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P2X receptors in peripheral neurons

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Abstract

P2X receptors are a family of ligand-gated ion channels, activated by extracellular ATP. The seven subunits cloned ($P2X_{1-7}$) can assemble to form homomeric and heteromeric receptors. Peripheral neurons of neural crest origin (e.g. those in dorsal root, trigeminal, sympathetic and enteric ganglia) and placodal origin (e.g. those in nodose and petrosal ganglia) express mRNAs for multiple P2X subunits. In this review, we summarize the molecular biological, electrophysiological and immunohistochemical evidence for P2X receptor subunits in sensory, sympathetic, parasympathetic, pelvic and myenteric neurons and adrenomedullary chromaffin cells. We consider the pharmacological properties of these native P2X receptors and their physiological roles. The responses of peripheral neurons to ATP show considerable heterogeneity between cells in the same ganglia, between ganglia and between species. Nevertheless, these responses can all be accounted for by the presence of $P2X_2$ and $P2X_3$ subunits, giving rise to varying proportions of homomeric and heteromeric receptors. While dorsal root ganglion neurons express predominantly $P2X_3$ and rat sympathetic neurons express mainly $P2X_2$ receptors, nodose and guinea-pig sympathetic neurons express mixed populations of $P2X_2$ and heteromeric $P2X_{2/3}$ receptors. P2X receptors are important for synaptic transmission in enteric ganglia, although their roles in sympathetic and parasympathetic ganglia are less clear. Their presence on sensory neurons is essential for some processes including detection of filling of the urinary bladder. The regulation of P2X receptor expression in development and in pathological conditions, along with the interactions between purinergic and other signalling systems, may reveal further physiological roles for P2X receptors in autonomic and sensory ganglia.

Contents

Abbreviations: αβ-meATP, α,β-methylene ATP; βγ-me-D-ATP, β,γ-methylene-D-ATP; βγ-me-L-ATP, β,γ-methylene-L-ATP; ACh, acetylcholine; Ap4A, diadenosine 5′,5‴-P^1,P^4-tetraphosphate; Ap5A, diadenosine 5′,5‴-P^1,P^5-pentaphosphate; ATP, adenosine 5′-triphosphate; ATPγS, adenosine 5′-*O*-(3-thiotriphosphate); BDNF, brain-derived neurotrophic factor; BzATP, 2′,3′-*O*-(4-benzoylbenzoyl)-ATP; cDNA, complimentary deoxyribose nucleic acid; CNS, central nervous system; DRG, dorsal root ganglion; EPSPs, excitatory postsynaptic potentials; fEPSPs, fast excitatory postsynaptic potentials; GABA, γ-aminobutyric acid; GDNF, glial cell line-derived neurotrophic factor; IB4, isolectin B4; Ip5I, diinosine pentaphosphate; 2-meSATP, 2-methylthio ATP; MNV, trigeminal mesencephalic nucleus; MPG, major pelvic ganglion; mRNA, messenger ribonucleic acid; nACh, nicotinic acetylcholine; NMDA, *N*-methyl-D-aspartate; P5P, pyridoxal-5-phosphate; PPADS, pyridoxalphosphate-6-azophenyl-2′,4′-disulfonic acid; RT-PCR, reverse transcriptase polymerase chain reaction; SCG, superior cervical ganglion; TNP-ATP, 2′,3′-*O*-trinitrophenyl-ATP; UTP, uridine 5′-triphosphate; VPG, vesical parasympathetic ganglion; YO-PRO-1, 4-[(3-methyl-2(3*H*)-benzoxazolylidene) methyl]-1-[3-(trimethylammino) propyl]-quinolinium diiodide; −/−, null mutant; +/+, wild type.

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1. Introduction

Neurons of the peripheral nervous system have many roles including conducting sensory information from peripheral organs, relaying commands from the central nervous system to the periphery, and controlling visceral organs. Among peripheral neurons, sensory neurons of the dorsal root ganglia (DRG) share with neurons of the sympathetic, parasympathetic and enteric ganglia, along with adrenomedullary chromaffin cells a common embryological origin in the neural crest. In contrast, cranial sensory neurons are derived from the placodes. While these sensory and autonomic neurons exhibit some common properties, they also show very diverse phenotypes commensurate with their diverse physiological roles. In this article, we examine the considerable and ever increasing information on the P2X receptors present on neurons of sensory, sympathetic, parasympathetic and enteric ganglia, as well as adrenomedullary chromaffin cells.

1.1. Discovery of purinergic receptors

As long ago as 1929, Drury and Szent-Gyorgyi had demonstrated that adenine compounds had potent extracellular actions (Drury and Szent-Györgyi, 1929), and in the 1950s, Holton demonstrated the release of ATP from nerve terminals (Holton, 1959). However, it was not until 1970 that Burnstock and his colleagues suggested that ATP or a related nucleotide might be a neurotransmitter released by non-adrenergic non-cholinergic neurons in the gut (Burnstock et al., 1970; Burnstock, 1972). Implicit in that proposal was the existence of specific purinergic receptors present in the post-junctional cell membrane.

Subsequent pharmacological studies, comparing agonist profiles in different tissues led to the division of these purinergic receptors first into P1 and P2 (Burnstock, 1978) and then subdivision of P2 into P2X and P2Y (Burnstock and Kennedy, 1985) subtypes. Other apparently distinct nucleotide receptors including $P2_Z$, $P2_U$ and $P2_T$ were subsequently identified before molecular cloning techniques brought about a rationalization of nucleotide receptor classification (see Abbracchio and Burnstock, 1994; Ralevic and Burnstock, 1998; North and Surprenant, 2000). However, the heteromeric assembly of subunits still complicates the characterization of native receptors. There are now two major P2 receptor families: P2Y receptors that are members of the G protein-coupled receptor superfamily and P2X receptors that are ligand-gated ion channels.

1.2. Structure of P2X receptors

To date, seven mammalian P2X receptor subunits ($P2X_1$–$P2X_7$) have been identified by molecular cloning (see Table 1; Buell et al., 1996a). In addition, an apparently distinct member of this family had been identified in the chicken and provisionally named $cP2X_8$ (Bo et al., 2000). The P2X subunits all share the same general structure (Fig. 1), having intracellular N- and C-termini, two membrane-spanning domains and a large extracellular loop containing 10 conserved cysteine residues. While the intracellular N-termini are of similar length, there is considerable variability in the length of the C-terminus from 30 residues in $P2X_6$ to 240 amino acids in $P2X_7$ (for reviews, see Buell et al., 1996a; MacKenzie et al., 1999).

Like all other ligand-gated ion channels, functional P2X receptors are formed by the assembly of a number of subunits. These may be identical, giving rise to homo-multimeric receptors, or non-identical forming hetero-oligomers. The number of subunits present in a P2X receptor is thought to be either three (Nicke et al., 1998; Stoop et al., 1999) or four (Kim et al., 1997; Ding and Sachs, 2000). However, the rules governing assembly are still poorly understood. Co-immunoprecipitation studies using human embryonic kidney cells (HEK) indicate that while the $P2X_7$ subunit is unlikely to assemble with other P2X subunits, $P2X_6$ is unlikely to form homo-multimers. Others such as $P2X_2$ and $P2X_5$ will readily heteropolymerize with most members of the P2X family (Torres et al., 1999; see Table 2). Thus, from these seven subunits, even if one assumes that all the subunits in the receptor must be compatible, there could be 33 distinct trimeric or 55 tetrameric receptors. However, even if all these possibilities exist, whether they can be distinguished by pharmacological, biophysical or other techniques is another matter. It is also possible that different heteropolymerization may occur in other cell types where different auxiliary proteins or processing are present. An additional level of complexity is generated by the existence of spliced variants of many of the P2X receptor subunits, which may produce receptors with modified properties (Brändle et al., 1997; Townsend-Nicholson et al., 1999; Chen et al., 2000), thus potentially producing even more distinct receptors.

Table 1
Tissues from which P2X receptor subunits were first cloned

Receptor	Tissue first cloned from	Reference
$P2X_1$	Vas deferens	Valera et al., 1994
$P2X_2$	Phaeochromocytoma cells (PC12)	Brake et al., 1994
$P2X_3$	Dorsal root ganglion	Chen et al., 1995
$P2X_4$	Brain (hippocampus)	Bo et al., 1995
$P2X_5$	Coeliac ganglion	Collo et al., 1996
$P2X_6$	Superior cervical ganglion	Collo et al., 1996
$P2X_7$	Superior cervical ganglion/brain (medial habenula)	Surprenant et al., 1996

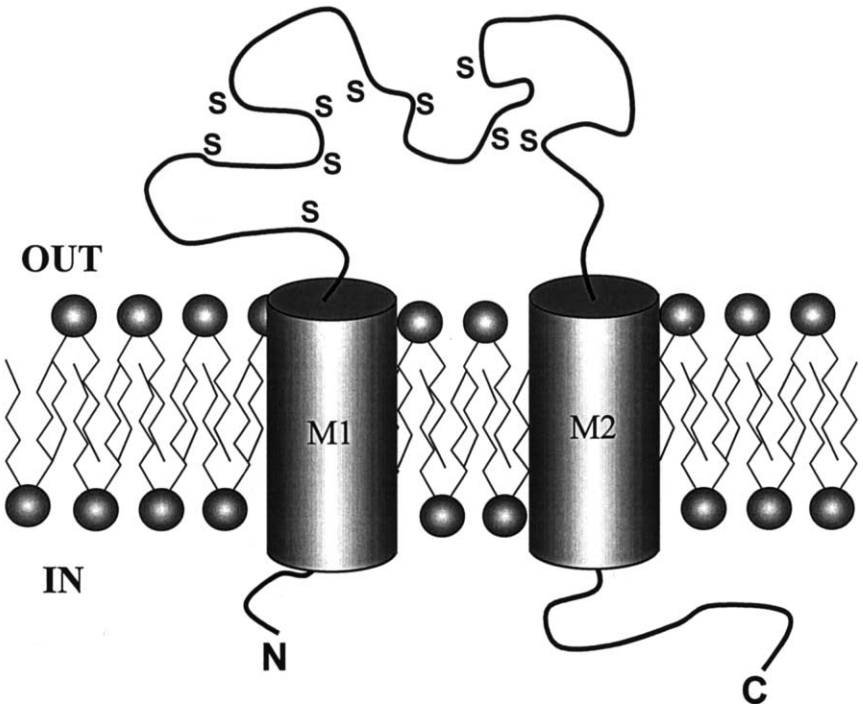

Fig. 1. Schematic representation of a P2X receptor subunit, having intracellular N- and C-termini, two transmembrane spanning domains (M1 and M2) and a large extracellular loop containing 10 conserved cysteine residues, which may form disulphide bonds.

1.3. Properties of recombinant P2X receptors

The seven homomeric P2X receptors, when studied in heterologous expression systems, exhibit different but overlapping properties in terms of agonist and antagonist sensitivities and rates of desensitization (see Table 3). Thus, $P2X_1$ and $P2X_3$ receptors are activated by α,β-methylene ATP (αβ-meATP), while $P2X_2$ is not. $P2X_1$ and $P2X_3$ receptors desensitize rapidly ($\tau \leq 1$ s) while $P2X_2$ receptors desensitize slowly ($\tau > 10$ s). Although the detailed mechanisms involved in the desensitization of P2X receptors have yet to be elucidated, it is clear that the rate of desensitization can be modified under some conditions, for example by phosphorylation (Boue-Grabot et al., 2000) or changes in the cytoskeleton (Parker, 1998). Consequently, caution should be exercised in its use for receptor characterization.

An interesting property of some P2X receptors, which was first described for $P2X_7$, is the ability to enable molecules up to 900 Da to enter the cell (Surprenant et al., 1996). While this has been attributed to the dilation of the $P2X_7$ ion channel (Virginio et al., 1999), an alternative explanation is that activation of the $P2X_7$ receptor leads to the opening of the same, large-diameter pore, which is opened by maitotoxin (Schilling et al., 1999). A similar increase in membrane permeability has now been reported to occur as a result of activating some neuronal P2X receptors (Virginio et al., 1999).

The expression of some pairs of P2X receptor subunits can give rise to the formation of receptors with novel pharmacological properties. Thus, coexpression of $P2X_2$ and $P2X_3$ subunits produces a receptor that responds to αβ-meATP (property of $P2X_3$) with a slowly desensitizing response (property of $P2X_2$) (Lewis et al., 1995). Other functional heteromeric receptors so far identified are $P2X_{4/6}$ (Lê et al., 1998), $P2X_{1/5}$ (Torres et al., 1998) and $P2X_{2/6}$ (King et al., 2000).

Table 2
P2X receptor subunit coassembly determined by co-immunoprecipitation (from Torres et al., 1999)[a]

	$P2X_1$	$P2X_2$	$P2X_3$	$P2X_4$	$P2X_5$	$P2X_6$	$P2X_7$
$P2X_1$	+	+	+	−	++[b]	+	−
$P2X_2$		+	++[c]	−	+	++[d]	−
$P2X_3$			+	−	+	−	−
$P2X_4$				+	+	++[e]	−
$P2X_5$					+	+	−
$P2X_6$						−	−
$P2X_7$							+

[a] + subunits coprecipitate; ++ coassembly demonstrated functionally; − subunits do not coprecipitate.
[b] Torres et al., 1998.
[c] Lewis et al., 1995.
[d] King et al., 2000.
[e] Lê et al., 1998.

