Naunyn-Schmiedeberg's Arch Pharmacol (2000) 362 : 324–339
DOI 10.1007/s002100000311

REVIEW ARTICLE

**Wolfgang Nörenberg · Peter Illes**

# Neuronal P2X receptors: localisation and functional properties

Published online: 17 August 2000


**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.

W. Nörenberg (✉) · P. Illes
Rudolf-Boehm-Institut für Pharmakologie und Toxikologie,
Medizinische Fakultät der Universität Leipzig,
Härtelstrasse 16–18, D-04107 Leipzig, Germany
e-mail: noerw@medizin.uni-leipzig.de,
Fax: +49-341-9724609

## 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 ($P2X_2$; 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 $P2X_2$ 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 $P2X_2$ and $P2X_3$ heterologously expressed in HEK293 cells (Lewis et al. 1995). ATP-gated currents, closely resembling those seen with the $P2X_{2/3}$ 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 $P2X_2$ and $P2X_3$ subunits (Table 1).

The tissue distribution of the seven P2X receptors ranges from abundant to discrete. Thus, $P2X_2$, $P2X_4$, and $P2X_6$ mRNAs or proteins have been detected throughout the brain and spinal cord, as well as in sensory and autonomic ganglia (Table 1). $P2X_1$ receptors were initially believed to be absent in the brain (Valera et al. 1994). However, later on it turned out that $P2X_1$ mRNA is localised in the cerebral cortex, striatum, hippocampus and cerebellum, where $P2X_1$ subunit proteins have also been detected (Table 1). Outside the brain, $P2X_1$ 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 $P2X_3$ 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, $P2X_3$ 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, $P2X_3$ was also found in the hypothalamic supraoptic nucleus, as well as in autonomic sympathetic ganglia (Table 1).

$P2X_5$ also showed a restricted localisation. In the CNS, $P2X_5$ mRNA was found only in the mesencephalic nucleus of the trigeminal nerve at which propioceptive primary afferents from muscles of mastication terminate, and in motoneurones of the spinal cord ventral horn (Table 1). In the peripheral nervous system, $P2X_5$ mRNA, $P2X_5$ 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 $P2X_5$ proteins (Xiang et al. 1998b).

It is generally believed that $P2X_7$ is not expressed in neuronal cells although it was originally cloned from superior cervical ganglia and brain tissue (Surprenant et al. 1996). Since $P2X_7$ 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 $P2X_2$ and $P2X_4$ 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 $P2X_7$ receptor (Virginio et al. 1999). Indeed, $P2X_7$ 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 $P2X_7$ 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 ($Na^+$, $K^+$, $Ca^{2+}$) 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-

**Table 1** Distribution of P2X receptor subunits in the rat nervous system. P2X mRNA was detected by in situ hybridisation using P2X-subtype-specific riboprobes (+) or by RT-PCR products from isolated cells (+++), and P2X proteins were detected by immunocytochemistry using subtype-specific antibodies (++; +? cerebellar cell layer not defined). Please note that the data given for the distribution of P2X subunits in brain were limited to those regions also mentioned elsewhere in the text. For more complete information see Collo et al. (1996) and Kanjhan et al. (1999)

| | $P2X_1$ | $P2X_2$ | $P2X_3$ | $P2X_4$ | $P2X_5$ | $P2X_6$ | $P2X_7$ |
|---|---|---|---|---|---|---|---|
| Brain | | | | | | | |
| Olfactory bulb | – | +[5]; ++[2, 7] | – | +[1, 6, 19]; ++[4] | – | +[1] | – |
| Cerebral cortex | +[3] | +[1–3, 5]; ++[2] | – | +[1, 6, 16]; ++[4] | – | +[1] | – |
| Caudate/putamen | +[3] | +[2, 3, 5]; ++[2] | – | +[1], ++[4] | – | +[1] | – |
| Hippocampal field CA1–CA3 | +[3] | +[2, 3, 5]; ++[2] | – | +[1, 6, 16, 19]; ++[4] | – | +[1] | – |
| Medial habenula nucleus | – | +[2, 3, 5]; ++[2] | – | +[1] | – | +[1] | – |
| Supraoptic nucleus | – | +[1, 2, 5]; ++[2, 12] | +[15] | +[1, 15]; ++[4] | – | +[1] | – |
| Tuberomammillary nucleus | – | +[2]; ++[2, 12] | – | +[1] | – | +[1] | – |
| Substantia nigra zona compacta | – | +[1, 2]; ++[2, 7] | – | +[1] | – | +[1] | – |
| Ventral tegmental area | – | +[1, 2]; ++[2, 7] | – | +[1] | – | +[1] | – |
| Mesencephalic trigeminal nucleus | – | +[2, 5]; ++[2] | – | +[1]; ++[4] | +[1] | +[1] | – |
| Locus coeruleus | – | +[1, 2, 5]; ++[2, 7] | – | +[1] | – | +[1] | – |
| Motor trigeminal nucleus | – | +[2]; ++[2] | – | +[1, 16] | – | +[1] | – |
| Vestibular nuclei | – | +[2]; ++[2] | – | – | – | – | – |
| Dorsal motor nucleus of vagus | – | +[1, 2]; ++[2] | – | +[1] | – | +[1] | – |
| Hypoglossal nucleus | – | +[1, 2]; ++[2] | – | +[1] | – | +[1] | – |
| Solitary tract nucleus | – | +[2]; ++[2, 7, 8] | ++[8, 14] | +[1] | – | +[1] | – |
| Rostral ventrolateral medulla | – | +[2]; ++[2] | – | – | – | – | – |
| Cerebellar Purkinje cells | +[3?]; ++[10] | +[2, 17]; ++[2, 17] | – | +[1, 6,16, 19]; ++[4] | – | +[1] | – |
| Cerebellar granular layer | +[3?]; ++[10] | +[2, 17]; ++[2, 17] | – | +[1, 6, 16]; ++[4] | – | +[1] | – |
| Spinal cord | | | | | | | |
| Dorsal horn | ++[7] | +[1]; ++[2, 7, 8] | ++[8, 14] | +[1]; ++[4] | – | +[1] | – |
| Ventral horn | +[1] | +[1]; ++[2] | – | +[1, 6, 19] | +[1] | +[1] | – |
| Intermediolateral region | – | ++[2, 8] | – | – | – | – | – |
| Sensory systems | | | | | | | |
| Trigeminal ganglion | +[1]; ++[9] | ++[9] | +[1, 15]; ++[9, 14] | ++[9] | +[1]; ++[9] | +[1]; ++[9] | – |
| Nodose ganglion | +[3, 13]; ++[9] | +[3, 5, 13]; ++[7–9] | +[1, 13]; ++[8, 9] | +[13, 16];++[8, 9] | ++[9] | ++[9] | – |
| Dorsal root ganglion | +[1, 13]; ++[9] | +[5, 13]; ++[8, 9] | +[1, 13]; ++[8, 9] | +[13]; ++[9] | +[1]; ++[9] | +[1]; ++[9] | – |
| Retinal ganglion cells | – | +[18]; ++[18] | – | – | – | – | ++[22] |
| Taste buds | – | ++[21] | ++[21] | – | – | – | – |
| Cochlear spiral ganglion | ++[24] | +[11, 25]; ++[24]; +++[23] | ++[24]; +++[23] | ++[24]; +++[23] | ++[24] | ++[24] | +++[23] |
| Autonomic ganglia | | | | | | | |
| Superior cervical | +[1, 3]; ++[9] | +[1, 3, 5, 20]; ++[9] | ++[9] | +[1, 16]; ++[9] | – | +[1]; ++[9] | – |
| Coeliac | +[1]; ++[9] | ++[9] | ++[9] | ++[9] | – | +[1]; ++[9] | – |
| Enteric system | | | | | | | |
| Myenteric plexus | – | ++[7] | – | – | – | – | – |

