Purines and Sensory Nerves

Geoffrey Burnstock

Contents

G. Burnstock
Autonomic Neuroscience Centre, Royal Free and University College Medical School, Rowland Hill Street, London, NW3 2PF, UK
g.burnstock@ucl.ac.uk

B.J. Canning and D. Spina (eds.), *Sensory Nerves*, Handbook of Experimental Pharmacology 194,
DOI: 10.1007/978-3-540-79090-7_10,

Abstract P2X and P2Y nucleotide receptors are described on sensory neurons and their peripheral and central terminals in dorsal root, nodose, trigeminal, petrosal, retinal and enteric ganglia. Peripheral terminals are activated by ATP released from local cells by mechanical deformation, hypoxia or various local agents in the carotid body, lung, gut, bladder, inner ear, eye, nasal organ, taste buds, skin, muscle and joints mediating reflex responses and nociception. Purinergic receptors on fibres in the dorsal spinal cord and brain stem are involved in reflex control of visceral and cardiovascular activity, as well as relaying nociceptive impulses to pain centres. Purinergic mechanisms are enhanced in inflammatory conditions and may be involved in migraine, pain, diseases of the special senses, bladder and gut, and the possibility that they are also implicated in arthritis, respiratory disorders and some central nervous system disorders is discussed. Finally, the development and evolution of purinergic sensory mechanisms are considered.

Keywords Bladder, Brain stem, Carotid body, Ganglion, Gut

1 Introduction

Review articles have been published concerned with P2X and P2Y receptors in sensory neurons (Burnstock 2000, 2007; Tsuda and Inoue 2006), purinergic sensory-motor neurotransmission (Rubino and Burnstock 1996) and purine-mediated signalling in pain (Burnstock and Wood 1996; Burnstock1996a, b, 2001a, 2006; McGaraughty and Jarvis 2006; Shieh et al. 2006; Inoue 2007). Au1

The first hint that ATP might be a neurotransmitter arose when it was proposed that ATP released from sensory nerve collaterals during antidromic nerve stimulation of the great auricular nerve caused vasodilatation of the rabbit ear artery (Holton 1959). ATP was shown early to excite mammalian dorsal root ganglia (DRG) neurons and some neurons in the dorsal horn of the spinal cord (~~Krishtal et al. 1983;~~ Jahr and Jessell 1983). Extracellular ATP was reported early to produce pain sensation in humans (Collier et al. 1966; Bleehen and Keele 1977) and to participate in pain pathways in the spinal cord (Fyffe and Perl 1984; Salter and Henry 1985).

Recent reviews about the current status of and pharmacological characterization of subtypes of receptors for purines and pyrimidines are available, including four subtypes of P1 (adenosine), seven subtypes of P2X ionotropic and eight subtypes of P2Y metabotropic receptors (North 2002; Abbracchio et al. 2006). A landmark discovery related to this chapter was the cloning of $P2X_3$ receptors and their localization on sensory nerves in 1995 (~~Lewis et al. 1995;~~ Chen et al. 1995~~a,~~ b). All P2X subtypes, except $P2X_7$, are found in sensory neurons, although the $P2X_3$ receptor has the highest level of expression [in terms of both messenger RNA (mRNA) and protein] and $P2X_{2/3}$ heteromultimers are particularly prominent in the nodose ganglion. $P2X_3$ and $P2X_{2/3}$ receptors are expressed on isolectin B4 (IB_4) binding subpopulations of small nociceptive neurons (Bradbury et al. 1998). P2Y receptors are also present on sensory neurons sometimes coexpressed with $P2X_3$ receptors (Burnstock 2007). It has been suggested that while $P2X_3$ receptor activation leads to increased firing of DRG neurons and subsequently to increased release of sensory transmitter from their central processes, $P2Y_1$ receptor activation may decrease the release of sensory transmitter onto spinal cord neurons and may thereby partly counterbalance the excitatory effect of ATP.