Table 3
General properties of recombinant rat P2X receptors[a]

	Kinetics		Agonist	Antagonist			Modulator		
	Activation	Desensitization	αβ-MeATP EC_{50}	Suramin IC_{50}	TNP-ATP IC_{50}	Ip5I[b] IC_{50}	H^+	Zn^{2+}[c]	Ivermectin[d] EC_{50}
$P2X_1$	Fast	Fast	1–3 μM	1 μM	6 nM	3 nM	Inhibition	–	–
$P2X_2$	Fast	Slow	>100 μM	10 μM	1 μM	Inactive	Potentiation	Increase	No effect
$P2X_3$	Fast	Fast	1 μM	3 μM	1 nM	3 μM	Inhibition		No effect
$P2X_4$	Biphasic[e]	Moderately slow	≫100 μM	>300 μM	15 μM	Increase EC_{50} 2 nM	Inhibition	Increase (<10 μM) Decrease (>30 μM)[f]	Increase ~250 nM
$P2X_5$	Fast	Slow	≫100 μM	4 μM	–	–	–	–	–
$P2X_7$	Biphasic[g]	None	≫300 μM	~500 μM	>30 μM	–	Inhibition	Decrease	No effect
$P2X_{2/3}$	Fast	Slow	1 μM	–	11 nM[h]	Inactive	Potentiation	–	No effect
$P2X_{1/5}$	Fast	Biphasic	5 μM	–	~200 nM	–	Inhibition[i]	–	–
$P2X_{4/6}$	Fast	Moderately slow	30 μM	–	–	–	Inhibition	Increase (10 μM)	Increase
$P2X_{2/6}$[j]	Biphasic	Biphasic	Inactive	6 μM	–	–	Potentiation (inhibition ph<6.5) Inhibition (3 μM)	Increase	–

[a] For more details and further references, see North and Surprenant (2000) and Khakh et al. (2001). Only additional references are listed here.
[b] See King et al. (1999) and Dunn et al. (2000).
[c] Effect of Zn^{2+}, without preincubation.
[d] See Khakh et al. (1999a).
[e] See Khakh et al. (1999b).
[f] See Wildman et al. (1999).
[g] See Nuttle and Dubyak (1994).
[h] See Liu et al. (2001).
[i] Inhibition by either lowering or raising extracellular pH.
[j] See King et al. (2000).

1.4. Neuronal responses to purines

As long ago as 1954, Feldberg and Sherwood demonstrated profound effects of intracerebroventricular injection of ATP, including ataxia and a tendency to sleep (Feldberg and Sherwood, 1954). Numerous subsequent studies have demonstrated biochemical and electrophysiological effects of purines on the central nervous system (CNS) including excitation of neurons in the chemoreceptor trigger zone (Borison et al., 1975) and the cerebral cortex (Phillis and Edstrom, 1976; for reviews, see Burnstock, 1977, 1997). Synaptic transmission in the brain, mediated by ATP, was first observed in the medial habenula (Edwards et al., 1992) and has now been detected in a number of other brain areas (Nieber et al., 1997; Pankratov et al., 1999).

The earliest report of an effect of ATP on autonomic ganglia was in 1948, when Feldberg and Hebb demonstrated that intra-arterial injection of ATP excited neurons in the cat superior cervical ganglion (SCG) (Feldberg and Hebb, 1948). Later work from de Groat's laboratory showed that in the cat vesical parasympathetic ganglia and SCG, purines inhibited synaptic transmission through P_1 receptors, but high doses of ATP depolarized and excited the post-ganglionic neurons (Theobald and de Groat, 1977, 1989).

The earliest intracellular recordings of an action of ATP on neurons were obtained in frog sympathetic ganglia. However, here, ATP produced a depolarization through a reduction in K^+ conductance (Siggins et al., 1977; Akasu et al., 1983), which was probably mediated through P2Y receptors.

In 1983, Jahr and Jessell, using intracellular recordings from cultured neurons, demonstrated that ATP could excite DRG neurons and some neurons from the dorsal horn of the spinal cord (Jahr and Jessell, 1983). These responses were associated with an increase in membrane conductance, which we now know to be due to the activation of P2X receptors.

1.5. Embryology of peripheral neurons

During early embryological development, the neural ectoderm folds to form the neural tube. Cells in the overlying ectoderm (the neural crest) then migrate within the ectoderm and into the mesoderm. The cells that follow this latter pathway differentiate and mature to become glial cells and neurons. Some become pri-

mary afferent neurons of the DRG, while others become the post-ganglionic neurons of the sympathetic and parasympathetic ganglia. A third group of cells go on to form the enteric nervous system. One group of potential sympathetic neurons become surrounded by developing adrenal cortical cells and develop into adrenomedullary chromaffin cells. The sensory neurons of cranial nerves, including those of nodose, petrosal and trigeminal ganglia, however, are derived partly or entirely from the neural placodes (Lindsay, 1996).

In view of their common embryological origins, it is perhaps not surprising that peripheral neurons and chromaffin cells possess some common properties, e.g. N-type calcium channels (Gross and Macdonald, 1987; Bossu et al., 1991; Zhu and Ikeda, 1993) and $GABA_A$ receptors (Adams and Brown, 1975; Bormann and Clapham, 1985; White, 1990). However, in keeping with their diverse roles, there are also many differences between them, e.g. only a distinct subpopulation of primary sensory afferents express the heat sensing vanilloid receptor (VR_1; Guo et al., 1999).

In the last few years, there has been a dramatic increase in our understanding of P2X receptors at the molecular level, and the discovery of new pharmacological tools with which to study them. In addition, demonstration of the coexistence of multiple P2X receptor subtypes in single neurons (Thomas et al., 1998; Grubb and Evans, 1999; Zhong et al., 2000a) and interspecies differences (Khakh et al., 1995; Zhong et al., 2000a,b) suggest a reappraisal of our knowledge of P2X receptors in peripheral neurons could be valuable.

2. Sensory neurons

2.1. Introduction

Since the early work by Krishtal et al. (1983) and Jahr and Jessell (1983), demonstrating a cation conductance activated by extracellular ATP in sensory neurons, there have been many additional reports characterizing the native P2X receptors in sensory neurons, including those from DRG, trigeminal, nodose, petrosal ganglia and the trigeminal mesencephalic nucleus (MNV). DRG and trigeminal ganglia contain primary somatosensory neurons, receiving nociceptive, mechanical and proprioceptive inputs. Nodose and petrosal ganglia, however, contain cell bodies of afferents to visceral organs (Lindsay, 1996). Although the MNV is located in the CNS, it contains cell bodies of sensory primary afferent neurons, which relay exclusively proprioceptive information (Liem et al., 1991). Thus, functionally, it is similar to peripheral sensory ganglia and is therefore included in this review. Primary afferent neurons display modality specific neurotrophin dependence in their development (Snider, 1994; Davies, 1996). Thus, during the phase of naturally occurring neuronal cell death during development, the nociceptors in DRG and trigeminal ganglia display nerve growth factor dependence. In contrast, cranial sensory neurons depend on brain-derived neurotrophic factor (BDNF) for survival, while proprioceptors (e.g. those in DRG and MNV) are dependent on neurotrophin-3 and BDNF (Snider, 1994; Davies, 1996). Using pharmacological, immunohistochemical and molecular biological tools, rapid progress has been made in recent years concerning the nature of the endogenous P2X receptors in sensory neurons. These studies have investigated not only the cell bodies, but also the peripheral and central terminals. More importantly, the physiological roles of P2X receptors in the afferent pathways are becoming clear (see Section 2.4; Burnstock, 2000).

2.2. Responses to P2X agonists

2.2.1. Actions of agonists on cell bodies

P2X receptors on the cell bodies of sensory neurons have been studied extensively using voltage-clamp recordings from dissociated neurons of DRG (Bean et al., 1990; Bouvier et al., 1991; Robertson et al., 1996; Rae et al., 1998; Burgard et al., 1999; Grubb and Evans, 1999; Li et al., 1999; Ueno et al., 1999; Petruska et al., 2000a,b,c,d), trigeminal ganglia (Cook et al., 1997), nodose ganglia (Li et al., 1993; Khakh et al., 1995; Lewis et al., 1995; Thomas et al., 1998), petrosal ganglia (Zhang et al., 2000) and the MNV (Cook et al., 1997; Khakh et al., 1997; Patel et al., 2001).

Rapid application of ATP to acutely dissociated or cultured sensory neurons evokes action potentials and, under voltage clamp, a fast-activating (<10 ms) inward current. The activation kinetics depend on the agonist concentration, and the currents decay rapidly when ATP is removed (Bean, 1990; Khakh et al., 1995). These currents show strong inward rectification and are non-selective for the cations Na^+, Ca^{2+} and K^+. The activation of P2X receptors results in depolarization of membrane potential and an increase in intracellular Ca^{2+} concentration that is dependent on the external calcium concentration and on the magnitude of the ATP-induced current response (Bean et al., 1990; Bouvier et al., 1991). The ion selectivity and single channel properties of these receptors have been reviewed elsewhere (Evans and Surprenant, 1996). During a longer ATP application (10–60 s), P2X channels on some nodose neurons (about 40%) progressively dilate to become permeable to much larger cations such as *N*-methyl-D-glucamine and the propidium analogue YO-PRO-1 (Virginio et al., 1999).

2.2.2. Actions of agonists on peripheral terminals

Since sensory neurons do not receive synaptic input to their soma, the P2X receptors of importance may be

those located on their peripheral or central terminals (but see Section 2.4). Topical application of ATP to the receptive field selectively evokes action potential in some afferent fibres (Dowd et al., 1998; Kirkup et al., 1999; Hamilton and McMahon, 2000; Rong et al., 2000). ATP and αβ-meATP induced firing in single units recorded from the saphenous nerve of the rat in an in-vitro skin-nerve preparation (Hamilton and McMahon, 2000). Among all C-fibre nociceptors recorded, 49% responded to ATP and αβ-meATP. The greatest response occurred in C-mechanoheat units (polymodal nociceptors), while significantly less responsiveness was observed for C-mechanonociceptors and Aδ-nociceptors. Low-threshold Aβ-fibre mechanoreceptors and D-hair receptors did not respond to either ATP or αβ-meATP. This suggests that ATP selectively activates cutaneous nociceptors.

ATP can also excite nociceptors in other tissues. Intra-arterial or intra-articular injection of ATP and αβ-meATP caused rapidly activating, short-lasting excitation of a subpopulation of C and Aδ nociceptive afferent nerves innervating rat knee joint (Dowd et al., 1998). In an in-vitro tongue/nerve preparation from adult rats, bolus close-arterial injection of ATP and αβ-meATP activated preferentially the trigeminal branch of the lingual nerve (which carries general sensory information including pain, temperature, touch, etc.), rather than the chorda tympani (which conduct taste sensation) (Rong et al., 2000). In contrast, no obvious P2X receptor-mediated actions at trigeminal nerves innervating the cornea (rat and cat) (Dowd et al., 1997) or at intradental nerves of the cat (Matthews et al., 1997) were found.

In addition to nociceptors, ATP has been shown to excite a variety of other primary afferent neurons. ATP released by oxygen sensing chemoreceptors in carotid body activates P2X receptors present on nerve endings of rat sinus nerve, and the hypoxic signalling in the carotid body is mediated by the corelease of ATP and acetylcholine (ACh) (Zhang et al., 2000). Intra-arterial injection of ATP and αβ-meATP excited mesenteric afferent nerves in the rat (Kirkup et al., 1999). Functional P2X receptors have also been demonstrated to be present on the nerve endings of afferent fibres in the pelvic nerve arising from the rat and mouse urinary bladder (Namasivayam et al., 1999; Vlaskovska et al., 2001), canine pulmonary vagal C fibres (Pelleg and Hurt, 1996), and vagal afferent nerves participating in the homeostatic mechanism for cardiovascular and respiratory regulation in the rat (McQueen et al., 1998).

2.2.3. Actions of agonists on central terminals

The activation of P2X receptors at peripheral terminals generates action potentials in primary afferent neurons, which propagate to their central terminals, and evoke release of transmitters onto postsynaptic neurons in the CNS. At central terminals of primary afferent neurons, ATP has been shown to act either presynaptically (Gu and MacDermott, 1997; Khakh and Henderson, 1998; Li et al., 1998), or postsynaptically (Fyffe and Perl, 1984; Salter and Henry, 1985; Sawynok and Sweeney, 1989; Li and Perl, 1995; Bardoni et al., 1997).

The facilitation of glutamate release by ATP acting on presynaptic P2X receptors has been described for nerve terminals of DRG neurons in dorsal horn (Gu and MacDermott, 1997), and for the MNV in the brain stem (Khakh and Henderson, 1998). In vivo, intrathecal administration of αβ-meATP produced a significant and dose-dependent thermal hyperalgesic response in rats (Tsuda et al., 1999a). This effect was blocked by the P2 antagonists pyridoxalphosphate-6-azophenyl-2′,4′-disulfonic acid (PPADS) and 2′,3′-*O*-trinitrophenyl-ATP (TNP-ATP). In addition, preventing Ca^{2+}-dependent exocytosis by administration of botulinum toxin, or blocking the *N*-methyl-D-aspartate (NMDA) receptors with dizocilpine, also prevented the hyperalgesia. This suggests that αβ-meATP may act via presynaptic P2X receptors on central terminals of DRG neurons to increase spinal glutamate release, which in turn activates NMDA receptors to generate thermal hyperalgesia.