References: [1]Collo et al. 1996; [2]Kanjhan et al. 1999; [3]Kidd et al. 1995; [4]Lê et al. 1998b; [5]Simon et al. 1997; [6]Soto et al. 1996; [7]Vulchanova et al. 1996; [8]Vulchanova et al. 1997; [9]Xiang et al. 1998b; [10]Loesch and Burnstock 1998; [11]Salih et al. 1998; [12]Xiang et al. 1998a; [13]Lewis et al. 1995; [14]Llewellyn-Smith and Burnstock 1998; [15]Shibuya et al. 1999; [16]Séguéla et al. 1996; [17]Khanjhan et al. 1996; [18]Greenwood et al. 1997; [19]Bo et al. 1995; [20]Brake et al. 1994; [21]Bo et al. 1999; [22]Brändle et al. 1998a; [23]Brändle et al. 1999; [24]Xiang et al. 1999; [25]Housley et al. 1998

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 outweighted 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 2** Functional characterisation of neuronal P2X receptors. P2X receptors were characterised in cell cultures, tissue slices or at the whole animal level either by microfluometric measurements (*MFM*) of intracellular free $Ca^{2+}$ (*$[Ca^{2+}]_i$*), whole-cell patch clamp (*WPC*), extracellular (*ECR*) and intracellular recordings (*ICR*) or microinjection (*MIJ*) of P2X agonists into identified central nervous system regions (*2MeSATP* 2-methylthio ATP, *α,βme-ATP* α,βmethylene ATP, *EPSC* excitatory postsynaptic current, *PPADS* pyridoxalphosphate-6-azophenyl-2',4'-disulfonic acid, *PSP* excitatory postsynaptic potential)

| | Preparation | Method | Effect | Agonists | Antagonists |
|---|---|---|---|---|---|
| Brain | | | | | |
| Cerebral cortex | Slice, rat[1, 2] | MFM | $[Ca^{2+}]_i\uparrow$ | ATP >> α,β-meATP | PPADS |
| Caudate/putamen | Slice, rat[3] | WPC | No effect | ATP | |
| Hippocampus | Culture, rat[4] | WPC | Inward current | 2MeSATP > ATP > α,β-meATP | Suramin |
| | Slice, rat[5] | WPC | Block of EPSC | | PPADS |
| Medial habenula nucleus | Slice, rat[6] | WPC | Block of EPSC | | Suramin |
| Hypothalamus | Culture, rat[7] | MFM | $[Ca^{2+}]_i\uparrow$ | ATP > α,βme-ATP | Suramin |
| Supraoptic nucleus | In vivo, rat[8] | ECR | Firing↑ | ATP = α,βme-ATP | Suramin |
| | Culture, rat[9] | ICR | Depolarization | α,βme-ATP > ATP > 2MeSATP | PPADS |
| | Slice, rat[10] | MFM | $[Ca^{2+}]_i\uparrow$ | 2MeSATP ≥ ATP >> α,βme-ATP | Suramin, PPADS |
| | Culture, rat[10] | WPC | Inward current | ATP | |
| Tuberomammillary nucleus | Culture, rat[11] | WPC | Inward current | ATP ≥ 2MeSATP >> α,βme-ATP | |
| Substantia nigra | Culture, rat[12] | WPC | Inward current | ATP | |
| Ventral tegmental area | Slice, rat[13] | ICR | No effect | 2MeSATP, α,βme-ATP | |
| Mesencephalic trigeminal nucleus | Slice, rat[15] | WPC | Inward current | ATP = α,βme-ATP | Suramin |
| Locus coeruleus | Slice, rat[14, 16–21] | ECR | Firing↑ | 2MeSATP = α,βme-ATP | Suramin, PPADS |
| | | ICR | Depolarization | ATP, α,βme-ATP | |
| | | | Block of PSP | | Suramin, PPADS |
| | | WPC | Inward current | 2MeSATP > ATP > α,βme-ATP | |
| Medial vestibular nucleus | Slice, rat[22] | ECR | Firing↑ | α,βme-ATP | Suramin, PPADS |
| Dorsal motor nucleus of vagus | Culture, rat[12] | WPC | Inward current | ATP, α,βme-ATP inactive | Suramin |
| Hypoglossal nucleus | Slice, mouse[23] | WPC | Inward current | ATP | Suramin |
| Solitary tract nucleus | Culture, rat[12, 24] | WPC | Inward current | ATP, 2MeSATP >> α,βme-ATP | |
| | In vivo, rat[25] | MIJ | Blood pressure↓ | α,βme-ATP ≥ ATP, 2MeSATP | Suramin |
| Ventrolateral medulla | In vivo, rat[26] | ECR | Firing↑ | ATP, α,βme-ATP | Suramin |
| | | MIJ | Blood pressure↓ | ATP | |
| | In vivo, rat[27] | MIJ | $CO_2$ sensitivity↓ | α,βme-ATP | Suramin |
| | In vivo, rabbit[28] | MIJ | Blood pressure↑↓ | α,βme-ATP | Suramin |
| Cerebellar Purkinje cells | Culture, rat[29, 30] | MFM | $[Ca^{2+}]_i\uparrow$ | ATP > 2MeSATP, α,βme-ATP inactive | Suramin, PPADS |
| Cerebellar granule cells | Culture, rat[29] | MFM | No effect | ATP, α,βme-ATP | |
| Spinal cord | | | | | |
| Dorsal horn | Slice, hamster[31] | WPC | Inward current | ATP | Suramin |
| | Culture, rat[32, 33] | WPC | Inward current | ATP | Suramin, PPADS |
| | | | Block of EPSC | | Suramin, PPADS |
| | | MFM | $[Ca^{2+}]_i\uparrow$ | ATP, α,βme-ATP inactive | Suramin, PPADS |
| | Slice, rat[32] | WPC | Block of EPSC | | Suramin, PPADS |
| Sensory systems | | | | | |
| Nodose ganglion | Culture, rat[34] | WPC | Inward current | ATP > α,βme-ATP | |
| | Culture, guinea pig[35] | WPC | Inward current | 2MeSATP ≥ ATP ≥ α,βme-ATP | Suramin, PPADS |

**Table 2** (continued)

| | Preparation | Method | Effect | Agonists | Antagonists |
|---|---|---|---|---|---|
| Dorsal root ganglion | Culture, rat[36–42] | WPC | Inward current | ATP = 2MeSATP ≥ α,βme-ATP | Suramin, PPADS |
| Retinal ganglion cells | Culture, rat[43] | WPC | Inward current | ATP, α,βme-ATP | Suramin |
| | | MFM | $[Ca^{2+}]_i\uparrow$ | ATP | |
| Mesenteric vagal afferent nerves | In vivo, rat[44] | ECR | Firing↑ | ATP, α,βme-ATP | Suramin, PPADS |
| Autonomic, sympathetic ganglia | | | | | |
| Superior cervical ganglion | Culture, rat[35, 45] | WPC | Inward current | ATP = 2MeSATP, α,βme-ATP inactive | Suramin, PPADS |
| Thoracolumbar ganglia | Culture, rat[46–48] | MFM | $[Ca^{2+}]_i\uparrow$ | ATP | |
| | | WPC | Inward current | ATP >> α,βme-ATP | Suramin, PPADS |
| | | | Depolarization | ATP | |
| Coeliac ganglion | Culture, guinea pig[35, 49, 50] | WPC | Inward current | 2MeSATP ≥ ATP ≥ α,βme-ATP | Suramin, PPADS |
| | Culture, rat[51] | WPC | Inward current | ATP, α,βme-ATP inactive | Suramin, PPADS |
| | Culture, mouse[51] | WPC | Inward current | ATP, α,βme-ATP inactive | Suramin, PPADS |
| Autonomic, parasympathetic ganglia | | | | | |
| Cardiac ganglia | Culture, rat[52] | WPC | Inward current | 2MeSATP = ATP >> α,βme-ATP | |
| Enteric system | | | | | |
| Myenteric plexus | Culture, guinea pig[53–55] | WPC | Inward current | ATP ≥ 2MeSATP >> α,βme-ATP | PPADS |