Au2

2 Peripheral Sensory Ganglionic Neurons

There have been many reports characterizing the native P2X receptors in sensory neurons, including those from DRG, trigeminal, nodose, petrosal and enteric ganglia (Burnstock 2000, 2007; Dunn et al. 2001). DRG and trigeminal ganglia contain primary somatosensory neurons, receiving nociceptive, mechanical and proprioceptive inputs. Nodose and petrosal ganglia, on the other hand, contain cell bodies of afferents to visceral organs.

All P2X subtypes, except $P2X_7$, are found in sensory neurons, and most prominent is the $P2X_3$ receptor. $P2Y_1$, $P2Y_2$, $P2Y_4$ and $P2Y_6$ receptors have also been described in sensory neurons (Burnstock and Knight 2004).

It has been shown that the sensory neurons have the machinery to form purinergic synapses on each other when placed in short-term tissue culture (Zarei et al. 2004). The resulting neurotransmitter release is calcium-dependent and uses synaptotagmin-containing vesicles; the postsynaptic receptor involved is a P2X subtype.

2.1 Dorsal Root Ganglia

The $P2X_3$ receptor subunit that was first cloned using a complementary DNA library from neonatal rat DRG neurons shows a selectively high level of expression in a subset of sensory neurons, including those in DRG. In DRG, the level of $P2X_3$ transcript is the highest, although mRNA transcripts of $P2X_{1–6}$ have been detected. In DRG, intensive $P2X_3$ immunoreactivity is found predominantly in a subset of small- and medium-diameter neurons, although it was absent from most large neurons. The $P2X_3$ subunit is predominantly located in the non-peptidergic subpopulation of nociceptors that binds IB_4, and is greatly reduced by neonatal capsaicin treatment. The $P2X_3$ subunit is present in an approximately equal number of neurons projecting to skin and viscera, but in very few of those innervating skeletal muscle (Bradbury et al. 1998). $P2X_2$ receptor immunoreactivity is observed in many small and large DRG neurons, although the level is lower than that of $P2X_3$. Some neurons show both $P2X_2$ and $P2X_3$ immunoreactivity, probably indicating a $P2X_{2/3}$ heteromultimer receptor. Variable levels of immunoreactivity for $P2X_1$, $P2X_2$, $P2X_4$, $P2X_5$ and $P2X_6$ receptors have also been detected in DRG neurons.

Both transient and sustained responses to P2 receptor agonists occur in DRG neurons (Dunn et al. 2001). The transient response in DRG neurons is activated by ATP, α,β-methylene-ATP (α,β-meATP) and 2-methylthio-ATP (2-MeSATP). The pharmacological evidence to date generally homomeric $P2X_3$ receptors. P2X receptors on the cell bodies of the sensory neurons have been studied extensively using voltage-clamp recordings from dissociated neurons of the DRG (Fig. 1a–c). Rapid application of ATP evokes action potentials and under voltage clamp, a fast-activating inward current (mediated by $P2X_3$ receptors), a sustained response (mediated by $P2X_2$ receptors) and a rapid response, followed by slow responses (mediated by $P2X_{2/3}$ receptors), as well as depolarization and an increase in intracellular Ca^{2+} concentration. Rapid reduction of the excitatory action of ATP on DRG neurons by GABA, probably via $GABA_A$ anionic receptors, and slow inhibition of ATP currents via metabotropic $GABA_B$ receptors appear to be additional mechanisms of sensory information processing. Oxytocin and 17β-oestradiol attenuate ATP-activated currents in DRG neurons. In contrast, neurokinin B potentiates ATP-activated currents in DRG neurons. Ω-Conotoxin GVIA, known as a selective blocker of N-type calcium channels, potently inhibits the currents mediated by P2X receptors in rat DRG neurons. There are species differences in the responses of DRG neurons to ATP. 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. In contrast, only sustained inward currents have been reported on DRG neurons from bullfrog. It has been claimed ~~recently~~ that release of ATP from neuronal cell bodies in DRG triggers neuron-satellite glial cell communication via $P2X_7$ receptors (Zhang et al. 2007~~a, b~~).