Within the spinal cord, P2X receptors are present in a subpopulation of dorsal horn neurons (Bardoni et al., 1997; Jo and Schlichter, 1999; Hugel and Schlichter, 2000; Rhee et al., 2000), and ATP is coreleased with γ-aminobutyric acid (GABA), but not with glutamate (Jo and Schlichter, 1999). In addition to acting as a fast excitatory synaptic transmitter, ATP facilitates excitatory transmission by increasing glutamate release and enhances inhibitory neurotransmission mediated by both GABA and glycine (Hugel and Schlichter, 2000; Rhee et al., 2000). Thus, ATP plays complex roles in spinal transmission and has been implicated in neurogenic and inflammatory pain pathways activated by formalin and capsaicin (Tsuda et al., 1999b). However, the underlying mechanisms are not clear.

2.3. Nature of the receptors

2.3.1. Molecular biology

The $P2X_3$ subunit was first cloned using a cDNA library from neonatal rat DRG neurons and shows a selectively high level of expression in a subset of sensory neurons, including those in DRG, trigeminal and nodose ganglia (Chen et al., 1995; Collo et al., 1996; Table 4). In DRG, although mRNA transcripts of $P2X_{1-6}$ have been detected, the level of $P2X_3$ transcript is the highest (Collo et al., 1996), and its level was substantially reduced in adult DRG following neonatal capsaicin treatment (Chen et al., 1995). A similar picture is found in trigeminal ganglia (Collo et al., 1996).

Sensory neurons from nodose ganglia express, in addition to $P2X_3$, significant levels of $P2X_{1,2,4}$ RNAs, and some of these RNAs are present in the same cell (Collo et al., 1996; Lewis et al., 1995). In the MNV, mRNAs for $P2X_2$, and $P2X_{4-6}$ were found to be present (Collo et al., 1996; Kanjhan et al., 1999). Interestingly, $P2X_4$ probe stained many sensory neurons of the trigeminal, nodose and DRG in adult rat, but the signal was absent in the embryo (Buell et al., 1996b). More data are needed to establish the expression pattern of P2X subunits in sensory ganglia during development.

2.3.2. Immunohistochemistry

Since a high level of $P2X_3$ mRNA is selectively present in a subpopulation of sensory neurons, its expression pattern in sensory ganglia has attracted much interest and has been studied by immunohistochemistry at both the light microscope (Vulchanova et al., 1997, 1998; Bradbury et al., 1998; Xiang et al., 1998; Barden and Bennett, 2000) and electron microscope (Llewellyn-Smith and Burnstock, 1998) levels.

In DRG, intensive $P2X_3$ immunoreactivity is found predominantly in a subset of small- and medium-diameter neurons, while absent from most large neurons (Vulchanova et al., 1997, 1998; Bradbury et al., 1998; Xiang et al., 1998; Novakovic et al., 1999). The $P2X_3$ subunit is predominantly located in the non-peptidergic subpopulation of nociceptors that bind the isolectin B4 (IB4), and is greatly reduced by neonatal capsaicin treatment (Bradbury et al., 1998; Vulchanova et al., 1998). The $P2X_3$ subunit is present in approximately equal numbers of neurons projecting to the skin and viscera but in very few of those innervating skeletal muscle (Bradbury et al., 1998). Following sciatic nerve axotomy, $P2X_3$ subunit expression decreases by about 50% in L4/5 DRG, and this downregulation is reversed by glial cell line-derived neurotrophic factor (GDNF) (Bradbury et al., 1998). In contrast, after chronic con-

Table 4
Summary of the distribution of P2X receptor transcripts and protein in rat sensory neurons[a]

		Cell body	Central terminal	Peripheral terminal
Dorsal root ganglia	Protein[b]	$P2X_3$-IR[c]: high level of expression in non-peptidergic small- to medium-diameter neurons, many bind IB4 $P2X_2$-IR: present in many small and large neurons $P2X_1$, $P2X_{4,5,6}$-IR: variable low level of expression in some cells	$P2X_3$-IR: present on axon terminals at inner lamina II of dorsal horn $P2X_2$-IR: present on axon terminals in lamina II and deeper layer, rarely overlap with $P2X_3$	$P2X_3$-IR: observed on nerve fibres in epidermis of glabrous skin, and in urothelium of urinary bladder
	mRNA transcripts	$P2X_{1-6}$ all present, with the level of $P2X_3$ transcript being the highest		
Trigeminal ganglia	Protein	$P2X_3$-IR: present in small and large neurons $P2X_{1,2,4,5,6}$-IR: variable and lower level compared with that of $P2X_3$	Both $P2X_2$- and $P2X_3$-IR are present in spinal trigeminal nucleus	$P2X_3$-IR: present on sensory nerve endings in tooth pulp and in circumvallate papillae of the tongue
	mRNA transcripts	$P2X_{1-6}$ are all present		
Nodose ganglia	Protein	$P2X_2$- and $P2X_3$-IR highly colocalize in cell body of many nodose neurons	$P2X_2$- and $P2X_3$-IR: colocalize in solitary tract and its nucleus (NTS)	
	mRNA transcripts	$P2X_{1,2,3,4}$ are present, colocalization of some mRNAs is observed in some neurons		
Petrosal ganglia	Protein	$P2X_2$-IR is present		
	mRNA transcripts	Not determined		
MNV	Protein	$P2X_2$-IR is present		
	mRNA transcripts	$P2X_{2,4,5,6}$ are present		

[a] See text for further details and references.
[b] Including findings in rat and mouse.
[c] IR: immunoreactivity.

striction injury to the sciatic nerve, the number of $P2X_3$ positive small and medium diameter DRG neurons appeared to have increased (Novakovic et al., 1999). The reason why these different forms of nerve damage produce opposite effects of $P2X_3$ subunit expression is at present unclear. $P2X_2$ receptor immunoreactivity is observed in many small and large DRG neurons, although the level is lower than that of $P2X_3$ (Vulchanova et al., 1997; Labrakakis et al., 2000; Petruska et al., 2000d). Some neurons seem to contain both $P2X_2$ and $P2X_3$ immunoreactivity. Although $P2X_3$ immunoreactivity is the predominant type detected, variable levels of immunoreactivity of $P2X_{1-2}$ and $P2X_{4-6}$ have also been detected in DRG neurons (Xiang et al., 1998; Barden and Bennett, 2000; Petruska et al., 2000a,d). These receptors take the form of clusters, 0.2–0.5 μm in diameter, and rarely appear to colocalize (Barden and Bennett, 2000).

In the spinal cord, $P2X_3$ immunoreactivity is apparent on the axon terminals of DRG neurons that extend across the entire mediolateral extent of inner lamina II of the dorsal horn (Vulchanova et al., 1997; Bradbury et al., 1998). This staining has a peripheral origin, as it was virtually eliminated by dorsal rhizotomy (Bradbury et al., 1998; Vulchanova et al., 1998). For the labelled nerve profiles in lamina II, $P2X_3$ receptors are located largely in terminals with ultrastructural characteristics of sensory afferent terminals (Llewellyn-Smith and Burnstock, 1998). In contrast, while $P2X_2$ immunoreactivity is most prominent in lamina II, it is also seen in deeper layers, and rarely overlaps with $P2X_3$ immunoreactivity (Vulchanova et al., 1997).

At the peripheral terminals of DRG neurons, $P2X_3$ immunoreactivity is observed in nerve fibres in rat and mouse glabrous skin, where the fibres appear to extend superficially and terminate in the epidermis (Vulchanova et al., 1998). $P2X_3$ immunoreactivity is also seen on the suburothelial nerve plexus of mouse urinary bladder, with positive staining on terminals of small nerve fibres that embed in the urothelium (Cockayne et al., 2000). Following sciatic nerve ligation, $P2X_3$ immunoreactivity accumulates proximal to the ligation site, indicating that $P2X_3$ is synthesized in the cell body, and transported along the processes into both central and peripheral terminals (Vulchanova et al., 1998; Novakovic et al., 1999).

The expression pattern of $P2X_3$ and $P2X_2$ in mouse DRG and spinal cord is very similar to that in the rat (Cockayne et al., 2000). Thus, $P2X_3$ immunoreactivity is present in small to medium neurons that bind IB4, while larger neurons contain $P2X_2$ immunoreactivity. Using antisera raised against rat P2X subunits, Vulchanova et al. (1997) described the expression pattern of $P2X_2$ and $P2X_3$ in monkey sensory neurons and their central terminals. Both $P2X_2$ and $P2X_3$ immunoreactivity was found in monkey DRG, while only $P2X_2$, but not $P2X_3$ immunoreactivity was detected in monkey spinal cord.

In trigeminal ganglia, $P2X_3$ immunoreactivity is found in the cell bodies of both small and large neurons (Cook et al., 1997; Llewellyn-Smith and Burnstock, 1998; Xiang et al., 1998). In addition, much lower levels of immunoreactivity to $P2X_{1,2}$ and $P2X_{4-6}$ appear to be present in these neurons (Xiang et al., 1998). Specific $P2X_3$ labelling is also observed at the sensory nerve endings in tooth pulp (Cook et al., 1997) and in the circumvallate papillae of the tongue (Bo et al., 1999). Both $P2X_3$ and $P2X_2$ labelling is seen in the spinal trigeminal nucleus (Vulchanova et al., 1996, 1997).

In nodose ganglia, $P2X_2$ and $P2X_3$ immunoreactivities are both present (Xiang et al., 1998) and are colocalized in the same neurons (Vulchanova et al., 1997). This is consistent with the colocalization of their mRNAs (Lewis et al., 1995). Colocalization of $P2X_2$ and $P2X_3$ immunoreactivity is also seen in the solitary tract and its nucleus (nucleus tractus solitarius) (Vulchanova et al., 1997). This is in contrast to the lack of overlap between $P2X_2$ and $P2X_3$ immunoreactivity at the dorsal horn of the spinal cord. These immunohistochemical data are therefore consistent with the differences in the pharmacological properties of P2X receptors expressed in nodose and DRG neurons (see Section 2.3.3). In the nucleus tractus solitarius, $P2X_3$-receptor positive boutons synapse on dendrites and cell bodies and have complex synaptic relationships with other axon terminals and dendrites (Llewellyn-Smith and Burnstock, 1998).

Relatively little is known about P2X receptor expression at protein level in the MNV or petrosal ganglia. Although $P2X_2$ immunoreactivity is found in both regions, expression of other subunits has not been determined (Kanjhan et al., 1999; Zhang et al., 2000).

2.3.3. Functional studies

2.3.3.1. Spectrum of response phenotype. Early studies on acutely dissociated or cultured sensory neurons of the rat showed that the ATP activated currents were either sustained or declined in the continued presence of agonist with a time constant of several seconds (Krishtal et al., 1983, 1988). Subsequent experiments revealed that while neurons of the nodose ganglion gave slowly desensitizing responses (Li et al., 1993; Khakh et al., 1995), those from DRG desensitized rapidly (<100–500 ms), and recovered slowly (>5–20 min) (Robertson et al., 1996; Rae et al., 1998). The time constant of desensitization is dependent on the concentrations of ATP, but the current level is typically $<10\%$ of peak at 500 ms after application of ATP 1 μM (Robertson et al., 1996; Rae et al., 1998). Moreover, recent studies on sensory neurons from rat DRG and trigeminal neurons revealed three types of responses, transient, sustained

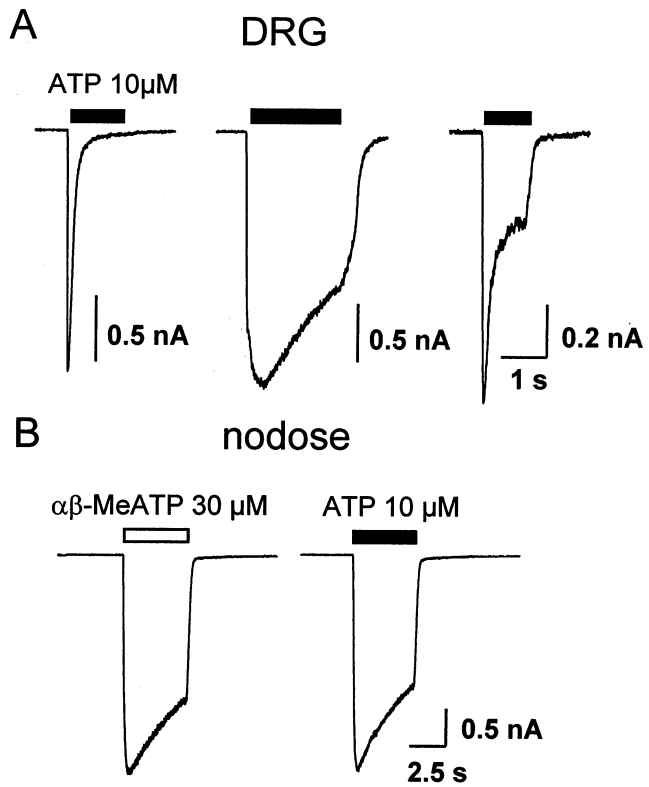

Fig. 2. Responses of sensory neurons from neonatal rats to ATP and α,β-methylene ATP (αβ-meATP). (A) Representative membrane currents evoked by 10 μM ATP recorded from three different dorsal root ganglia (DRG) neurons having respectively transient, sustained and biphasic responses. (B) Membrane currents recorded from a single nodose ganglion neuron in response to αβ-meATP (30 μM) and ATP (10 μM). Over 99% of nodose ganglion neurons respond to both agonists with a slowly desensitizing current. (P. Dunn and Y. Zhong, unpublished data).

and biphasic, comprising both transient and sustained components (Cook et al., 1997; Burgard et al., 1999; Grubb and Evans, 1999; Li et al., 1999; Ueno et al., 1999; Petruska et al., 2000a,c,d; Liu et al., 2001; see Fig. 2). However, the relative proportions of the three types of response observed vary considerably between studies, with between 28 and 80% neurons giving transient responses. Neurons from the petrosal ganglion (Zhang et al., 2000) and the mesencephalic nucleus of the trigeminal nerve (Cook et al., 1997; Khakh et al., 1997) appear to exhibit exclusively slowly desensitizing ATP responses.