References: [1]Lalo et al. 1998; [2]Lalo and Kostyuk 1998; [3]Nörenberg et al. 1997; [4]Balachandran and Bennett 1996; [5]Pankratov et al. 1998; [6]Edwards et al. 1992; [7]Chen et al. 1994; [8]Day et al. 1993; [9]Hiruma and Bourque 1995; [10]Shibuya et al. 1999; [11]Furukawa et al. 1994; [12]Nabekura et al. 1995; [13]Poelchen et al. 1998; [14]Shen and North 1993; [15]Khakh et al. 1997; [16]Tschöpl et al. 1992; [17]Harms et al. 1992;[18]Fröhlich et al. 1996; [19]Nieber et al. 1997; [20]Sansum et al. 1998; [21]Wirkner et al. 1998; [22]Chessel et al. 1997; [23]Funk et al. 1997; [24]Ueno et al. 1992; [25]Ergene et al. 1994; [26]Sun et al. 1992; [27]Thomas et al. 1999; [28]Horiuchi et al. 1999; [29]Mateo et al. 1998; [30]García-Lecea et al. 1999; [31]Li and Perl 1995; [32]Bardoni et al. 1997; [33]Jo and Schlichter 1999; [34]Thomas et al. 1998; [35]Khakh et al. 1995; [36]Bean 1990; [37]Robertson et al. 1996; [38]Rae et al. 1998; [39]Stebbing et al. 1998; [40]Ueno et al. 1999; [41]Grubb and Evans 1999; [42]Burgard et al. 1999; [43]Taschenberger et al. 1999; [44]Kirkup et al. 1999; [45]Boehm 1999; [46]Von Kügelgen et al. 1997; [47]Von Kügelgen et al. 1999a; [48]Nörenberg et al. 1999; [49]Evans et al. 1992; [50]Silinsky and Gerzanich 1993; [51]Zhong et al. 2000a; [52]Fieber and Adams 1991; [53]Barajas-López et al. 1993; [54]Zhou and Galligan 1996; [55]Barajas-López et al. 1996

are taken. Hence, $Ca^{2+}$ entry through voltage-gated $Ca^{2+}$ channels, as well as depletion of intracellular $Ca^{2+}$ stores, should be avoided. Under these conditions, P2X receptors would increase the intracellular free $Ca^{2+}$ concentration (measured by $Ca^{2+}$-sensitive fluorescent dyes such as fura-2/AM) in a manner absolutely dependent on extracellular $Ca^{2+}$. 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 (Tschöpl 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 ($EC_{50}$≥100 μM) or even inactive at recombinant $P2X_2$ and $P2X_{4–7}$ 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 1994) before it reached its site of action. Alternatively, 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 ($P2X_1$) tissues functionally expressed as homomeric ($P2X_{1–7}$) or heteromeric receptors ($P2X_{2/3}$, $P2X_{1/5}$, $P2X_{4/6}$). P2X cDNAs were microinjected into Xenopus oocytes (Brake et al. 1994; Valera et al. 1994; Bo et al. 1995; Chen et al. 1995; Evans et al. 1995; Séguéla et al. 1996; Soto et al. 1996; Wang et al. 1996; Simon et al. 1997; Lê et al. 1998a; Lê 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

| | Agonists[a] | Antagonists[a] | Desensitization[b] |
|---|---|---|---|
| $P2X_1$[1–4] | ATP (0.9 μM) ≥ 2MeSATP (1 μM) > α,β-meATP (2.2 μM) | PPADS (1 μM) = suramin (1 μM) | Rapid |
| $P2X_2$[2–5] | 2MeSATP (3 μM) > ATP (8 μM); α,β-meATP inactive[c] | PPADS (1 μM) > suramin (8 μM) | Slow |
| $P2X_3$[4, 7, 8] | 2MeSATP (0.3 μM) > ATP (0.6 μM) ≥ α,β-meATP (0.7 μM) | PPADS (1.5 μM) > suramin (3 μM) | Rapid |
| $P2X_4$[9–13] | ATP (5.5 μM) >> 2MeSATP[d] (12.7 μM) >> α,β-meATP | PPADS (100 μM) > suramin (>100 μM) | Slow |
| $P2X_5$[14, 15] | ATP (15.4 μM) > 2MesATP (20 μM); α,β-meATP inactive[c] | PPADS (2.6 μM) > suramin (4 μM) | Slow |
| $P2X_6$[14] | 2MeSATP (9 μM) > ATP (12 μM); α,β-meATP inactive[c] | PPADS (>100 μM); suramin (>100 μM) | Slow |
| $P2X_7$[16, e] | ATP[d] (115 μM) > 2MeSATP[d]; α,β-meATP inactive[c] | PPADS (45 μM) > suramin (>300 μM) | Slow |
| $P2X_{2/3}$[4, 8] | 2MeSATP (1 μM) > ATP (2 μM) > α,β-meATP (3 μM) | 80% inhibition by 10 μM PPADS | Slow |
| $P2X_{1/5}$[17–19] | ATP (0.7 μM) > 2MeSATP (1.3 μM) > α,β-meATP[d] (3.1 μM) | PPADS (0.6 μM) > suramin (1.6 μM) | Biphasic |
| $P2X_{4/6}$[20] | ATP (6.3 μM) ≥ 2MeSATP[d] (7.7 μM) > α,β-meATP[d] (12 μM) | 40% inhibition by 10 μM PPADS and 10 μM suramin | Slow |

[a]Numbers *in parentheses* are $EC_{50}$ and $IC_{50}$ values of agonists and antagonists, respectively, where available
[b]Desensitisation is used descriptively for the decay in current amplitude during continuous agonist application ($EC_{90}$ to $EC_{100}$) 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 $P2X_{1/5}$ heterooligomer displayed a composite phenotype consisting of a transient peak current followed by a sustained plateau
[c]No effect at 100 μM
[d]Partial agonist
[e]Measured in the presence of extracellular divalent cations (4-benzoyl benzoyl ATP is a more potent full agonist with an $EC_{50}$ of 7 μM)
References: [1]Valera et al. 1994; [2]Evans et al. 1995; [3]Surprenant 1996; [4]Evans and Surprenant 1996; [5]Brake et al. 1994; [6]Simon et al. 1997; [7]Chen et al. 1995; [8]Lewis et al. 1995; [9]Bo et al. 1995; [10]Séguéla et al. 1996; [11]Soto et al. 1996; [12]Wang et al. 1996; [13]Jones et al. 2000; [14]Collo et al. 1996; [15]Garcia-Guzman et al. 1996; [16]Surprenant et al. 1996; [17]Torres et al. 1998; [18]Haines et al. 1999; [19]Lê et al. 1999; [20]Lê et al. 1998a