Au3

Au4

Neurons and glial cells differentially express P2Y receptor subtype mRNA in rat DRG (Kobayashi et al. 2006). $P2Y_1$ and $P2Y_2$ receptor mRNA was expressed in

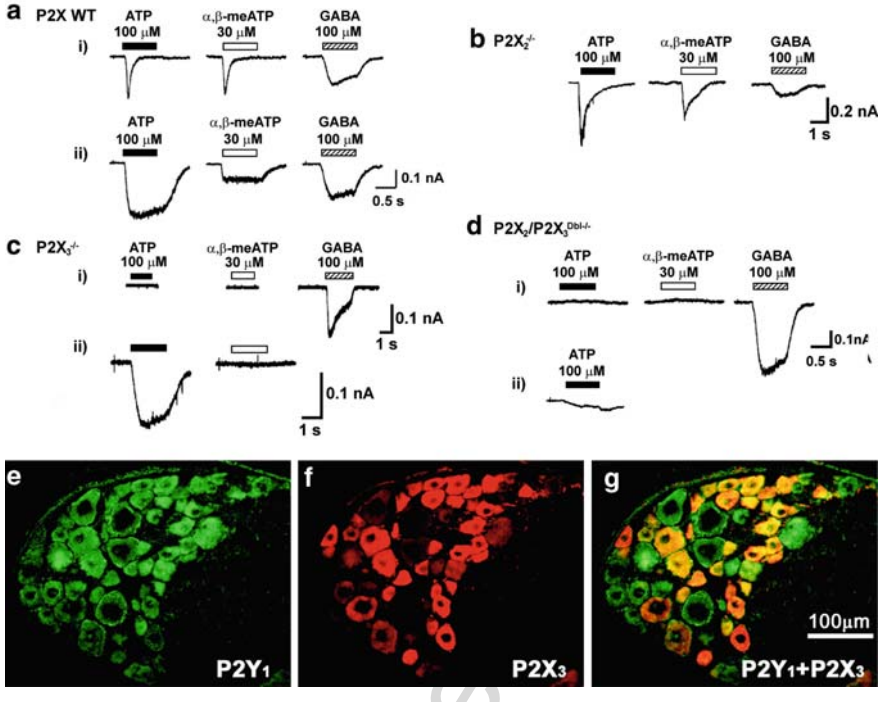


Fig. 1 Dorsal root ganglion (DRG). (**a**–**d**) Whole-cell patch-clamp recordings of DRG neurons from $P2X_2^{-/-}$, $P2X_3^{-/-}$ and $P2X_2/P2X_3^{Dbl-/-}$ mice in response to P2X agonists. (**a**) Wild-type DRG neurons responded to ATP and α,β-methylene-ATP (α,β-meATP) with either rapidly desensitizing (*i*) or sustained (*ii*) responses; a composite response having both rapidly and slowly desensitizing components was also observed in some neurons (data not shown). All DRG neurons examined responded to 100 μM GABA with a sustained inward current. (**b**) In $P2X_2^{-/-}$ mice, DRG neurons all responded to ATP and α,β-meATP with rapidly desensitizing transient responses. (**c**) In $P2X_3^{-/-}$ mice, many DRG neurons failed to respond to either ATP or α,β-meATP, but did respond to 100 μM GABA (*i*). Other $P2X_3^{-/-}$ neurons responded to ATP with a sustained inward current, but failed to respond to α,β-meATP (*ii*). (**d**) In $P2X_2/P2X_3^{Dbl-/-}$ mice, most DRG neurons failed to respond to ATP or α,β-meATP, but did respond to 100 μM GABA (*i*). A small percentage of neurons in double knockout mice gave small, very low amplitude responses to ATP (*ii*), but did not respond to α,β-meATP. (**e**–**g**) Colocalization (**g**) (*yellow/orange*) of $P2Y_1$ receptor immunoreactivity (**e**) (*green*) with $P2X_3$ receptor immunoreactivity (**f**) (*red*) in rat DRG. Examples of double-labelled neurons, $P2X_3$ receptor positive cells that are not double labelled and $P2Y_1$ receptor positive cells that are not $P2X_3$ receptor immunoreactive are shown in **g**. (**a**–**d** Reproduced from Cockayne et al. 2005, with permission from Blackwell Publishing; **e**–**g** reproduced from Ruan and Burnstock 2003, with permission from Springer-Verlag)