It has been suggested that P2X receptors on neonatal DRG neurons desensitize rapidly, while those on adult DRG neurons desensitize slowly (Evans and Surprenant, 1996). However, this does not appear to be the case since all three types of response have been observed in both neonatal (Dunn et al., 2000) and adult (Burgard et al., 1999; Grubb and Evans, 1999; Petruska et al., 2000a,c) as well as cultured embryonic (Labrakakis et al., 2000) rat DRG neurons, while both adult (Li et al., 1993; Khakh et al., 1995) and neonatal (Dunn et al., 2000) rat nodose neurons respond to ATP with a persistent responses.

2.3.3.2. Pharmacology—transient responses. The transient response in DRG neurons is activated by ATP, αβ-meATP and 2-methylthio ATP (2-meSATP) with EC_{50}s of about 1 μM (Robertson et al., 1996; see Table 5). β,γ-Methylene-D-ATP (βγ-me-D-ATP) and diadenosine 5′,5‴-P^1,P^5-pentaphosphate (Ap_5A) also appear to be full agonists, but with a lower potency than ATP (Rae et al., 1998), while diadenosine 5′,5‴-P^1,P^4-tetraphosphate (Ap_4A) and β,γ-methylene-L-ATP (βγ-me-L-ATP) appear to be weak agonists (Rae et al., 1998; Grubb and Evans, 1999). Although UTP has been reported to be a potent agonist (Robertson et al., 1996; Rae et al., 1998), other studies have been unable to confirm these results (Grubb and Evans, 1999; P. Dunn, unpublished observation). TNP-ATP, diinosine

Table 5
Summary of the pharmacology of P2X receptors in rat and mouse sensory ganglia[a]

Response type/α,βMeATP sensitivity	Ganglia where they are frequently encountered	Full agonist(EC_{50})	Antagonist(pIC_{50})	Modulators	Presumed identity of receptor
Transient/sensitive	DRG, trigeminal	ATP = αβmeATP = 2-meSATP (1 μM), βγ-me-D-ATP (13 μM), Ap5A (3 μM)	TNP-ATP (9.5), Ip_5I (7.1), Suramin (6.5), PPADS (6.4)	H^+ inhibition $Ca^{2+}_{(o)}$ potentiation	$P2X_3$ homomer
Persistent/sensitive	Nodose, petrosal, DRG, trigeminal	ATP (3 μM), αβmeATP (9 μM), 2-meSATP (0.4 μM)	Suramin, PPADS, Cibacron blue, TNP-ATP($\approx$ 8.7)	Ca^{2+} inhibition	$P2X_{2/3}$ heteromer
Persistent/insensitive	Nodose	ATP	Cibacron blue, TNP-ATP ($<$6.7)	Zn^{2+} potentiation, H^+ potentiation	$P2X_2$ homomer
Persistent/sensitivity	MNV	ATP, ATPγS, αβmeATP (254 μM)	Suramin, PPADS, TNP-ATP (partial)	Zn^{2+} potentiation Ca^{2+} partial inhibition	Novel heteromeric receptor?

[a] See text for further details and references.

pentaphosphate (Ip_5I), suramin and PPADS inhibit the rapidly desensitizing P2X receptor (Robertson et al., 1996; Cook et al., 1997; Rae et al., 1998; Burgard et al., 1999) with pIC_{50} values of 9.5, 7.1, 6.5 and 6.4, respectively (Grubb and Evans, 1999; Dunn et al., 2000). Reducing the extracellular pH decreases the amplitude of the transient response (Burgard et al., 1999), while elevating extracellular Ca^{2+} increases the response (Cook and McCleskey, 1997) and speeds up the recovery from desensitization (Cook et al., 1998). The pharmacological evidence to date generally supports the hypothesis that this rapidly desensitizing response is mediated by homomeric $P2X_3$ receptors. Although the immunoreactivity of $P2X_1$ either alone or together with that of $P2X_3$ has been observed in adult rat DRG neurons, which gave transient responses to ATP (Petruska et al., 2000b,d), the complete loss of αβ-meATP sensitivity and of the transient response to ATP in $P2X_3$-null mutants (Cockayne et al., 2000; Souslova et al., 2000) would argue against the contribution by $P2X_1$ subunit to functional P2X receptors with fast kinetics, at least in mouse DRG neurons.

2.3.3.3. Pharmacology—persistent responses. ATP, αβ-meATP and 2-meSATP evoke rapidly activating and sustained currents in rat nodose neurons, with EC_{50}s of 3, 9 and 0.4 μM, respectively (Khakh et al., 1995). Compared with ATP, 2-meSATP appears to be a full agonist, while adenosine 5′-*O*-(3-thiotriphosphate) (ATPγS) is a partial agonist on these neurons (Lewis et al., 1995). These responses are inhibited by suramin, PPADS, Cibacron blue, TNP-ATP and Ca^{2+} (Khakh et al., 1995; Thomas et al., 1998; Virginio et al., 1998), but not by Ip_5I at concentrations up to 100 μM (Dunn et al., 2000). Therefore, the αβ-meATP-sensitive persistent responses in nodose neurons resemble the recombinant $P2X_{2/3}$ receptors (Lewis et al., 1995). However, the biphasic inhibition curve by TNP-ATP, when ATP is used as the agonist, reveals the presence of multiple P2X receptors in the same nodose neurons (Thomas et al., 1998). The responses evoked by ATP, in nodose neurons are potentiated by H^+, Zn^{2+} and Cu^{2+} and inhibited by Mg^{2+} (Li et al., 1993, 1996a,b, 1997a,b; Wright and Li, 1995). However, because more than one receptor subtype is present on these cells, caution must be taken in interpreting these data obtained with ATP as the agonist, and it remains to be seen how the individual subpopulations of receptors are modulated by these chemicals.

Neurons of the mouse nodose ganglion give persistent responses to both ATP and αβ-meATP similar to those seen in the rat (Cockayne et al., 2000; Souslova et al., 2000). In $P2X_3$-deficient mice, no nodose neurons respond to αβ-meATP at concentrations up to 100 μM, while the response to ATP is significantly reduced. The residual persistent responses to ATP have all the characteristics of recombinant $P2X_2$ homomers. They desensitize slowly, are not sensitive to αβ-meATP, and are inhibited by Cibacron blue. While the responses are potentiated by Zn^{2+} and acidification, they are not enhanced by ivermectin or Ip_5I (Fig. 3). Thus, the pharmacological evidence to date is consistent with the

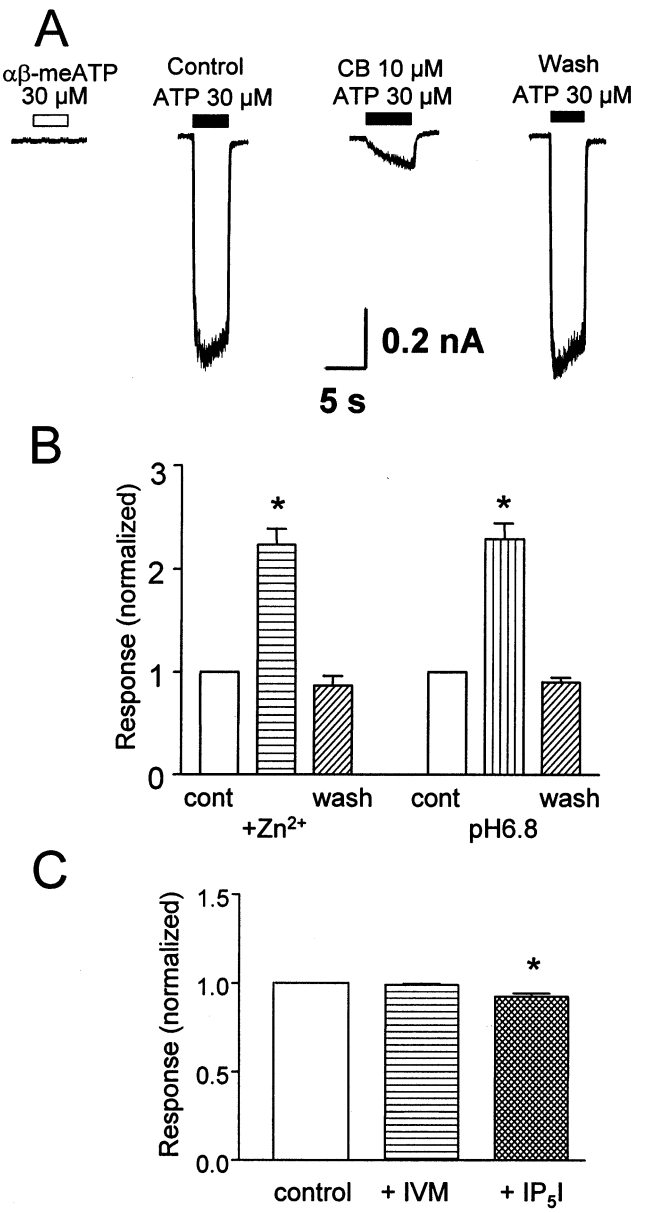

Fig. 3. Pharmacological properties of P2X receptors on nodose ganglion neurons from $P2X_3$-deficient mice. (A) These neurons respond to ATP with a slowly desensitizing inward current, but α,β-methylene ATP (α,βmeATP) fails to evoke a response. The response to ATP is almost abolished when neurons are first incubated with Cibacron blue (CB, 10 μM). (B) Combined data demonstrating the reversible potentiation of the ATP response by 10 μM Zn^{2+} and by lowering the pH to 6.8 ($n = 5$). (C) Ivermectin (IVM, 1 μM) had no effect on the response to 10 μM ATP, while diinosine pentaphosphate (Ip_5I) produced a small reduction in the response ($n = 5$). $^*P < 0.05$ by Student's *t*-test (Y. Zhong and P. Dunn unpublished).

notion that both heteromeric $P2X_{2/3}$ and homomeric $P2X_2$ receptors are present in significant amounts in nodose neurons, although the proportions may vary from cell to cell.

The receptors underlying persistent P2X currents observed in DRG neurons can be divided into αβ-meATP-sensitive and -insensitive subtypes (Petruska et al., 2000d). A small proportion of DRG neurons from $P2X_3$-deficient mice gave αβ-meATP-insensitive sustained responses to ATP (Cockayne et al., 2000), which may be attributed to homomeric $P2X_2$ receptors (as occurs in nodose ganglion neurons; see above). The αβ-meATP-sensitive persistent P2X currents observed in DRG and trigeminal neurons are activated by ATP and αβ-meATP. Suramin and TNP-ATP are effective antagonists at these receptors (Cook et al., 1997; Burgard et al., 1999; Dunn et al., 2000). Their properties are therefore very similar to the αβ-meATP-sensitive responses found on nodose ganglion neurons, and may be attributed to heteromeric $P2X_{2/3}$ receptors. Fast perfusion of ATP and αβ-meATP over petrosal ganglion neurons evokes slowly desensitizing, suramin-sensitive inward currents. While this is consistent with the presence of heteromeric $P2X_{2/3}$ receptors (Zhang et al., 2000), it remains to be seen whether additional P2X receptors are also present.

On neurons of the trigeminal mesencephalic nucleus, ATP, ATPγS and αβ-meATP are full agonists (Khakh et al., 1997; Patel et al., 2001). While ATPγS is the most potent, αβ-meATP displays an unusually low potency (EC_{50}, 254 μM). Interestingly, both TNP-ATP and raising the extracellular Ca^{2+} concentration inhibit only part of the response to ATPγS. This has led to the suggestion that there are two populations of P2X receptors present on these neurons (Patel et al., 2001). Although some of the properties of these receptors are consistent with those of $P2X_2$ and $P2X_{2/3}$, others cannot be accounted for by any cloned homomeric or heteromeric receptors so far described (for reviews, see North and Surprenant, 2000; Khakh et al., 2001). Since neurons of the MNV contain $P2X_2$, $P2X_4$, $P2X_5$ and $P2X_6$ mRNAs (Collo et al., 1996; Kanjhan et al., 1999), it is possible that heteromeric receptors incorporating two or more of these subunits are involved.

2.3.3.4. Heterogeneity of P2X receptors in sensory neurons. The observation of transient, sustained and biphasic ATP responses in DRG and trigeminal ganglion neurons (see above) suggests the presence of multiple receptor types. However, the rates of desensitization of P2X receptors may be modified by changes in cytoskeleton (Parker, 1998), phosphorylation (Boue-Grabot et al., 2000), and alternative splicing (Brändle et al., 1997). The presence of multiple receptors has, however, been confirmed by the use of cross-desensitization (Burgard et al., 1999; Grubb and Evans, 1999) and the selective antagonist Ip_5I (Dunn et al., 2000), which have clearly demonstrated that the transient and sustained components of the biphasic response are indeed mediated by distinct receptor subtypes. Furthermore, lowering extracellular pH inhibits the peak of the transient response, yet potentiates the persistent response to αβ-meATP (Burgard et al., 1999). Thus, the transient component appears to be mediated by $P2X_3$ receptors, while the sustained component is mediated by heteromeric $P2X_{2/3}$ receptors (with a small contribution from homomeric $P2X_2$ receptors, see above).

