate opposing responses. The net effect depends on the sum of the individual components. Thirdly, both native and recombinant receptors may show large interspecies and developmental differences with respect to their agonist- and antagonist-sensitivities.

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