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,βme-ATP, 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 tegemental area neurones in their termination field in the nucleus accumbens (Krügel et al. 1999). Hence, a possible explanation may be that in ventral tegemental 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 $P2X_1$ 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 receptor-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 during continuous agonist application) or rundown (decay of the response upon repeated agonist application) as e.g. demonstrated in neurones from the cerebral cortex (Lalo 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 (Tschöpl 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, $P2X_2$, $P2X_5$, $P2X_6$ and $P2X_7$ were insensitive to α,β-meATP but only $P2X_2$ and $P2X_5$ were blocked by suramin and PPADS at low micromolar concentrations (Table 3). Since $P2X_5$ 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, $P2X_2$ 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 ($P2X_1$, $P2X_3$) or were rather insensitive to suramin and PPADS ($P2X_4$; 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 $P2X_1$, $P2X_2$ and $P2X_3$ 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 $P2X_4$ and $P2X_6$ subunits can co-assemble into functional ATP-gated channels (Lê et al. 1998a). $P2X_6$, 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). $P2X_4$ 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). $P2X_{4/6}$ heteromeric receptors were activated by intermediate concentrations of α,βme-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 $P2X_{4/6}$, in good agreement with the abundant occurrence and co-localisation of $P2X_4$/$P2X_6$ 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 sympathoexcitatory or sympathoinhibitory neurones were activated (Horiuchi et al. 1999). These effects were antagonised by suramin (Table 2). Hence, α,β-meATP-sensitive, $P2X_{4/6}$-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 $P2X_4$, $P2X_6$ or $P2X_7$ receptors (Table 3), although $P2X_4$, $P2X_6$ and $P2X_7$ subunits have been detected in rat sensory ganglia, and $P2X_4$ as well as $P2X_6$ 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 $P2X_1$–$P2X_6$ (Table 1). Up to date, in rat retinal ganglion cells only $P2X_2$ subunit proteins have been demonstrated unequivocally (Table 1) but RT-PCR experiments showed the additional presence of $P2X_3$–$P2X_5$ 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 $P2X_1$/$P2X_4$, $P2X_2$/$P2X_4$, $P2X_3$/$P2X_4$, $P2X_3$/$P2X_6$, or for any combination containing $P2X_7$ subunits could not be detected (Torres et al. 1999).

With regard to the hitherto functionally expressed recombinant heteromeric receptors, either $P2X_{2/3}$ or $P2X_{4/6}$ may account for the slowly desensitising P2X receptors in sensory ganglia (Table 3). However, two observations strongly argue against $P2X_{4/6}$. 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 $P2X_{4/6}$ 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 $P2X_{4/6}$ receptors (Lê et al. 1998a).

Hence, $P2X_{2/3}$ 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 $P2X_2$ and $P2X_3$ 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 $P2X_{2/3}$ antagonist trinitrophenyl-ATP (Virginio 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 $P2X_2$ and heteromeric $P2X_{2/3}$ but not fast desensitising homomeric $P2X_3$ 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 $P2X_{2/3}$ 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, $P2X_1$, as well as $P2X_3$, fulfil the criteria required for the fast-desensitising response (Table 3). However, the receptor in dorsal root ganglia appears to be $P2X_3$, since β,γ-me-L-ATP (β,γ-methylene-L-ATP), which is a potent agonist at recombinant $P2X_1$ receptors (Evans et al. 1995), as well as at prototypic native $P2X_1$ 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 $P2X_3$ (Chen et al. 1995; Lewis et al. 1995) but not $P2X_1$ receptors (Evans and Surprenant 1996), also supports this conclusion. Accordingly, the receptor in retinal ganglion neurones may also belong to the $P2X_3$ subtype, since ocular tissue lacked $P2X_1$ mRNA (Brändle et al. 1998b). As a reason of the biphasic response seen in some dorsal root neurones, it was proposed that $P2X_3$ homomeric (fast-desensitising) together with $P2X_{2/3}$ 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 $P2X_1$ and $P2X_5$ (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 $P2X_{1/5}$ 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 $P2X_5$ and $P2X_7$ have been detected in sympathetic ganglia (Table 1). Hence, $P2X_2$ 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 ($EC_{50}$ 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 $P2X_{2/3}$ (cf. Table 3). In line with this assumption, the likely existence of functional heteromeric $P2X_{2/3}$ receptors has been demonstrated in neurones from guinea-pig superior cervical ganglia, where they may co-exist with homomeric $P2X_2$ 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 $P2X_1$ or $P2X_3$ (cf. Table 3), whereas the greater proportion of neurones may bear $P2X_2$-like receptors (Zhou and Galligan 1996), in agreement with the detection of $P2X_2$ 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 $P2X_4$ 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 $P2X_4$-mediated responses were potentiated by suramin or by two other P2 antagonists, 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 $P2X_4$ 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, $P2X_2$ and $P2X_5$ showed a high sensitivity to PPADS as well as a slow desensitisation during agonist application (Table 3). Since $P2X_4$ co-assembled with $P2X_5$ but not with $P2X_2$ (Torres et al. 1999), it would be interesting to investigate whether possible recombinant $P2X_{4/5}$ 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 $P2X_3$ and $P2X_2$ 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 $Ca^{2+}$ permeability, as well as rectification properties of the AMPA receptor heteromeric complex (Verdoorn et al. 1991; Geiger et al. 1995). The properties of the $P2X_{2/3}$ heteromeric channel, where the slowly desensitising characteristics of $P2X_2$ 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 $P2X_2$ subunit have been detected of which $P2X_{2(b)}$ (alternative name $P2X_{2\text{-}2}$) formed functional homomeric ATP-gated channels, which differed from prototypic $P2X_2$ ($P2X_{2(a)}$) in that $P2X_{2(b)}$-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 ionotropic P2X receptors together with metabotropic P2Y and P1 (adenosine) receptors which may also influence the neuronal response to ATP (e.g. locus coeruleus; Tschöpl 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 somatodentritic 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 $Ca^{2+}$ 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 $P2X_1$, $P2X_2$, $P2X_3$ and $P2X_4$ subunits.

By means of the first approach, $P2X_1$ 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, $P2X_3$ 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), $P2X_4$ 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 $P2X_2$ immunoreactivity in the nucleus of the solitary tract whereas ipsilateral removal of the dorsal root ganglia decreased the intensity of $P2X_2$ and $P2X_3$ 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 $Ca^{2+}$ in synaptic terminals prepared from the human cortex, which was absolutely dependent on transmembrane $Ca^{2+}$ entry and was antagonised by PPADS, but only partially affected by the blockade of voltage-operated $Ca^{2+}$ 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 $Ca^{2+}$, which was sensitive to suramin and PPADS, was abolished by the withdrawal of extracellular $Ca^{2+}$ and was not affected by the blockade of voltage-operated $Ca^{2+}$ channels (Troadec 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-nitroquinoxaline-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 $Ca^{2+}$ or co-application of PPADS but were partially resistant to the blockade of voltage-operated $Ca^{2+}$ 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 ($P2X_2$, $P2X_3$ and $P2X_4$ proteins were described at this location; see preceding section) and most likely also at their peripheral endings ($P2X_3$ 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 gelatinosa 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 $Ca^{2+}$-free extracellular medium. Hence, presynaptic P2X receptors of glycinergic terminals ending at substantia gelatinosa 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 $Ca^{2+}$-free extracellular medium, but not by depletion of intracellular $Ca^{2+}$ 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 [$^3$H]noradrenaline (Boehm 1994; von Kügelgen et al. 1997). This effect depended on the presence of extracellular $Ca^{2+}$, and was only partially inhibited, when action potential propagation or voltage-operated $Ca^{2+}$ 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 somatodentritic 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 of $Ca^{2+}$ through the plasma membrane (Fu and Poo 1991). Moreover, ATP, as well as α,β-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 presynaptic 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 α,β-meATP and PPADS (≤100 μM), and only marginally desensitised (Gu and McDermott 1997; Khakh and Henderson 1998; Troadec 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 nodose 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 spiral 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 neurones contained in brain slices were much larger in 18- to 23-day-old than in 8- to 14-day-old rats. In contrast, $\alpha_2$-adrenoceptor-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 $\alpha_2$-adrenoceptors, 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 $P2X_3$ see Ralevic and Burnstock 1998).