Since nodose neurons express both $P2X_2$ and $P2X_3$ subunits in their plasma membrane (Vulchanova et al., 1997), mixed populations of receptors made from $P2X_2$ and $P2X_3$ subunits (i.e. the multiple forms of $2_n3_{(N-n)}$, where N = the total number of subunits in the receptor) may be present (Thomas et al., 1998). The properties of the αβ-meATP-sensitive persistent currents are consistent with those of the heteromeric $P2X_{2/3}$ receptors (Lewis et al., 1995), while the phenotype of αβ-meATP-insensitive persistent currents retained in $P2X_3$ -null mutant mice suggests that they may be $P2X_2$ homomers (Cockayne et al., 2000; Y. Zhong and P. Dunn, unpublished observations). The pharmacological evidence suggests that both $P2X_{2/3}$ and $P2X_2$ receptors are present in rat nodose neurons. However, we cannot rule out the possibility of multiple forms of heteromeric receptors, or the involvement of splice variants. Very occasionally ($\ll 1\%$), we have observed transient and biphasic currents in nodose neurons of rat and mouse, indicating the presence of $P2X_3$ homomers in a very small percentage of these neurons (Y. Zhong, unpublished observations).

Molecular biology and immunohistochemistry studies suggest that the mRNAs and proteins for $P2X_{1-6}$ subunits are present in sensory ganglia. At the functional level, it is now clear that mixed receptor populations coexist in the same neurons of rat DRG, trigeminal and nodose ganglia. The expression of these receptors shows great neuron–neuron variability, which has brought considerable complexity to pharmacological characterization, and has pointed to the need for re-evaluation of the pharmacological characterization in some of the early studies. To date, the pharmacological properties of the P2X receptors expressed by sensory neurons resemble those of the $P2X_3$, $P2X_2$ homomers and $P2X_{2/3}$ heteromers. The apparent discrepancy between functional and molecular studies may be due to the limitation in distinguishing heteromeric receptors with currently available pharmacological tools. Alternatively, the properties of the heteromeric receptors are dominated by $P2X_2$ and $P2X_3$ subunits. It is also possible that distinct P2X receptors may be differentially distributed at cell soma and nerve terminals of the same neuron (for a review, see Khakh and Henderson, 2000). However, the time course of ATP-

evoked transmitter release from the nerve terminal correlates with that of the ATP-gated current recorded at the cell bodies of DRG neurons, suggesting that similar P2X receptors are present on the soma and their associated terminals (Labrakakis et al., 2000). The fourth possibility is that, some of the P2X mRNAs may not contribute significantly to functional receptors. Subclasses of rat DRG neurons show distint P2X currents (Petruska et al., 2000c). However, more data are needed to establish the physiological significance of the heterogeneity in P2X receptor expression in sensory neurons.

2.3.3.5. Difference between nociceptors/non-nociceptors. Although there are heterogeneous populations of P2X receptors within each sensory ganglion, there appears to be a clear difference between those containing nociceptors (DRG and trigeminal ganglia) and those that do not (nodose, petrosal ganglia and MNV) (see above). Direct evidence supporting this suggestion comes from the study of Cook et al. (1997). Using a retrograde-labelling technique, they found that ATP and αβ-meATP evoked three types of responses (transient, persistent and biphasic) on tooth pulp innervating trigeminal nociceptors, while ATP (and weakly αβ-meATP) evoked only sustained responses in proprioceptors from mesencephalic nucleus of trigeminal nerve, which innervate the jaw muscles.

Adult rat DRG neurons can be subclassified into several major groups according to their cell size, histochemical markers and the patterns of capsaicin-, proton- and ATP-activated currents (Petruska et al., 2000a,c). However, the correlation between capsaicin and ATP responses appears to be more complicated than previously supposed. Furthermore, capsaicin has been shown to cross-desensitize the persistent P2X responses (Piper and Docherty, 2000). Nevertheless, neonatal capsaicin treatment was found to greatly reduce the percentage of cells showing transient response, but left the percentage with persistent responses unaltered (Tsuda et al., 2000). (In fact, this equates to a loss of approximately 50% of the neurons with sustained ATP responses).

2.3.3.6. Species differences. Transient responses are the predominant type evoked by P2X agonists from DRG neurons of rat and mouse, with persistent and biphasic types seen less frequently (Burgard et al., 1999; Grubb and Evans, 1999; Cockayne et al., 2000). In contrast, only sustained inward currents have been reported on DRG neurons from bullfrog (Bean, 1990; Li et al., 1997c). However, nearly all of the nodose neurons from the rat (Li et al., 1993; Khakh et al., 1995), the mouse (Cockayne et al., 2000) and guinea-pig (Zhong et al., 2001) responded to ATP and αβ-meATP with persistent responses.

In addition, a species difference is evident for some pharmacological properties of these neuronal P2X receptors. Thus, while the potency of ATP is increased by Zn^{2+} on nodose neurons from rat and mouse (Li et al., 1993; Y. Zhong, unpublished observations), the ATP responses of nodose neurons from guinea-pig and DRG neurons from bullfrog are inhibited by Zn^{2+} (Li et al., 1997c; Zhong et al., 2001). The mechanisms underlying these differences are at present unclear.

2.4. Physiological roles

2.4.1. General aspects

ATP is present in millimolar concentrations in virtually all cells and can be released by exocytosis or following cell lysis (see Burnstock and Wood, 1996; Hamilton and McMahon, 2000). The activation of P2X receptors present at both peripheral and central nerve terminals may result in the generation of action potentials and an increase in intracellular Ca^{2+} concentration in primary afferent neurons (see Section 2.2). Hence, ATP has been suggested to play important roles in normal sensory transduction, nociception, thermal hyperalgesia, and mechanical allodynia. Although sensory neurons do not receive synaptic input to their cell body, P2X receptors on the soma may play important roles under certain pathological conditions. For example, in neuropathic pain states, sympathetic axons can sprout into DRG and make close contact with neurons in DRG (e.g. Ramer and Bisby, 1997). Thus, ATP released by these sympathetic nerve endings (for a review, see Burnstock, 2000) may activate P2X receptors expressed on the soma, and play a part in neuropathic pain.

While 50–96% dissociated DRG neurons responded to ATP (Bean, 1990; Grubb and Evans, 1999), neurons in intact DRG failed to respond to ATP or αβ-meATP (Stebbing et al., 1998). This has led to the suggestion that enzymic dissociation and cell-culture condition may dramatically upregulate the expression of P2X receptors. However, several lines of evidence would argue against this. Immunohistochemical studies of both intact ganglia and acutely dissociated rat DRG neurons have demonstrated the presence of P2X receptor proteins (Vulchanova et al., 1997; Xiang et al., 1998; Barden and Bennett, 2000). More importantly, application of ATP to both peripheral and central nerve terminals of primary afferent fibres evokes a physiological response (see Section 2.2). The accessibility of drug in an intact preparation, the fast-desensitizing phenotype of $P2X_3$ receptors, and the rapid internalization of receptors on exposure to ATP (Dutton et al., 2000) may contribute to the failure to detect responses to ATP in intact DRG.

ATP selectively excites primary afferent fibres, including those in the pelvic nerve from the urinary

bladder (Namasivayam et al., 1999; Vlaskovska et al., 2001), mesenteric afferent fibres (Kirkup et al., 1999), sinus nerve (Zhang et al., 2000), vagal afferent fibres (Pelleg and Hurt, 1996; McQueen et al., 1998), lingual nerve in the tongue (Rong et al., 2000) and nociceptors in the knee (Dowd et al., 1998). When ATP was applied to a blister base or injected intradermally, it caused pain in humans (Bleehen and Keele, 1977; Coutts et al., 1981). Furthermore, a pain sensation described as modest and burning was experienced when ATP was delivered to the volar forearm skin of humans using the non-invasive method of iontophoresis (Hamilton et al., 2000). The pain produced by ATP was dependent on capsaicin-sensitive sensory neurons as it was virtually abolished in skin treated repeatedly with topical capsaicin. Conversely, the pain-producing effects of ATP were greatly potentiated in hyperalgesia induced by acute capsaicin treatment and UV irradiation (Hamilton et al., 2000). In animal models, subplantar injection of ATP and 2′,3′-*O*-(4-benzoylbenzoyl)-ATP (BzATP) produced nocifensive behaviour (hindpaw lifting and licking) in the rat and mouse (Bland-Ward and Humphrey, 1997; Hamilton et al., 1999; Cockayne et al., 2000; Jarvis et al., 2001). This effect was dose-related, blockable by PPADS and TNP-ATP, but potentiated by Cibacron blue (Hamilton et al., 1999; Jarvis et al., 2001). Furthermore, the threshold was significantly lower in inflamed skin (Hamilton et al., 1999). In addition, low concentrations of ATP may act in concert with other mediators recruited by the inflammatory process to augment the pain signal, so ATP may play a more important role in inflammatory pain (Sawynok and Reid, 1997; Hamilton et al., 1999; Bland-Ward and Humphrey, 2000). Indeed, the concentrations of ATP needed to induce pain in humans were greatly reduced when tested on blister base rather than normal skin (Bleehen and Keele, 1977; Coutts et al., 1981), and fractions of cell cytosol also caused pain when applied to blister bases (Bleehen et al., 1976).

In addition to its direct activation of P2X receptors on peripheral terminals and synergistic effects with other mediators, ATP can sensitize the peripheral nerve terminals to other stimuli. The rise in intracellular Ca^{2+} concentration following P2X receptor activation increases the sensitivity of DRG neurons to heat (Kress and Guenther, 1999), and P2X agonists sensitize mechanoreceptors on vagal afferent fibres in acute oesophagitis (Page et al., 2000). The activation of heteromeric $P2X_{2/3}$ receptors in peripheral terminals of capsaicin-insensitive primary afferent fibres may lead to the induction of mechanical allodynia (Tsuda et al., 2000). Within the dorsal horn of the spinal cord, ATP has been suggested to act on presynaptic P2X receptors near central terminals of DRG neurons to facilitate glutamate release, and so ATP may be responsible for thermal hyperalgesia in inflamed skin (Hamilton et al., 1999; Tsuda et al., 1999a). However, the effect of spinal endogenous ATP is likely to be complex, since ATP is coreleased with the inhibitory transmitter GABA and may facilitate both inhibitory and excitatory synaptic transmission (see Section 2.2).

2.4.2. Transgenic studies

Studies of transgenic mice lacking the $P2X_3$ subunit provided direct evidence for the physiological roles of homo- and or heteromeric P2X receptors containing the $P2X_3$ subunit (Cockayne et al., 2000; Souslova et al., 2000). $P2X_3$-null mutant mice are viable and outwardly normal. They respond normally to acute mechanical and thermal stimuli, but with an attenuated response in both phases of the formalin test. This is consistent with previous findings using antagonists (Sawynok and Reid, 1997), and points to a role for ATP activation of $P2X_3$ in mediating nociceptive response to tissue damage. In addition, the $P2X_3$ subunit within the dorsal horn may play a regulatory role in persistent inflammatory pain (Souslova et al., 2000).

The most significant phenotype of $P2X_3^{-/-}$ mice, however, is a profound urinary bladder hyporeflexia, which suggests an essential role for $P2X_3$ in regulating micturition reflex excitability (Cockayne et al., 2000) (Fig. 4). It is likely that ATP, released from urothelium of the bladder in response to stretch during filling (Ferguson et al., 1997), directly activates P2X receptors on the terminal of afferent fibres (Namasivayam et al., 1999) and thus excites the primary afferents and generates the micturition reflex. In the $P2X_3$-null mutant mice, the loss of $P2X_3$ may significantly impair sensory neuron excitability during bladder filling, resulting in increased volume threshold and decreased voiding frequency.

It will be interesting to see how the primary afferent pathways is affected in transgenic mice lacking other P2X subunits, for example the $P2X_2$-deficient mice, and animals lacking both $P2X_2$ and $P2X_3$ subunits.