about 20% of neurons; Schwann cells expressed $P2Y_2$ mRNA and non-neuronal satellite cells expressed $P2Y_{12}$ and $P2Y_{14}$ mRNA. ATP and UTP produce slow and sustained excitation of sensory neurons in DRG via $P2Y_2$ receptors. $P2Y_1$, $P2Y_2$, $P2Y_4$ and $P2Y_6$ mRNA is expressed on neurons of rat DRG and receptor protein for $P2Y_1$ is localized on over 80% of mostly small neurons (Ruan and Burnstock 2003). Double immunolabelling showed that 73–84% of $P2X_3$ receptor positive neurons

also stained for the $P2Y_1$ receptor (Fig. 1e–g), while 25–35% also stained for the $P2Y_4$ receptor. The findings of patch-clamp studies of cultured neurons from DRG were consistent with both $P2X_3$ and $P2Y_1$ receptors being present in a subpopulation of DRG neurons. Inhibition of N-type voltage-activated calcium channels in DRG neurons by P2Y receptors has been proposed as a mechanism of ADP-induced analgesia. $P2Y_2$ and $P2Y_4$ receptors were strongly expressed in DRG of the cat, as were $P2X_3$ receptors (Ruan et al. 2005). However, there was low expression of $P2Y_1$ receptors compared with more than 80% of $P2Y_1$ receptor positive neurons in rat DRG. Green fluorescent protein studies have shown that there is ADP-induced endocytosis and internalization of P2Y receptors in DRG neurons (Wang et al. 2006).

Adenosine 5′-*O*-(3-thiotriphosphate) enhances nerve growth factor (NGF)-promoted neurite formation in DRG neurons, perhaps via its ability to increase NGF-promoted TrkA activation (Arthur et al. 2005). NTPDase2 has been shown to be present in satellite glial cells in DRG, consistent with evidence for a functional role for ATP in satellite glial cells. Functional expression of $P2X_7$ receptors on non-neuronal glial cells, but not on small-diameter neurons from rat DRG, has been reported.

2.2 Nodose Ganglia

$P2X_2$ and $P2X_3$ receptors are expressed in rat nodose ganglia. ATP, α,β-meATP and 2-MeSATP evoke sustained currents in rat nodose neurons. These responses are inhibited by suramin, pyridoxal phosphate-6-azopheyl-2′,4′-disulphonic acid (PPADS), Cibacron blue and trinitrophenyl ~~(TNP)~~ ATP, but not by diinosine pentaphosphate. Therefore, the α,β-meATP-sensitive persistent responses in nodose neurons resemble the recombinant $P2X_{2/3}$ receptors. Neurons of the mouse nodose ganglion give persistent responses to both ATP and α,β-meATP similar to those seen in the rat and guinea pig. In $P2X_3$ receptor deficient mice, no nodose neurons respond to α,β-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. Thus, the pharmacological evidence is consistent with the 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 (Cockayne et al. 2005). Subpopulations of rat nodose neurons expressed $P2X_{1/3}$ and $P2X_{2/3}$ heteromultimers. Sensory neurons from nodose ganglia express, in addition to $P2X_3$ receptor mRNA, significant levels of $P2X_1$, $P2X_2$, and $P2X_4$ receptor mRNAs, and some of these mRNAs are present in the same cell.

$P2Y_1$ receptors have been demonstrated immunohistochemically in rat and human nodose ganglia. Coexistence of functional P2Y receptors (acting via the

inositol 1,4,5-trisphosphate pathway) and ryanodine receptors and their activation by ATP have been demonstrated in vagal sensory neurons from the rabbit nodose ganglion. Reverse transcription PCR (RT-PCR) has shown $P2Y_1$, $P2Y_2$, $P2Y_4$ and $P2Y_6$ receptor mRNA in rat nodose ganglia (Ruan and Burnstock 2003). $P2Y_1$ receptor immunoreactivity was found in over 80% of the sensory neurons, particularly small-diameter (neurofilament-negative) neurons, while $P2Y_4$ receptors were expressed in more medium- and large-diameter neurons. About 80% of the $P2X_3$ receptor immunoreactive neurons also stained for $P2Y_1$ receptors, while about 30% of the neurons showed colocalization of $P2Y_4$ with $P2X_3$ receptors.