Another example relates to glycinergic nerve terminals projecting to substantia gelatinosa 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 α,β-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 α,β-meATP-sensitivity or a new α,β-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 mulatta*) 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 possiblity 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 $hP2X_3$ were similar to those found in their rat 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 neurones from guinea-pig coeliac ganglia were dissimilar to P2X receptors in neurones 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 $P2X_{2/3}$-like and $P2X_2$-like receptors, respectively. Other examples relate to sensory ganglia. While bullfrog dorsal root neurones responded to ATP always with non-desensitising 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 neurones (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 characterisitics 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.

**Acknowledgements** We are grateful to Dr. J. Gal for linguistic corrections.

## References

Abbracchio M, Burnstock G (1994) Purinoceptors: are there families of $P_{2X}$ and $P_{2Y}$ purinoceptors? Pharmacol Ther 64:445–475

Balachandran C, Bennett MR (1996) ATP-activated cationic and anionic conductances in cultured rat hippocampal neurons. Neurosci Lett 204:73–76

Barajas-López C, Barrientos M, Espinosa-Luna R (1993) Suramin increases the efficacy of ATP to activate an inward current in myenteric neurons from guinea-pig ileum. Eur J Pharmacol 250:141–145

Barajas-López C, Espinosa-Luna R, Gerzanich V (1994) ATP closes a potassium and opens a cationic conductance through different receptors in neurons of guinea pig submucous plexus. J Pharmacol Exp Ther 268:1397–1402

Barajas-López C, Huizinga JD, Collins SM, Gerzanich V, Espinosa-Luna R, Peres AL (1996) $P_{2X}$-purinoceptors of myenteric neurones from the guinea-pig ileum and their unusual properties. Br J Pharmacol 119:1541–1548

Bardoni R, Goldstein PA, Lee CJ, Gu JG, MacDermott AB (1997) ATP P2X receptors mediate fast synaptic transmission in the dorsal horn of the rat spinal cord. J Neurosci 17:5297–5304

Bean BP (1990) ATP-activated channels in rat and bullfrog sensory neurons: concentration dependence and kinetics. J Neurosci 10:1–10

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

Bo X, Alavi A, Xiang Z, Oglesby I, Ford A, Burnstock G (1999) Localization of ATP-gated $P2X_2$ and $P2X_3$ receptor immunoreactive nerves in rat taste buds. Neuroreport 10:1107–1111

Bo X, Schoepfer R, Burnstock G (2000) Molecular cloning and characterization of a novel ATP P2X receptor subbtype from embryonic chick skeletal muscle. J Biol Chem 275:14401–14407

Boehm S (1994) Noradrenaline release from rat sympathetic neurons evoked by $P_2$-purinoceptor activation. Naunyn-Schmiedeberg's Arch Pharmacol 350:454–458

Boehm S (1999) ATP stimulates sympathetic transmitter release via presynaptic P2X purinoceptors. J Neurosci 19:737–746

Brake AJ, Wagenbach MJ, Julius D (1994) New structural motif for ligand-gated ion channels defined by an ionotropic ATP receptor. Nature 371:519–523

Brändle U, Kohler K, Wheeler-Schilling TH (1998a) Expression of the $P2X_7$-receptor subunit in neurons of the retina. Brain Res Mol Brain Res 62:106–109

Brändle U, Guenther E, Irrle C, Wheeler-Schilling TH (1998b) Gene expression of the P2X receptors in the rat retina. Brain Res Mol Brain Res 59:269–272

Brändle U, Zenner HP, Ruppersberg JP (1999) Gene expression of P2X-receptors in the developing inner ear of the rat. Neurosci Lett 273:105–108

Burgard EC, Niforatos W, Van Biesen T, Lynch KJ, Touma E, Metzger RE, Kowaluk EA, Jarvis MF (1999) P2X receptor mediated currents in dorsal root ganglion neurons. J Neurophysiol 82:1590–1598

Burnstock G (1986) The changing face of autonomic neurotransmission. Acta Physiol Scand 126:67–91

Chen ZP, Levy A, Lightman SL (1994) Activation of specific ATP receptors induces a rapid increase in intracellular calcium ions in rat hypothalamic neurons. Brain Res 641:249–256

Chen CC, Akopian AN, Sivilotti L, Colquhoun D, Burnstock G, Wood JN (1995) A P2X purinoceptor expressed by a subset of sensory neurons. Nature 377:428–431

Chessel IP, Michel AD, Humphrey PPA (1997) Functional evidence for multiple purinoceptor subtypes in the rat medial vestibular nucleus. Neuroscience 77:783–791

Chessel IP, Simon J, Hibell AD, Michel AD, Barnard EA, Humphrey PPA (1998) Cloning and functional characterisation of the mouse $P2X_7$ receptor. FEBS Lett 439:26–30

Collo G, North RA, Kawashima E, Merlo-Pich E, Neidhart S, Surprenant A, Buell G (1996) Cloning of $P2X_5$ and $P2X_6$ receptors and the distribution and properties of an extended family of ATP-gated ion channels. J Neurosci 16:2495–2507

Collo G, Neidhart S, Kawashima E, Kosco-Vilbois M, North RA, Buell G (1997) Tissue distribution of $P2X_7$ receptors. Neuropharmacology 36:1277–1283

Day TA, Sibbald JR, Khanna S (1993) ATP mediates an excitatory noradrenergic neuron input to supraoptic vasopressin cells. Brain Res 607:341–344

Edwards FA, Gibb AJ, Colquhoun D (1992) ATP receptor mediated synaptic currents in the central nervous system. Nature 359:144–147

Ergene E, Dunbar JC, O'Leary DS, Barraco RA (1994) Activation of P2-purinoceptors in the nucleus tractus solitarius mediates depressor response. Neurosci Lett 174:188–192

Evans RJ, Surprenant A (1996) P2X receptors in autonomic and sensory neurons. Semin Neurosci 8:217–223

Evans RJ, Derkach V, Surprenant A (1992) ATP mediates fast synaptic transmission in mammalian neurons. Nature 357:503–505

Evans RJ, Lewis C, Buell G, Valera S, North RA, Surprenant A (1995) Pharmacological characterization of heterologously expressed ATP-gated cation channels ($P_{2X}$ purinoceptors). Mol Pharmacol 48:178–183

Evans RJ, Lewis C, Virginio C, Lundstrom K, Buell G, Surprenant A, North RA (1996) Ionic permeability of, and divalent cation effects on, two ATP-gated channels (P2X receptors) expressed in mammalian cells. J Physiol (Lond) 497:413–422