2.5. Summary

Immunohistochemistry and molecular biology indicate that, while the main subunits expressed by sensory neurons are $P2X_3$ and $P2X_2$, a number of other subunits may also be present. Nevertheless, with perhaps the exception of the MNV, all the data from pharmacological experiments to date can be accounted for by the presence of homomeric $P2X_2$ and $P2X_3$ and heteromeric $P2X_{2/3}$ receptors on cell bodies of sensory neurons. It remains to be determined whether nerve terminals express P2X receptors distinct from those of the cell soma. While the expression of $P2X_3$ can be modified by nerve injury, little is known about the factors that determine the relative proportions of homomeric and heteromeric receptors. Understanding the

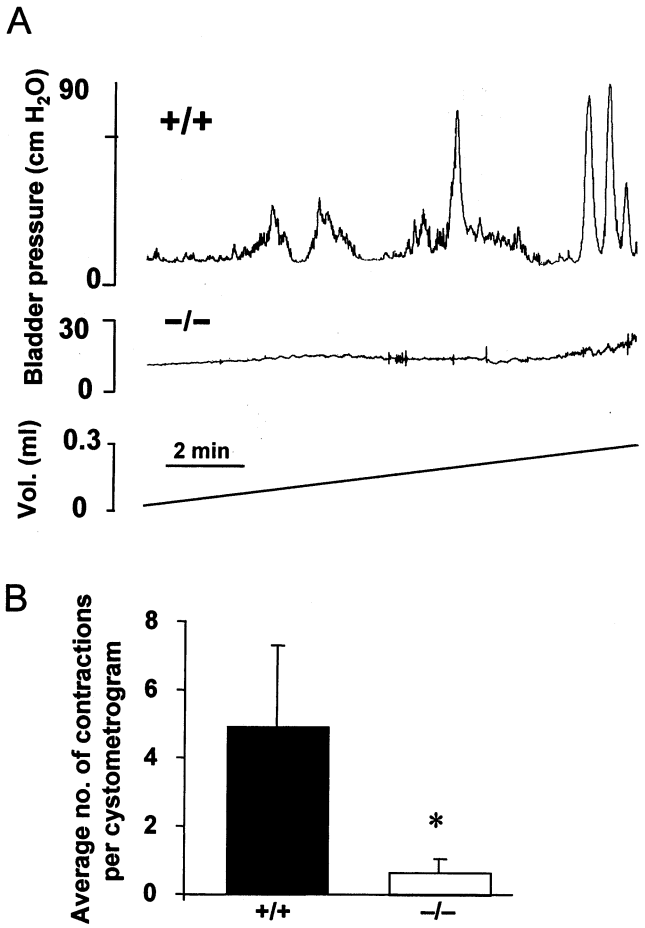

Fig. 4. Bladder reflexes in wild-type and $P2X_3$-deficient mice. (A) Measurement of intravesicular pressure recorded from anaesthetized mice, during a constant rate infusion of 0.3 ml saline over 15 min. In the wild-type (+/+) animal, this results in a series of reflex bladder contractions; no such contractions are seen in the null mutant (−/−) $P2X_3$-deficient animal. (B) Histogram of the averaged number of contractions per cystometrogram for eight wild-type and 11 null mutant mice. $^*P < 0.05$ by Student's t-test (reproduced with permission from Cockayne et al., 2000 Nature 407, 1011–1015, http://www.nature.com/

functional relevance of this heterogeneity and its regulatory factors may shed new light on mechanisms of primary afferent transmission.

3. Sympathetic and parasympathetic neurons

3.1. Introduction

The earliest report of an action of ATP on sympathetic neurons came in 1948, when Feldberg and Hebb observed that intra-arterial perfusion of the cat superior cervical ganglia (SCG) with ATP evoked contraction of the nictitating membrane (Feldberg and Hebb, 1948). Since similar responses were produced by comparable concentrations of pyrophosphate and adenylic acid, the underlying mechanism remains unclear. Following on from the suggestion that ATP might act as a neurotransmitter (Burnstock et al., 1970; Burnstock, 1972), intracellular recordings from frog sympathetic ganglion neurons revealed a depolarizing action of ATP, which was subsequently shown to result from inhibition of the M-current, and presumably mediated through P2Y receptors (Siggins et al., 1977; Akasu et al., 1983). Demonstration of depolarization and excitation of cat autonomic neurons (Theobald and de Groat, 1977, 1989; Akasu et al., 1984), and subsequent recording of single channels (Fieber and Adams, 1991; Cloues et al., 1993) confirmed the presence of what we now know to be P2X receptors.

3.2. Sympathetic neurons

3.2.1. Immunohistochemistry

A number of studies have now demonstrated the presence of P2X receptors in sympathetic ganglia by immunohistochemistry. Xiang et al. (1998) detected immunoreactivity for $P2X_{1-4}$ and $P2X_6$ receptors in SCG and coeliac ganglia of the rat. In a study of cultured SCG neurons, $P2X_2$ was the most highly expressed receptor, while lower, though detectable, levels of all the other subunits except $P2X_4$ were present (Li et al., 2000). However, it is at present unclear to what extent the expression of P2X receptors may be influenced by tissue culture conditions. In general agreement with these observations, $P2X_2$ but not $P2X_1$ immunoreactivity was detected in perivascular nerves in the gut (Vulchanova et al., 1996). In a study of the guinea-pig SGG, $P2X_2$ and $P2X_3$ immunoreactivity was detected using antibodies raised against the corresponding rat receptor epitopes. However neither $P2X_1$ nor $P2X_{4-6}$ appeared to be present in guinea-pig SCG, although the antibodies did detect immunoreactivity in other guinea-pig tissues (Zhong et al., 2000a).

3.2.2. Molecular biology

In keeping with the histochemical evidence, mRNA for most P2X subunits has been detected in sympathetic neurons. $P2X_5$ and $P2X_6$ receptors were first isolated by PCR from coeliac and SCG mRNAs, respectively (Collo et al., 1996). Fragments of $P2X_3$ and $P2X_4$ receptors have also been cloned from a rat SCG cDNA library (Lewis et al., 1995; Buell et al., 1996b). Three splice variants of the rat $P2X_2$ receptor have been cloned, and all three were detected in SCG neurons by in-situ hybridization (Simon et al., 1997). Other in-situ studies have detected $P2X_{1,4,6}$ mRNA in rat SCG neurons (Buell et al., 1996b; Collo et al., 1996).

3.2.3. Pharmacology

Almost 40 years after the first report of an action of ATP on sympathetic ganglia (Feldberg and Hebb,

1948), it was demonstrated that purinergic synaptic transmission occurred between sympathetic neurons in culture (Evans et al., 1992; Silinsky and Gerzanich, 1993). A number of subsequent studies have characterized the receptors present on sympathetic neurons, and it is now clear that there is a species difference between rat and guinea-pig. Some studies using extracellular recording techniques on intact ganglia have demonstrated the presence of multiple receptor types (Brown et al., 1979; Connolly and Harrison, 1994). However, these techniques detect responses mediated by both P2X and non-P2X receptors (Bofill-Cardona et al., 2000). Consequently, it is electrophysiological studies on dissociated neurons that have provided the most useful information. However, even with these studies, care must be taken to avoid complications arising from the activation of P2Y receptors. Numerous studies have demonstrated an inward current in response to micromolar concentrations of ATP, due to the opening of a non-selective cation current and which can be blocked by suramin (Evans et al., 1992; Cloues et al., 1993; Nakazawa, 1994; Khakh et al., 1995; Rogers et al., 1997).

The ATP receptors present on sympathetic neurons are activated by ATP with an EC_{50} of 40–80 μM (Khakh et al., 1995; Zhong et al., 2000a,b). 2-MeSATP is also an effective agonist with a similar affinity and efficacy (Khakh et al., 1995). The sensitivity to αβ-meATP varies with species. Thus, in the guinea-pig, αβ-meATP is an effective agonist on SCG (Reekie and Burnstock, 1994; Zhong et al., 2000a;but see below) and coeliac ganglion neurons (Khakh et al., 1995). In contrast, αβ-meATP evoked only a small, slowly desensitizing response in a proportion of neurons from rat SCG (Cloues et al., 1993; Khakh et al., 1995; Rogers et al., 1997). However in a study of rat and mouse coeliac ganglion neurons, no responses to 100 μM αβ-meATP were detected (Zhong et al., 2000b). Recent work from our laboratory indicates that there may be a loss of αβ-meATP-sensitive receptors from rat sympathetic neurons during development (J. Geever and P. Dunn, unpublished observation; see Section 5.3).

ATP responses in rat sympathetic neurons are antagonized by suramin with an IC_{50} of 10–100 μM (Khakh et al., 1995; Zhong et al., 2000b). Cibacron blue is a more potent antagonist and, when used at low concentrations, is completely reversible (Zhong et al., 2000b). In contrast, pyridoxal-5-phosphate (P5P) and PPADS produce an irreversible or slowly reversible antagonism (Khakh et al., 1995; Zhong et al., 2000b). Although the early study of Khakh et al. (1995) reported the responses of guinea-pig coeliac neurons to be sensitive to the antagonists suramin, Cibacron blue and P5P, more recent studies on guinea-pig pelvic ganglion and SCG neurons (see below and Fig. 5) indicate that two or more different receptors, with different antagonist sensitivities, may coexist on these neurons. Thus, responses to αβ-meATP on guinea-pig SCG neurons are antagonized by TNP-ATP with an IC_{50} of 70 nM, while

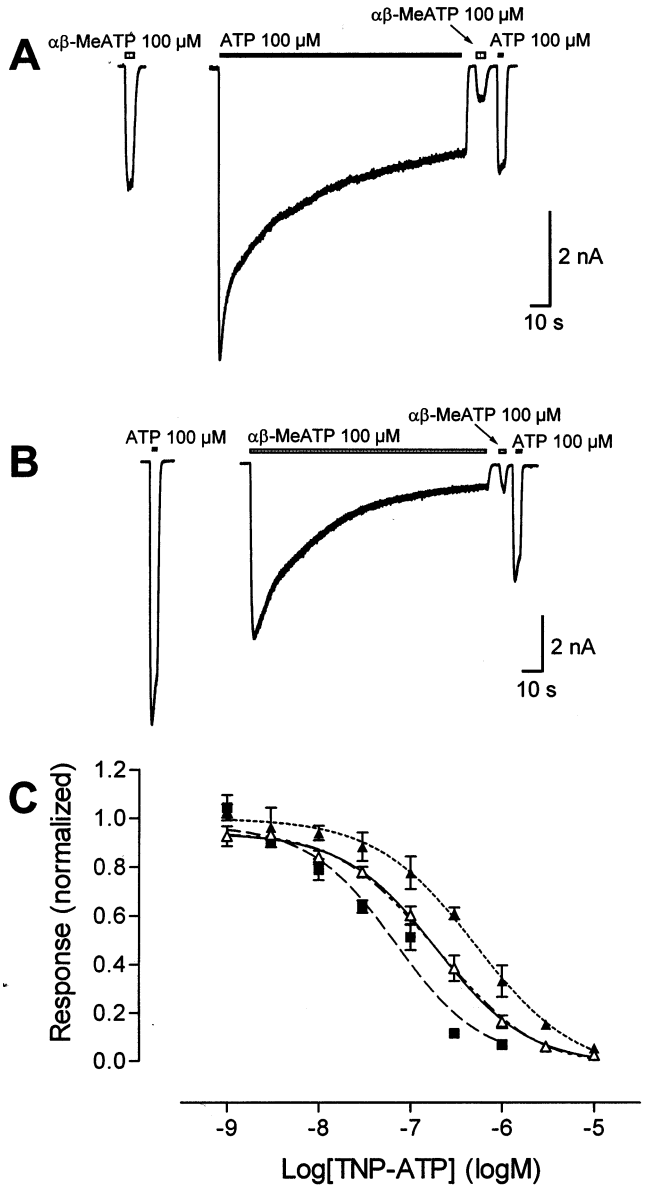

Fig. 5. Pharmacological evidence that guinea-pig SCG neurons express two P2X receptors. (A, B) Membrane currents recorded from two guinea-pig SCG neurons, showing the effect of cross-desensitization. In (A), desensitization by prolonged application of ATP reduced the response to ATP and αβ-meATP to 30 and 27% of the control, respectively. For the cell shown in (B), desensitization by αβ-meATP reduced the response to 14% of control, while that to ATP was only reduced to 44%. (C) Inhibition curves for the action of TNP-ATP on guinea-pig SCG neurons. When using αβ-meATP as the agonist (■), TNP-ATP was approximately 10 times more potent than when ATP was used on cells lacking αβ-meATP responses (▲). When ATP was used as the agonist on cells having equal proportions of αβ-meATP-sensitive and -insensitive receptors (△), the potency of TNP-ATP lay between the two, consistent with the presence of equal proportions of high- and low-affinity receptors (reproduced with permission from Zhong et al., 2000a).

responses to ATP (on neurons not responding to αβ-meATP) were inhibited with an IC_{50} of 500 nM (Zhong et al., 2000a).

ATP-evoked noradrenaline release has now been detected from both rat (Boehm, 1999) and guinea-pig (Sperlágh et al., 2000) sympathetic nerve terminals. The properties of the receptors involved are broadly in keeping with those determined in electrophysiological studies. Thus, ATP-evoked noradrenaline release from rat sympathetic nerve terminals could be blocked by suramin and PPADS, while the agonist profile for this response was ATP > 2-meSATP > ATPγS ≫ αβ-meATP (Boehm, 1999). P2X agonists stimulated release from the guinea-pig heart with a potency order of ATP > 2-meSATP > αβ-meATP = ADP. This response was antagonized by TNP-ATP, PPADS and suramin, but not by Reactive blue 2. Interestingly, the response was potentiated by 50 μM Zn^{2+}, which conflicts with the electrophysiological data of Zhong et al. (2001).

ATP responses in rat and mouse sympathetic neurons are potentiated by micromolar concentrations of Zn^{2+} (Cloues, 1995; Zhong et al., 2000b). This is due to an increase in the affinity of the receptor for ATP, and is seen at the single channel level as an increased duration of the burst of channel openings (Cloues, 1995). The response of sympathetic neurons to ATP is potentiated by acidification, and inhibited by raising the pH (Zhong et al., 2000b), which is a property of the recombinant $P2X_2$ receptor (King et al., 1996; Stoop et al., 1997).