2.3 Trigeminal Ganglia

Most of the facial sensory innervation is provided by nerve fibres originating in the trigeminal ganglion, comprising neurons that transduce mechanical, thermal and chemical stimuli, probably including odorant molecules. In trigeminal ganglia, $P2X_3$ receptor immunoreactivity is found in the cell bodies of both small and large neurons. Lower levels of immunoreactivity to $P2X_1$, $P2X_2$, $P2X_4$ and $P2X_6$ receptors appear to be present in these neurons. Forty percent of $P2X_2$ and 64% of $P2X_3$ receptor expressing cells were IB_4-positive and 33% of $P2X_2$ and 31% of $P2X_3$ receptor expressing cells were NF200-positive (Staikopoulos et al. 2007). About 40% of cells expressing $P2X_2$ receptors also expressed $P2X_3$ receptors and vice versa. Chronically applied NGF upregulated the function of $P2X_3$ receptors in trigeminal neurons without changing transient receptor potential vanilloid 1 (TRPV1) activity. IB_4-positive neurons release ATP by faster exocytosis compared with IB_4-negative neurons which release neuropeptides (Matsuka et al. 2007). Au5
Whole-cell patch-clamp studies of trigeminal neurons showed ATP-activated (both fast and slow) desensitizing currents in the majority of cells examined, but outward or biphasic currents also occurred in a small number of cells (Gu et al. 2006). Different types of cells show different types of ATP-activated currents related to different P2X subunit assemblies (Luo et al. 2006).

$P2Y_1$ and $P2Y_4$ receptor mRNA and protein are also expressed in rat trigeminal ganglia, with many neurons showing colocalization with $P2X_3$ receptors (Ruan and Burnstock 2003). In particular, only a small percentage of IB_4-binding neurons express $P2X_3$ receptors in trigeminal ganglia, whereas many peptidergic neurons express $P2X_3$ receptors.

Satellite glial cells in mouse trigeminal ganglia express P2Y receptors (possibly the $P2Y_1$ subtype). Single-cell calcium imaging demonstrated that both $P2Y_1$ and, to a lesser extent, $P2Y_{2,4,6,12,13}$ receptors on satellite glial cells contribute to ATP-induced calcium-dependent signalling in mixed neuron-glia primary cultures from mouse trigeminal ganglia (Ceruti et al. 2006).

2.4 Petrosal Ganglia

The petrosal ganglion provides sensory innervation of the carotid sinus and carotid body through the carotid sinus nerve. Acetylcholine (ACh) and ATP act as excitatory transmitters between cat glomus cells and petrosal ganglion neurons (Alcayaga et al. 2007), but independently of each other. ATP activates rat, cat and rabbit petrosal ganglia neurons in vitro via P2X receptors and evokes ventilatory reflexes in situ, which are abolished after bilateral chemosensory denervation. Dopamine inhibits ATP-induced responses of neurons of the cat petrosal ganglia.

2.5 Retinal Ganglia

Retinal ganglion cells on the eye receive information from both rods and cones and early papers about purinergic transmission in the retina have been reviewed (Pintor 2000). $P2X_2$ receptors have been identified in retinal ganglion cells, particularly within cone pathways (Puthussery and Fletcher 2006), while $P2X_3$ receptors are associated with both rod and cone bipolar cell axon terminals in the inner plexiform layer (Puthussery and Fletcher 2007). Functional studies have also identified $P2X_{2/3}$ heteromultimeric receptors in cultured rat retinal ganglion cells. $P2X_2$ receptors are also expressed on cholinergic amacrine cells of mouse retina and also GABAergic amacrine cells.