Fieber LA, Adams DJ (1991) Adenosine triphosphate-evoked currents in cultured neurones dissociated from rat parasympathetic cardiac ganglia. J Physiol (Lond) 434:239–256

Fredholm BB, Abbracchio MP, Burnstock G, Dubyak GR, Harden TK, Jacobson KA, Schwabe U, Williams M (1997) Towards a revised nomenclature for P1 and P2 receptors. Trends Pharmacol Sci 18:79–82

Fröhlich R, Boehm S, Illes P (1996) Pharmacological characterization of $P_2$ purinoceptor types in rat locus coeruleus neurons. Eur J Pharmacol 315:255–261

Fu W-M, Poo M-M (1991) ATP potentiates spontaneous transmitter release at developing neuromuscular synapses. Neuron 6: 837–843

Funk GD, Kanjhan R, Walsh C, Lipski J, Comer AM, Parkis MA, Housley GD (1997) P2 receptor excitation of rodent hypoglossal motoneuron activity in vitro and in vivo: a molecular physiological analysis. J Neurosci 17:6325–6337

Furukawa K, Ishibashi H, Akaike N (1994) ATP-induced inward current in neurons freshly dissociated from the tuberomammillary nucleus. J Neurophysiol 71:868–873

Galligan JJ, Bertrand PP (1994) ATP mediates fast synaptic potentials in enteric neurons. J Neurosci 14:7563–7571

Garcia-Guzman M, Soto F, Laube B, Stühmer W (1996) Molecular cloning and functional expression of a novel rat heart P2X purinoceptor. FEBS Lett 388:123–127

Garcia-Guzman M, Stühmer W, Soto F (1997a) Molecular cloning and pharmacological properties of the human $P2X_3$ purinoceptor. Brain Res Mol Brain Res 47:59–66

Garcia-Guzman M, Soto F, Gomez-Hernandez JM, Lund PE, Stuhmer W (1997b) Characterization of recombinant human $P2X_4$ receptor reveals pharmacological differences to the rat homologue. Mol Pharmacol 51:109–118

García-Lecea M, Delicado EG, Miras-Portugal 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

Geiger JR, Melcher T, Koh DS, Sakmann B, Seeburg PH, Jonas P, Monyer H (1995) Relative abundance of subunit mRNAs determines gating and $Ca^{2+}$ permeability of AMPA receptors in principal neurons and interneurons in rat CNS. Neuron 15:193–204

Greenwood D, Yao WP, Housley GD (1997) Expression of the $P2X_2$ receptor subunit of the ATP-gated ion channnel in the retina. Neuroreport 8:1083–1088

Grubb BD, Evans RJ (1999) Characterization of cultured dorsal root ganglion neuron P2X receptors. Eur J Neurosci 11:149–154

Gu JG, MacDermott AB (1997) Activation of ATP P2X receptors elicits glutamate release from sensory neuron synapses. Nature 389:749–753

Haines WR, Torres GE, Voigt MM, Egan TM (1999) Properties of the novel ATP-gated ionotropic receptor composed of the $P2X_1$ and $P2X_5$ isoforms. Mol Pharmacol 56:720–727

Harms L, Finta EP, Tschöpl M, Illes P (1992) Depolarization of rat locus coeruleus neurons by adenosine 5'-triphosphate. Neuroscience 48:941–952

Hiruma H, Bourque CW (1995) P2 purinoceptor-mediated depolarization of rat supraoptic neurosecretory cells in vitro. J Physiol (Lond) 489:805–811

Horiuchi J, Potts PD, Tagawa T, Dampney RAL (1999) Effects of activation and blockade of $P_{2X}$ receptors in the ventrolateral medulla on arterial pressure and sympathetic activity. J Auton Nerv Syst 76:118–126

Housley GD, Luo L, Ryan AF (1998) Localization of mRNA encoding the $P2X_2$ receptor subunit of the adenosine 5'-triphosphate-gated ion channel in the adult and developing rat inner ear by in situ hybridization. J Comp Neurol 393:403–414

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

Jahr CE, Jessel TM (1983) ATP excites a subpopulation of rat dorsal horn neurones. Nature 304:730–733

Jang I-S, Rhee J-S, Akaike N, Akaike N (2000) Developmental changes of P2X receptor on glycinergic presynaptic nerve terminals projecting to substantia gelatinosa neuron of the rat spinal cord. J Neurosci (in press)

Jo YH, Schlichter R (1999) Synaptic corelease of ATP and GABA in cultured spinal neurons. Nat Neurosci 2:241–245

Jones CA, Chessell IP, Simon J, Barnard EA, Miller KJ, Michel AD, Humphrey PPA (2000) Functional characterization of the $P2_{X4}$ receptor orthologues. Br J Pharmacol 129:388–394

Kanjhan R, Housley GD, Thorne PR, Christie DL, Palmer DJ, Luo L, Ryan AF (1996) Localization of ATP-gated ion channels in cerebellum using $P2_{X2}R$ subunit-specific antisera. Neuroreport 7:2665–2669

Kanjhan R, Housley GD, Burton LD, Christie DL, Kippenberger A, Thorne PR, Luo L, Ryan AF (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

Kawaguchi Y, Wilson CJ, Augood SJ, Emson PC (1995) Striatal interneurones: chemical, physiological and morphological characterization. Trends Neurosci 18:527–525

Kennedy C, Leff P (1995) How should $P_{2X}$ purinoceptors be classified pharmacologically. Trends Pharmacol Sci 16:168–174

Khakh BS, Henderson G (1998) ATP receptor-mediated enhancement of fast excitatory neurotransmitter release in the brain. Mol Pharmacol 54:372–378

Khakh BS, Humphrey PPA, Surprenant A (1995) Electrophysiological properties of $P_{2X}$-purinoceptors in rat superior cervical, nodose and guinea-pig coeliac neurones. J Physiol (Lond) 484: 385–395

Khakh BS, Humphrey PPA, Henderson G (1997) ATP-gated cation channels (P2X purinoceptors) in trigeminal mesencephalic nucleus neurones of the rat. J Physiol (Lond) 498: 709–715

Kidd EJ, Grahames CBA, Simon J, Michel AD, Barnard EA, Humphrey PPA (1995) Localization of $P_{2X}$ purinoceptor transcripts in the rat nervous system. Mol Pharmacol 48:569–573

Kidd EJ, Miller KJ, Sansum AJ, Humphrey BP (1998) Evidence for $P2X_3$ receptors in the developing rat brain. Neuroscience 87:533–539

Kirkup AJ, Booth CE, Chessel IP, Humphrey PPA, Grundy D (1999) Excitatory effects of P2X receptor activation on mesenteric afferent nerves in the anaesthetised rat. J Physiol (Lond) 520:551–563

Krishtal OA, Marchenko SM, Pidoplichko VI (1983) Receptor für ATP in the membrane of mammalian sensory neurones. Neurosci Lett 35:41–45

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

Lalo U, Kostyuk P (1998) Developemtal changes in purinergic calcium signalling in rat neocortical neurones. Brain Res Dev Brain Res 111:43–50

Lalo U, Voitenko N, Kostyuk P (1998) Iono- and metabotropically induced purinergic calcium signalling in rat neocortical neurons. Brain Res 799:285–291

Lê KT, Paquet M, Nouel D, Babinski K, Séguéla P (1997) Primary structure and expression of a naturally truncated human P2X ATP receptor subunit from brain and immune system. FEBS Lett 418:195–199

Lê KT, Babinski K, Séguéla P (1998a) Central $P2X_4$ and $P2X_6$ channel subunits coassemble into a novel heterotrimeric ATP receptor. J Neurosci 18:7152–7159