Most of the properties described for P2X receptors in rat sympathetic neurons (kinetics, agonist and antagonist profile, effect of Zn^{2+} and pH) are consistent with those of the recombinant $P2X_2$ receptor. The presence of a small slowly desensitizing αβ-meATP response in rat SCG neurons can be explained most easily by the coexistence of some heteromeric $P2X_{2/3}$ receptors. The receptors present on guinea-pig sympathetic neurons have been less thoroughly characterized. Furthermore, little is known about recombinant receptors from this species. However from the combination of immunohistochemical and functional data, it is tempting to speculate that these neurons also possess a mixture of $P2X_2$ and $P2X_{2/3}$ receptors.

3.3. Parasympathetic neurons

3.3.1. Immunohistochemistry and molecular biology

Because of their smaller size and more diffuse location, parasympathetic ganglia are much harder to study. Consequently, there appears to be little or no immunohistochemical or molecular biological information on the presence of P2X receptors in these neurons.

3.3.2. Pharmacology

Prior to the cloning of P2X receptors, large doses of ATP were found to produce excitation in the vesical parasympathetic ganglion of the cat (Theobald and de Groat, 1989). Responses to ATP have been recorded from dissociated neurons from the chick ciliary ganglia (Abe et al., 1995), rabbit vesical parasympathetic ganglion (Nishimura and Tokimasa, 1996), intramural ganglia from the guinea-pig urinary bladder (Burnstock et al., 1987) and guinea-pig and rat cardiac neurons in culture (Allen and Burnstock, 1990; Fieber and Adams, 1991). In general, the results are very similar to those obtained in sympathetic neurons. Thus, application of ATP evokes a rapid depolarization or inward current through the activation of P2X receptors. While 2-meSATP is approximately equipotent with ATP, αβ-meATP evoked only small responses when applied at high concentrations to rat neurons. On guinea-pig neurons, the response to αβ-meATP varied from cell to cell between 0 and 75% of that to ATP. On rat cardiac ganglion neurons, the response to ATP was antagonized by Reactive blue 2 (Cibacron blue 3GA) with an IC_{50} of approximately 1 μM (Fieber and Adams, 1991), while on guinea-pig neurons, responses were potentiated by this drug at concentrations up to 30 μM (Allen and Burnstock, 1990).

3.4. Pelvic ganglion neurons

The neurons providing motor innervation to the bladder and other pelvic organs originate in the pelvic plexus. In the rat and mouse, this plexus consists of a pair of major pelvic ganglia (MPG) and a number of small accessory ganglia (Purinton et al., 1973). In the guinea-pig, there are additional intramural ganglia within the wall of the bladder. The MPG receives sympathetic and parasympathetic inputs from pre-ganglionic axons within the hypogastric and pelvic nerves, respectively (Keast, 1995), and is therefore unique in being neither sympathetic nor parasympathetic.

3.4.1. Immunohistochemistry

Using polyclonal antibodies specific for P2X receptor subunits, $P2X_2$ immunoreactivity has been detected in MPG neurons of the rat (Zhong et al., 1998). While many neurons showed low levels of staining, a small percentage showed strong, and specific staining. However, no significant staining was observed with antibodies to $P2X_1$ or $P2X_{3-6}$ subunits (Y. Zhong, unpublished observations). In keeping with these observations, $P2X_2$ but not $P2X_1$ immunoreactivity was detected in axons and nerve terminals in the vas deferens (Vulchanova et al., 1996). In the guinea-pig pelvic ganglion, in addition to staining for the $P2X_2$ subunit, $P2X_3$ immunoreactivity has also been detected (Zhong et al., 2000a).

3.4.2. Molecular biology

Studies using in-situ hybridization have detected high levels of $P2X_2$ mRNA in rat MPG neurons (Zhong et al., 1998). While some $P2X_4$ message was also detectable, no staining was observed using probes directed against $P2X_1$ and $P2X_3$ mRNA.

3.4.3. Pharmacology

P2X receptors in rat MPG neurons have an agonist profile of ATP ≥ 2-meSATP = ATPγS > BzATP, and αβ-meATP is inactive at concentrations up to 100 μM (Zhong et al., 1998). The receptors are antagonized reversibly by suramin (pA_2 5.6) and Cibacron blue (IC_{50} 0.7 μM), while PPADS is an irreversible or slowly reversible antagonist. Micromolar concentrations of Zn^{2+} and H^+ increase the agonist affinity of the receptor. The properties of the receptor present on mouse pelvic ganglion neurons are almost identical to those of the rat (Zhong et al., 2000b). The situation in the guinea-pig is much more complex. There are at least three distinct receptor subtypes present, which occur in varying proportions from cell to cell. One receptor is rapidly desensitizing and activated by αβ-meATP. Another receptor is activated by αβ-meATP, but is slowly desensitizing. This receptor is antagonized by suramin but is only weakly inhibited by Cibacron blue (Zhong et al., 2001). The third receptor is slowly desensitizing and insensitive to αβ-meATP. This receptor is not antagonized by either suramin or Cibacron blue. Both slowly desensitizing receptors are antagonized by PPADS, potentiated by low pH, and inhibited by high micromolar concentrations of Zn^{2+} (Zhong et al., 2001).

The pharmacological properties of the P2X receptors present on rat MPG neurons are consistent with those of the recombinant $P2X_2$ receptor. The properties of the three different receptors present on guinea-pig pelvic neurons, in particular the antagonist sensitivity of the slowly desensitizing ones, are harder to account for. However, the presence of $P2X_2$ and $P2X_3$ subunits has been demonstrated by immunohistochemistry. Thus, it may be that the properties of some guinea-pig P2X receptors differ appreciably from their rat orthologues, and that the receptors present are in fact $P2X_2$, $P2X_3$ and $P2X_{2/3}$.

3.5. Chromaffin cells

Chromaffin cells of the adrenal medulla can be regarded as a highly specialized form of sympathetic neuron. Although the $P2X_2$ receptor was originally cloned from PC12 cells which are a rat phaeochromacytoma cell line, adrenomedullary chromaffin cells have received relatively little attention.

3.5.1. Immunohistochemistry

In one of the first immunohistochemical studies of P2X receptors, using antibodies raised against $P2X_1$ and $P2X_2$ receptors, Vulchanova and colleagues observed both $P2X_1$ and $P2X_2$ immunoreactivity in both differentiated PC12 cells and chromaffin cells of the adrenal medulla (Vulchanova et al., 1996). This observation is in marked contrast to functional studies (see below), and was not substantiated in more recent studies (Afework and Burnstock, 1999, 2000a) in which only limited expression of $P2X_5$ and $P2X_7$ was detected in rat chromaffin cells, while $P2X_6$ immunoreactivity was detected in the guinea-pig.

3.5.2. Molecular biology

Brake and colleagues cloned the $P2X_2$ receptor from PC12 cells and detected weak expression of the mRNA in the adrenal gland by Northern blotting (Brake et al., 1994). $P2X_4$ mRNA has also been detected (Bo et al., 1995). However, in both studies, it is not certain whether the RNA was present in the medulla or cortical cells.

3.5.3. Pharmacology

ATP can produce at least three different effects on adrenal chromaffin cells: inhibition of voltage gated Ca^{2+} channels (Diverse-Pierluissi et al., 1991; Currie and Fox, 1996), release of Ca^{2+} from internal stores (Reichsman et al., 1995) and activation of a non-selective cation channel (Otsuguro et al., 1995; Liu et al., 1999). While the first two effects are most probably mediated by P2Y receptors, the third effect has the characteristics for the activation of P2X receptors.

Functional studies have demonstrated the presence of P2X receptors on bovine (Reichsman et al., 1995) and guinea-pig (Otsuguro et al., 1995; Liu et al., 1999) chromaffin cells. However, these receptors appear to be absent in the rat (Hollins and Ikeda, 1997; Liu et al., 1999). The P2X receptor present on chromaffin cells can be activated by ATP and 2-meSATP, but is much less sensitive or insensitive to αβ-meATP (Reichsman et al., 1995; Liu et al., 1999). To date, the only detailed pharmacological study of P2X receptors on chromaffin cells has been carried out on the guinea-pig. Here, the receptor is antagonized by PPADS, but suramin and Cibacron blue are quite weak antagonists. The response is potentiated by low pH, but inhibited by Zn^{2+}. Thus, while this receptor has some properties in common with the rat $P2X_2$ receptor (agonist profile, effect of pH), the lack of potentiation by Zn^{2+} and the low sensitivity to the antagonists suramin and Cibacron blue are not. Although three spliced variants of the guinea-pig $P2X_2$ receptor have been cloned, and some pharmacological characterization has been carried out (Parker, 1998; Chen et al., 2000), there is at present insufficient information to confirm the identity of the native P2X receptor present on guinea-pig chromaffin cells.

The pharmacological properties of the P2X receptor present on guinea-pig chromaffin cells are very similar to that of the αβ-meATP-insensitive receptor found on pelvic ganglion neurons. It therefore seems likely that it is in fact the homomeric $P2X_2$ receptor.

3.6. Physiological roles

The presence of P2X receptors on autonomic ganglion neurons (see above) and the release of ATP from preganglionic nerve fibres (Vizi et al., 1997) suggests a possible role for ATP as a ganglionic neurotransmitter. Synaptic transmission in the rat MPG is relatively resistant to nicotinic antagonists (Felix et al., 1998), and non-cholinergic excitatory postsynaptic potentials (EPSPs) have been recorded in neonatal rat cardiac ganglia (Seabrook et al., 1990). Furthermore, purinergic synapses form between ganglion neurons in culture (Evans et al., 1992; Silinsky and Gerzanich, 1993). Although direct evidence is still lacking (Inokuchi and McLachlan, 1995), the level of P2X receptor expression and receptor subtypes vary considerably from cell to cell, between ganglia and between species. Thus, the existence of purinergic transmission in some autonomic ganglia remains a distinct possibility.

3.7. Summary

P2X receptors have been detected in almost all sympathetic and parasympathetic ganglia studied. However, their physiological significance is still unclear. While the immunohistochemical, molecular biological and functional information on P2X receptors in autonomic neurons may not agree perfectly, the majority of information supports the following hypothesis: autonomic neurons express $P2X_2$ and $P2X_3$ subunits, which can coassemble to form three functionally identifiable receptors. However, the level of $P2X_3$ expression varies greatly between species (rat vs. guinea pig) and even from cell to cell. Thus, rat autonomic neurons express almost exclusively homomeric $P2X_2$ receptors, while guinea-pig neurons express a mixture of homomeric $P2X_2$, heteromeric $P2X_{2/3}$ and, in some cases, homomeric $P2X_3$ receptors. Adrenomedullary chromaffin cells appear to be an exception to this general pattern since those of the rat lack P2X receptors, while those in the guinea-pig appear to express only the $P2X_2$ subtype.

4. Enteric neurons

4.1. Immunohistochemistry

To date, little has been published on the presence of P2X immunoreactivity in the enteric nervous system. $P2X_2$ but not $P2X_1$ immunoreactivity is present in submucous plexus neurons of the guinea-pig ileum (Vulchanova et al., 1996). $P2X_3$ immunoreactivity has been detected in the human myenteric plexus (Yiangou et al., 2000), and these neurons also show positive staining for $P2X_2$ (O. Fajobi, personal communication).

4.2. Molecular biology

The diffuse nature of the enteric plexuses make them unattractive subjects for molecular biology. Although $P2X_4$ mRNA has been detected in rat gut by Northern blot and RT-PCR (Soto et al., 1996), it is unclear whether it is present in the neurons, muscle or epithelium. Similarly, the inability to detect $P2X_3$ mRNA in rat ileum by Northern blot (Chen et al., 1995) might be because only very low amounts of neuronal RNA were present.

4.3. Pharmacology

Early studies using intracellular microelectrode recording from intact myenteric and submucosal ganglia revealed that ATP could evoke a slow hyperpolarization, or depolarization, and inhibit synaptic transmission (Katayama and Morita, 1989; Barajas-López et al., 1994; Kamiji et al., 1994). However, these effects are mediated through P1 and P2Y receptors. Subsequent experiments using patch clamp recordings from dissociated and cultured ganglion neurons revealed an additional fast depolarization (observed as an inward current under voltage clamp) due to the activation of P2X receptors (Barajas-López et al., 1994; Glushakov et al., 1996; Zhou and Galligan, 1996). In addition, elevation of intracellular Ca^{2+}, measured by microfluorimetry has observed responses mediated through both P2X and non-P2X receptors (Kimball and Mulholland, 1995; Kimball et al., 1996; Christofi et al., 1997).

The pharmacological properties of the P2X receptors present on guinea-pig myenteric and submucosal ganglion neurons have been investigated in a number of studies. While the majority of neurons gave slowly desensitizing responses, approximately 10% of neurons responded to ATP and αβ-meATP with a rapidly desensitizing response (Zhou and Galligan, 1996; Glushakov et al., 1998). The agonist profile reported for the slowly desensitizing receptor is ATPγS ≥ 2-meSATP ≥ ATP ≫ αβ-meATP ≥ βγ-meATP, with UTP and adenosine being inactive (Barajas-López et al., 1994, 1996; Zhou and Galligan, 1996). However, the activity of αβ-meATP varies considerably from cell to cell. Thus, in some neurons, it evokes no response, while in others, the maximum response is approximately 50% of that to ATP (Barajas-López et al., 1994; Zhou and Galligan, 1996). The possible coexistance of two or more populations of P2X receptors on these

neurons complicates the interpretation of this data. In those cells where αβ-meATP does not act as an agonist, it can inhibit the response to ATP (Glushakov et al., 1996; Zhou and Galligan, 1996; LePard et al., 1997), but when αβ-meATP is a weak agonist, it does not seem to affect the response to ATP (Barajas-López et al., 1996).