It was proposed that ATP, coreleased with ACh from retinal neurons, modulates light-evoked release of ACh by stimulating a glycinergic inhibitory feedback loop (Neal and Cunningham 1994). RT-PCR at the single-cell level revealed expression of $P2X_2$, $P2X_3$, $P2X_4$ and $P2X_5$ receptor mRNA in approximately one third of the bipolar cells (Wheeler-Schilling et al. 2001), $P2X_7$ receptors were identified on both inner and outer retinal ganglion cell layers of the primate and rat, and electron microscope analysis suggested that these receptors were localized in synapses. Stimulation of $P2X_7$ receptors elevated Ca^{2+} levels and killed retinal ganglion cells (Zhang et al. 2005) and may be involved in retinal cholinergic neuron density regulation.

$P2X_3$ receptors are present on Müller cells. Müller cells release ATP during Ca^{2+} wave propagation. While the potent $P2X_7$ agonist 3′-O-(4-benzoyl)benzoyl ATP killed retinal ganglion cells, this was prevented by the breakdown product, adenosine, via A_3 receptors (Zhang et al. 2006). Evidence has been presented for the involvement of $P2X_7$ receptors in outer retinal processing: $P2X_7$ receptors are expressed postsynaptically on horizontal cell processes as well as presynaptically on photoreceptor synaptic terminals in both rat and marmoset retinas (Puthussery et al. 2006).

2.6 *Intramural Enteric Sensory Neurons*

Most of the data about enteric sensory transmission are based on studies of the guinea pig ileum (Furness et al. 1998). The after hyperpolarization (AH) defined neurons appear to be the enteric sensory neurons, which represent about 30% of the neurons in the myenteric plexus. About 90% of Dogiel type II neurons in the guinea pig ileum exhibit slow AHs and many express the calcium-binding protein calbindin. These neurons are distinct from Dogiel type I, S neurons, which are motor neurons or interneurons. The functional properties of Dogiel type II (AH) sensory neurons have been reviewed recently (Blackshaw et al. 2007).

Several laboratories have studied purinergic signalling in the guinea pig myenteric and submucous neurons (Burnstock 2007). Exogenous and endogenous ATP, released during increase in intraluminal pressure, inhibits intestinal peristalsis in guinea pig. Exogenous ATP depresses peristalsis mostly ~~probably~~ via suramin- and PPADS-insensitive $P2X_4$ receptors, whereas endogenous purines probably act via $P2X_2$ and/or $P2X_3$ and/or $P2X_{2/3}$ receptors sensitive to both suramin and PPADS initiate peristalsis (Bian et al. 2003). ATP plays a major role in excitatory neuro-neuronal transmission in both ascending and descending reflex pathways to the longitudinal and circular muscles of the guinea pig ileum triggered by mucosal stimulation. Experiments with $P2X_2$ and $P2X_3$ receptor knockout mice showed that peristalsis is impaired in the small intestine. $P2X_3$ receptors are dominant on neurons in the submucosal plexus of the rat ileum and distal colon and up to 70% of the neurons express calbindin, a marker for enteric sensory neurons (Xiang and Burnstock 2004a). $P2X_3$ receptor immunoreactivity has also been shown on sensory neurons in the *human* myenteric plexus.

Intracellular recordings from myenteric and submucosal neurons in guinea pig small intestine showed that ATP induced a transient depolarization of most AH-type neurons (Bertrand and Bornstein 2002; Monro et al. 2004) (Fig. 2a, c, d). Fast and slow depolarizations and Ca^{2+} responses of cultured guinea pig ileal submucosal neurons to ATP were mediated by P2X and P2Y receptors respectively. Slow excitatory postsynaptic potentials were mediated by $P2Y_1$ receptors in neurons in the submucosal plexus of guinea pig small intestine. ATP plays a major excitatory role, probably largely via $P2X_2$ receptors, in rat myenteric neurons, whether sensory neurons, motor neurons or interneurons. A $P2Y_1$ receptor has been cloned and characterized from guinea pig submucosa (Gao et al. 2006). About 40–60% of $P2X_3$ receptor immunoreactive neurons were immunoreactive for $P2Y_2$ receptors in the myenteric plexus and all $P2X_3$ receptor immunoreactive neurons expressed $P2Y_2$ receptors in the submucosal plexus (Xiang and Burnstock 2006). About 28–35% of $P2Y_6$ receptor immunoreactive neurons coexist with nitric oxide synthase (NOS), but not with calbindin, while all $P2Y_{12}$ receptor immunoreactive neurons were immunopositive for calbindin and appear to be AH intrinsic primary afferent neurons.