Lê KT, Villeneuve P, Ramjaun AR, McPherson PS, Beaudet A, Séguéla P (1998b) Sensory presynaptic and widespread somatodendritic immunolocalization of central ionotropic P2X ATP receptors. Neuroscience 83:177–190

Lê KT, Boué-Grabot É, Archambault V, Séguéla P (1999) Functional and biochemical evidence for heteromeric ATP-gated channels composed of $P2X_1$ and $P2X_5$ subunits. J Biol Chem 274:15415–15419

LePard KJ, Messori E, Galligan JJ (1997) Purinergic fast excitatory postsynaptic potentials in myenteric neurons of guinea pig: distribution and pharmacology. Gastroenterology 113: 1522–1534

Lewis C, Neidhart S, Holy C, North RA, 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 J, Perl ER (1995) ATP modulation of synaptic transmission in the spinal substantia gelatinosa. J Neurosci 15:3357–3365

Li C, Peoples RW, Weight FF (1996) Proton potentiation of ATP-gated ion channel responses to ATP and zinc in rat nodose ganglion neurons. J Neurophysiol 76:3048–3058

Li C, Peoples RW, Weight FF (1997) Inhibition of ATP-activated current by zinc in dorsal root ganglion neurones of bullfrog. J Physiol (Lond) 505:641–653

Li P, Amelita A, Zhuo M (1998) ATP P2X receptors and sensory synaptic transmission between primary afferent fibers and spinal dorsal horn neurons in rats. J Neurophysiol 80:3356–3360

Llewellyn-Smith IJ, Burnstock G (1998) Ultrastructural localization of $P2X_3$ receptors in rat sensory neurons. Neuroreport 9: 2545–2550

Loesch A, Burnstock G (1998) Electron-immunocytochemical localization of $P2X_1$ receptors in the rat cerebellum. Cell Tissue Res 294:253–260

Lynch KJ, Touma E, Niforatos W, Kage KL, Burgard EC, Van Biesen T, Kowaluk EA, Jarvis MF (1999) Molecular and functional characterization of human $P2X_2$ receptors. Mol Pharmacol 56:1171–1181

Mateo J, García-Lecea M, Miras-Portugal T, Castro E (1998) $Ca^{2+}$ signals mediated by P2X-type purinoceptors in cultured cerebellar Purkinje cells. J Neurosci 18:1704–1712

Miller KJ, Michel AD, Chessell IP, Humphrey PPA (1998) Cibacron blue allosterically modulates the rat $P2X_4$ receptor. Neuropharmacology 37:1579–1586

Nabekura J, Ueno S, Ogawa T, Akaike N (1995) Colocalization of ATP and nicotinic Ach receptors in the identified vagal preganglionic neurone of rat. J Physiol (Lond) 489:519–527

Nicke A, Bäumer HG, 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

Nieber K, Poelchen W, Illes P (1997) Role of ATP in fast excitatory synaptic potentials in locus coeruleus neurones of the rat. Br J Pharmacol 122:423–430

Nörenberg W, Wirkner K, Illes P (1997) Effect of adenosine and some of its structural analogues on the conductance of NMDA receptor channels in a subset of rat neostriatal neurones. Br J Pharmacol 122:71–80

Nörenberg W, Kügelgen I von, Meyer A, Illes P(1999) Elextrophysiological analysis of P2-receptor mechanisms in rat sympathetic neurones. In: Illes P, Zimmermann H (eds) Nucleotides and their receptors in the nervous system. Progress in brain research, vol 120. Elsevier, Amsterdam, pp 209–221

North RA, Barnard EA (1997) Nucleotide receptors. Curr Opin Neurobiol 7:346–357

Pankratov Y, Castro E, Miras-Portugal MT, Krishtal O (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

Pintor J, Díaz-Hernández M, Bustamante C, Gualix J, Gomez de Terreros FJ, Miras-Portugal MT (1999) Presence of dinucleotide and ATP receptors in human cerebrocortical synaptic terminals. Eur J Pharmacol 366:159–165

Poelchen W, Sieler D, Inoue K, Illes P (1998) Effect of extracellular adenosine 5'-triphophate on principal neurons of the rat ventral tegmental area. Brain Res 800:170–173

Rae MG, Rowan EG, Kennedy C (1998) Pharmacological properties of $P2X_3$-receptors present in neurones of the rat dorsal root ganglia. Br J Pharmacol 124:176–180

Ralevic V, Burnstock G (1998) Receptors for purines and pyrimidines. Pharmacol Rev 50:413–492

Rassendren F, Buell GN, Virginio C, Collo G, North RA, Surprenant A (1997) The permeabilizing ATP receptor, $P2X_7$. J Biol Chem 272:5842–5486

Rhee JS, Wang ZM, Nabekura J, Inoue K, Akaike N (2000) ATP facilitates spontaneous glycinergic IPSC frequency at dissociated rat dorsal horn interneuron synapses. J Physiol (Lond) 524:471–483

Robertson SJ, Rae MG, Rowan EG, Kennedy C (1996) Characterization of a $P_{2X}$-purinoceptor in cultured neurones of the rat dorsal root ganglia. Br J Pharmacol 118:951–956

Rogers M, Dani JA (1995) Comparison of quantitative calcium flux through NMDA, ATP, and ACh receptor channels. Biophys J 68:501–506

Salih SG, Housley GD, Burton LD, Greenwood D (1998) P2X2 receptor subunit expression in a subpopulation of cochlear type I spiral ganglion neurones. Neuroreport 9:279–282

Sansum AJ, Chessel IP, Hicks GA, Trezise DJ, Humphrey PPA (1998) Evidence that P2X purinoceptors mediate the excitatory effects of α,β-methylene-ADP in rat locus coeruleus neurones. Neuropharmacology 37:875–885

Séguéla P, Haghighi A, Soghomonian JJ, Cooper E (1996) A novel neuronal $P_{2X}$ ATP receptor ion channel with widespread distribution in the brain. J Neurosci 16:448–455

Shen KZ, North RA (1993) Excitation of rat locus coeruleus neurons by adenosine 5'-triphosphate: ionic mechanism and receptor characterization. J Neurosci 13:894–899

Shibuya I, Tanaka K, Hattori Y, Uezono Y, Harayama N, Noguchi J, Ueta Y, Izumi F, Yamashita H (1999) Evidence that multiple P2X purinoceptors are functionally expressed in rat supraoptic neurones. J Physiol (Lond) 514:351–367

Silinsky EM, Gerzanich V (1993) On the excitatory effects of ATP and its role as a neurotransmitter in coeliac neurons of the guinea-pig. J Physiol (Lond) 464:197–212

Silinsky EM, Gerzanich V, Vanner SM (1992) ATP mediates excitatory synaptic transmission in mammalian neurones. Br J Pharmacol 106:762–763

Simon J, Kidd EJ, Smith FM, Chessel IP, Murrell-Lagnado R, Humphrey PPA, Barnard EA (1997) Localization and functional expression of splice variants of the $P2X_2$ receptor. Mol Pharmacol 52:237–248

Soto F, Garcia-Guzman M, Gomez-Hernandez JM, Hollmann M, Karschin C, Stühmer W (1996) $P2X_4$: an ATP-activated ionotropic receptor cloned from rat brain. Proc Natl Acad Sci USA 93:3684–3688

Stebbing MJ, McLachlan EM, Sah P (1998) Are there functional P2X receptors on cell bodies in intact dorsal root ganglia of rats? Neuroscience 86:1235–1244