The slowly desensitizing P2X receptors on enteric neurons are antagonized by micromolar concentrations of PPADS, with an IC_{50} of approximately 3 μM (Barajas-López et al., 1996; Zhou and Galligan, 1996; LePard et al., 1997). However, suramin has been reported either to antagonize (Galligan and Bertrand, 1994), have no effect (Glushakov et al., 1998) or potentiate (Barajas-López et al., 1993, 1996) responses to ATP. Since similar potentiation was observed when αβ-meATP was used as the agonist (Barajas-López et al., 1996), this effect is unlikely to result from blockade of ecto-ATPase by suramin. Cibacron blue is similarly ineffective at antagonizing the slowly desensitizing receptor in enteric neurons or produces a modest potentiation of responses to ATP (Barajas-López et al., 1996; Glushakov et al., 1996).

4.4. Physiological roles

In the guinea-pig myenteric plexus, approximately 30% of fast excitatory postsynaptic potentials (fEPSPs) can be abolished by the nicotinic antagonist hexamethonium. However, fEPSPs remaining in the presence of hexamethonium could be further reduced by the P2 antagonists suramin and PPADS (Galligan and Bertrand, 1994; LePard et al., 1997), indicating the presence of a purinergic component to the fEPSP. Although fEPSPs with a purinergic component can be found throughout the length of the gut, they are more common in the duodenum (80%) and least frequently seen in the gastric corpus. Surgical denervation studies have demonstrated that the fEPSPs with a purinergic component arise from circumferentially and aborally projecting neurons (LePard and Galligan, 1999). However, experiments on reflex responses to mucosal stimulation suggest that while descending excitation is almost exclusively purinergic (Clark et al., 1996; Spencer et al., 2000), both purinergic and nicotinic transmission are important for ascending excitation (Spencer et al., 2000).

4.5. Summary

Purinergic synaptic transmission in enteric ganglia is particularly important for descending reflex pathways in the guinea-pig (Spencer et al., 2000). Our knowledge of the receptors involved, however, is at present incomplete. The picture that emerges appears similar to that in guinea-pig sympathetic and pelvic ganglion neurons (see Sections 3.2 and 3.4), with a subpopulation of neurons expressing transient responses, while the majority have slowly desensitizing responses, and express varying levels of αβ-meATP sensitive receptors. While the most prudent explanation is that these neurons express varying proportions of $P2X_2$, $P2X_3$ and $P2X_{2/3}$ receptors, more detailed studies are required to rule out the involvement of other subunits. It also remains to be determined whether similar receptor expression, and the involvement of purinergic transmission in gastro-intestinal reflex pathways occurs in other mammalian species including humans.

5. Concluding comments

5.1. Interaction with other receptors

Autonomic and sensory neurons possess a plethora of receptors, giving rise to possibilities of interaction between different intercellular signalling pathways at the level of the receptor and at subsequent downstream events. For example, the activation of muscarinic receptors will increase the excitability of autonomic ganglion neurons by inhibition of the M-current (Adams et al., 1982). This will then enhance the excitation produced by the activation of nicotinic ACh receptors.

5.1.1. Interaction with metabotropic receptors

Many peripheral neurons possess both ionotropic P2X and metabotropic P2Y receptors. Furthermore, at least the $P2X_2$ receptor can be regulated by phosphorylation (Chow and Wang, 1998; Boue-Grabot et al., 2000). Thus, activation of P2Y receptors (or other metabotropic receptors) could modulate P2X receptor function. However, to date, no such interaction has been demonstrated.

5.1.2. Interaction with nACh receptors

Autonomic ganglion neurons express both nicotinic ACh (nACh) and P2X receptors. A number of studies have now demonstrated inhibitory interactions between these two populations of receptors. In most cases, non-additive behaviour between maximally effective concentrations of a P2X and a nACh agonist has been described (Nakazawa et al., 1991; Nakazawa, 1994; Glushakov et al., 1996; Barajas-López et al., 1998; Zhou and Galligan, 1998). However, the study of Searl et al. (1998) described cross-inhibition, where ATP responses were reduced by low concentrations of nicotine and vice versa. Furthermore, some studies have reported additive behaviour between nACh and P2X responses (Rogers et al., 1997), and even in the work of Searl et al. (1998), additivity was observed in a small number (3/29) of experiments.

The non-additive behaviour appears to be quite specific for nicotinic and purinergic receptors, since additive behaviour was observed between GABA or 5-hydroxytryptamine when coapplied with either ATP or ACh (Zhou and Galligan, 1998). Although the precise mechanisms underlying these interactions remain to be elucidated, they appear to depend upon the inward flow of current but do not require the influx of Ca^{2+}. Cytoplasmic second messengers do not appear to be involved since the effects can be observed in isolated, outside-out membrane patches. The effect has also been observed in a recombinant system, in which the nicotinic α3β4 receptor was coexpressed with $P2X_2$ receptors in *Xenopus* oocytes (Khakh et al., 2000). However, it is at present unclear which combinations of nACh and P2X receptor subunits will permit these interactions to occur.

ATP is coreleased with ACh from the terminals of preganglionic autonomic neurons (Vizi et al., 1997). Consequently, this interaction could have quite a significant role in ganglionic transmission. One possibility is that it produces a ceiling effect on the amplitude of the EPSP. Under such circumstances, a decrease in the amount of transmitter released would result in less 'cross-inhibition', so the amplitude of the EPSP might remain almost unchanged.

5.2. Plasticity and regulation

Many autonomic and sensory neurons express at least two P2X receptor subunits, giving rise to a mixture of homomeric and heteromeric receptors, with different neurons expressing them in different proportions. Whether these receptors form by random assembly of subunits or whether the process is regulated in any way is at present unclear. In addition, it is not known whether the same complement of P2X receptors is always expressed in a given neuron, or whether it changes with time. It has been shown that the expression of $P2X_3$ receptors in DRG neurons can be modified by chronic nerve injury (Novakovic et al., 1999), axotomy, and by neurotrophins (Bradbury et al., 1998). However, the extent to which such changes alter the functional response to ATP is at present unclear.

5.3. Developmental aspects

Messenger RNA for the $P2X_4$ subunit is not present in autonomic ganglia of embryonic rats, although it is present in the adult (Buell et al., 1996b). Interestingly, during this period of development, there appears to be a reduction in the expression of functional P2X receptors, with almost complete loss of $P2X_3$ subunits (J. Geever and P. Dunn, unpublished information; see Fig. 6). Post-natal changes in the expression of P2X receptors have also been reported in chromaffin cells of the adrenal medulla (Afework and Burnstock, 2000b). Furthermore, it is tempting to speculate that the fast synaptic response in neonatal cardiac ganglia which was resistant to cholinergic antagonists (Seabrook et al., 1990) was mediated by ATP. Thus, P2X receptors may have an important role in neuronal development and synaptogenesis. Further studies are required to address this possibility.

5.4. Summary

The properties of P2X receptors in peripheral ganglion neurons are summarized in Table 6. Although molecular biology and immunohistochemistry indicate the expression of a wide variety of P2X receptor subunits in peripheral neurons, functional studies have so far only provided conclusive evidence for the involvement of $P2X_2$ and $P2X_3$ subunits. Of course, it may be that the other subunits are present but in heteromeric complexes, the properties of which are either dominated by $P2X_2$ and $P2X_3$ subunits, or that they cannot at present be distinguished by the available pharmacologi-

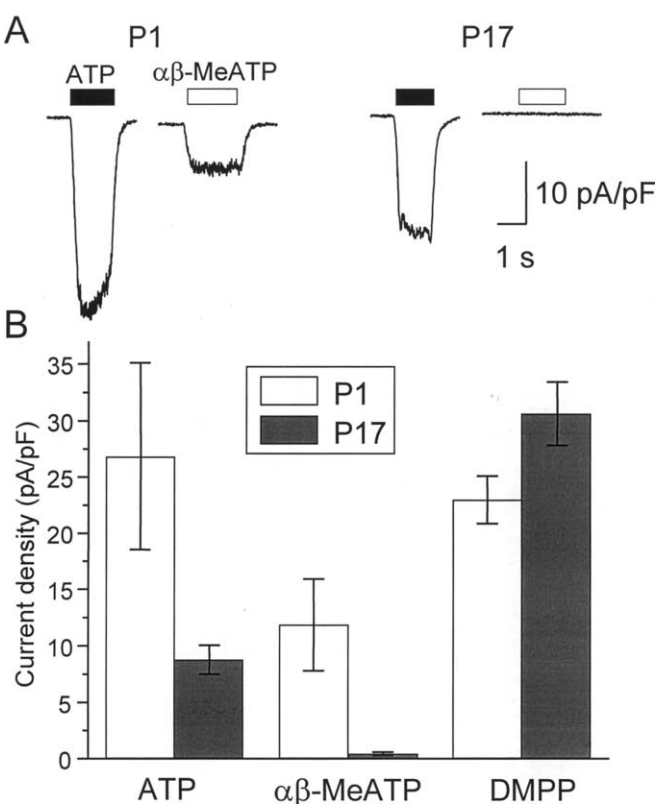

Fig. 6. Developmental changes in P2X responses in rat superior cervical ganglion (SCG) neurons. (A) Representative traces showing membrane currents evoked by 100 μM ATP and 100 μM αβ-meATP recorded from SCG neurons dissociated from newborn (P1) and 17-day-old (P17) rats. (B) Histogram comparing the current density for responses to ATP 100 μM, αβ-meATP 100 μM and the nicotinic receptor agonist dimethylphenylpiperazine (DMPP, 10 μM) in SCG neurons at these two ages (J. Geever and P. Dunn, unpublished data).

Table 6
Summary of the properties of the predominant P2X receptors present in peripheral ganglion neurons and chromaffin cells of rat, mouse and guinea-pig

Species	Ganglion	Number of receptor populations	Properties of each P2X receptor	
			αβ-meATP sensitivity	Kinetics of the current
Rat	DRG	Two	Type 1, Yes	Transient
			Type 2, Yes	Persistent
	Trigeminal	Two	Type 1, Yes	Transient
			Type 2, Yes	Persistent
	Nodose	Two	Type 1, Yes	Persistent
			Type 2, No	Persistent
	Petrosal	One	Yes	Persistent
	MNV	Two	Type 1, Weak[a]	Persistent
			Type 2, Very weak	Persistent
	SCG	One	No[b]	Persistent
	Coeliac	One	No	Persistent
	Pelvic	One	No	Persistent
	Cardiac	One	No[c]	Persistent
	Chromaffin cells	None		
Mouse	DRG	Three	Type 1, Yes	Transient
			Type 2, Yes	Persistent
			Type 3, No	Persistent
	Nodose	Two[d]	Type 1, Yes	Persistent
			Type 2, No	Persistent
	Coeliac	One	No	Persistent
	Pelvic	One	No	Persistent
Guinea-pig	SCG	Two	Type 1, Yes	Persistent
			Type 2, No	Persistent
	Pelvic	Three	Type 1, Yes	Transient
			Type 2, Yes	Persistent
			Type 3, No	Persistent
	Enteric	Two	Type 1, Yes	Transient
			Type 2, No	Persistent
	Chromaffin cells	One	No	Persistent

[a] $EC_{50} = 254$ μM.
[b] But see Section 5.3 and Fig. 6.
[c] Small responses at high concentrations have been reported.
[d] A third (transient) responses is rarely observed.

cal tools. An alternative explanation is that different P2X receptors are localized at different sites on the neuron such as the axon or nerve terminals. Since most functional studies have involved electrophysiological recording from the cell body, different receptors located elsewhere on the neuron might not be detected. Further studies using new techniques will be required to test this hypothesis.

Although this general expression pattern of $P2X_2$ and $P2X_3$ subunits is present throughout the neurons of neural crest origin, there is considerable heterogeneity between species, between different ganglia, and even between neurons in the same ganglia. For example, rat adrenomedullary chromaffin cells express no P2X receptors, while those of the guinea-pig express only $P2X_2$ receptors; sensory neurons in DRG express predominantly homomeric $P2X_3$ receptors, while neurons in the nodose ganglion express a mixture of $P2X_2$ and $P2X_{2/3}$. The factors underlying this variation in receptor expression and its possible change over time are at present poorly understood. Receptor expression can be modified by neurotrophins (Bradbury et al., 1998), and there is clearly some regulation of receptor expression by developmental factors and under some pathological conditions (see Abbracchio and Burnstock, 1998). Furthermore, apart from one study on the expression of P2X receptors on DRG and spinal cord of monkey (Vulchanova et al., 1997), little is known about the pattern of P2X receptor expression in peripheral neurons of other species such as primates and humans.

The physiological role of ATP and P2X receptors in synaptic transmission in the enteric nervous system is well established. More recent work has identified an important role for ATP acting via $P2X_3$ receptors in the detection of filling in the urinary bladder of the mouse (Cockayne et al., 2000). In view of the widespread distribution of P2X receptors in peripheral neurons, the increased research into purinergic signalling generated

by these observations is likely to find additional roles, possibly during development or under pathological conditions, when P2X receptor expression may be altered.

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