Stoop R, Thomas S, Rassendren F, Kawashima E, Buell G, Surprenant A, North RA (1999) Contribution of individual subunits to multimeric $P2X_2$ receptor: estimates based on methanethiosulfonate block at T336C. Mol Pharmacol 56:973–981

Sun MK, Wahlestedt C, Reis DJ (1992) Action of externally applied ATP on rat reticulospinal vasomotor neurons. Eur J Pharmacol 224:93–96

Sun XP, Stanley EF (1996) An ATP-activated, ligand-gated ion channel on a cholinergic presynaptic nerve terminal. Proc Natl Acad Sci USA 93:1859–1863

Surprenant A (1996) Functional properties of native and cloned P2X receptors. In: Chadwick DJ, Goode JA (eds) P2 purinoceptors: localization, function and transduction mechanism. Wiley, Chichester, pp 208–222

Surprenant A, Rassendren F, Kawashima E, North RA, Buell G (1996) The cytolytic $P_{2Z}$ receptor for extracellular ATP identified as P2X receptor ($P2X_7$). Science 272:735–738

Taschenberger H, Jüttner R, Grantyn R (1999) $Ca^{2+}$-permeable P2X receptor channels in cultured rat retinal ganglion cells. J Neurosci 19:3353–3366

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

Thomas T, Ralevic V, Gadd CA, Spyer KM (1999) Central $CO_2$ chemoreception: a mechanism involving P2 purinoceptors localized in the ventrolateral medulla of the anaesthetized rat. J Physiol (Lond) 517:899–905

Torres GE, Haines WR, Egan TM, Voigt MM (1998) Co-expression of $P2X_1$ and $P2X_5$ receptor subunits reveals a novel ATP-gated ion channel. Mol Pharmacol 54:989–993

Torres GE, Egan TM, Voigt MM (1999) Hetero-oligomeric assembly of P2X receptor subunits. J Biol Chem 274:6653–6659

Trezise DJ, Michel AD, Grahames CBA, Khakh BS, Surprenant A, Humphrey PPA (1995) The selective P2X purinoceptor agonist, β,γ-methylene-L-adenosine 5'-triphosphate, discriminates between smooth muscle and neuronal P2X purinoceptors. Naunyn-Schmiedeberg's Arch Pharmacol 351:603–609

Troadec JD, Thirion S, Nicaise G, Lemos JR, Dayanithi G (1998) ATP-evoked increases in $[Ca^{2+}]_i$ and peptide release from rat isolated neurohypophysial terminals via a $P_{2X2}$ purinoceptor. J Physiol (Lond) 511:89–103

Tschöpl M, Harms L, Nörenberg W, Illes P (1992) Excitatory effects of adenosine 5'-triphosphate on rat locus coeruleus neurones. Eur J Pharmacol 213:71–77

Ueno S, Harata N, Inoue K, Akaike N (1992) ATP-gated current in dissociated rat nucleus solitarii neurons. J Neurophysiol 68: 778–785

Ueno S, Tsuda M, Iwanaga T, Inoue K (1999) Cell type-specific ATP-activated responses in rat dorsal root ganglion neurons. Br J Pharmacol 126:429–436

Urano T, Nishimori H, Han HJ, Furuhata T, Kimura Y, Nakamura Y, Tokino T (1997) Cloning of *P2XM*, a novel human P2X receptor gene regulated by p53. Cancer Res 57:3281–3287

Valera S, Hussy N, Evans RJ, Adami N, North RA, Surprenant A, Buell G (1994) A new class of ligand-gated ion channels defined by $P_{2X}$ receptors for extracellular ATP. Nature 371:516–519

Valera S, Talabot F, Evans RJ, Gos A, Atonarakis SE, Morris MA, Buell GN (1995) Characterization and chromosomal localization of a human P2X receptor from the urinary bladder. Receptors Channels 3:283–289

Verdoorn TA, Burnashev N, Monyer H, Seeburg PH, Sakmann B (1991) Structural determinants of ion flow through recombinant glutamate receptor channels. Science 252:1715–1718

Virginio C, Robertson G, Surprenant A, North RA (1998) Trinitrophenyl-substituted nucleotides are potent antagonists selective for $P2X_1$, $P2X_3$ and heteromeric $P2X_{2/3}$ receptors. Mol Pharmacol 53:969–973

Virginio C, MacKenzie A, Rassendren FA, North RA, Surprenant A (1999) Pore dilation of neuronal P2X receptors. Nat Neurosci 2:315–321

Von Kügelgen I, Starke K (1991) Noradrenaline-ATP cotransmission in the sympathetic nervous system. Trends Pharmacol Sci 12:319–324

Von Kügelgen I, Nörenberg W, Illes P, Schobert A, Starke K (1997) Differences in the mode of stimulation of cultured rat sympathetic neurons between ATP and UDP. Neuroscience 78:935–941

Von Kügelgen I, Nörenberg W, Meyer A, Illes P, Starke K (1999a) Role of action potentials and calcium influx in ATP- and UDP-induced noradrenaline release from rat cultured sympathetic neurones. Naunyn-Schmiedeberg's Arch Pharmacol 359:360–369

Von Kügelgen I, Nörenberg W, Koch H, Meyer A, Illes P, Starke K (1999b) P2-receptors controlling neurotransmitter release from postganglionic sympathetic neurones. In: Illes P, Zimmermann H (eds) Nucleotides and their receptors in the nervous system. Progress in brain research, vol 120. Elsevier, Amsterdam, pp 173–182

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

Vulchanova L, Riedl MS, Shuster SJ, Buell G, Surprenant A, North RA, 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

Vulchanova L, Riedl MS, Shuster SJ, Stone LS, Hargreaves KM, Buell G, Surprenant A, North RA, Elde R (1998) $P2X_3$ is expressed by DRG neurons that terminate in inner lamina II. Eur J Neurosci 10:3470–3478

Wang CZ, Namba N, Gonoi T, Inagaki N, Seino S (1996) Cloning and pharmacological characterization of a fourth P2X receptor subtype widely expressed in brain and peripheral tissues including various endocrine tissues. Biochem Biophys Res Commun 220:196–202

Wirkner K, Franke H, Inoue K, Illes P (1998) Differential age-dependent expression of $\alpha_2$ adrenoceptor- and P2 purinoceptor-functions in rat locus coeruleus neurons. Naunyn-Schmiedeberg's Arch Pharmacol 357:186–189

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

Xiang Z, Bo X, Burnstock G (1998b) Localization of ATP-gated P2X receptor immunoreactivity in rat sensory and sympathetic ganglia. Neurosci Lett 256:105–108

Xiang Z, Bo X, Burnstock G (1999) P2X receptor immunoreactivity in the rat cochlea, vestibular ganglion and cochlear nucleus. Hear Res 128:190–196

Zhong Y, Dunn PM, Burnstock G (2000a) Pharmacological comparison of P2X receptors on rat coeliac, mouse coeliac and mouse pelvic ganglion neurons. Neuropharmacology 39:172–180

Zhong Y, Dunn PM, Burnstock G (2000b) Guinea-pig sympathetic neurons express varying proportions of two distinct P2X receptors. J Physiol (Lond) 523:391–402

Zhou X, Galligan JJ (1996) P2X purinoceptors in cultured myenteric neurons of guinea-pig small intestine. J Physiol (Lond) 496:719–729

Zimmermann H (1994) Signalling via ATP in the nervous system. Trends Neurosci 17:420–426