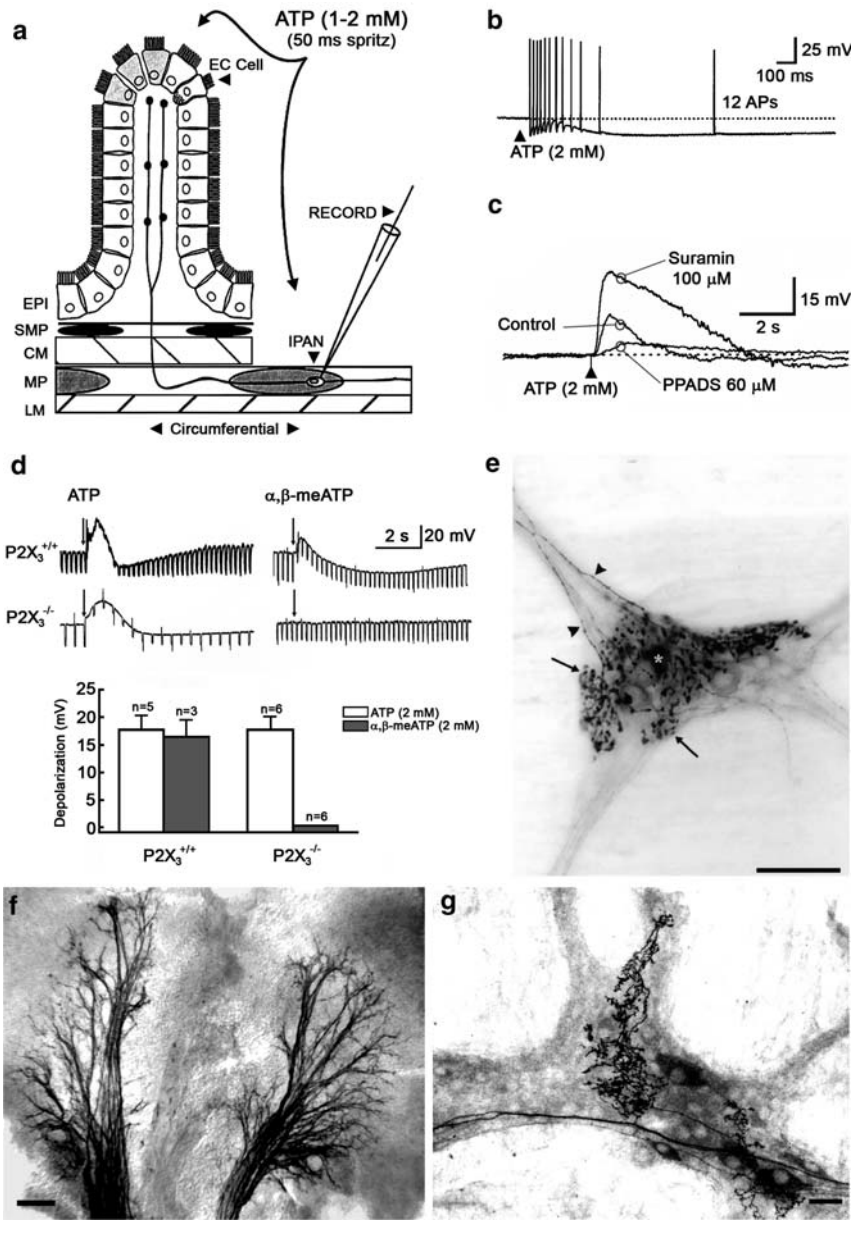


Fig. 2 Enteric sensory neurons. (**a**) Illustration of the experimental arrangement and the relation of the epithelium and the after hyperpolarization (Dogiel type II) sensory nerve terminals. *LM* longitudinal muscle, *MP* myenteric plexus, *CM* circular muscle, *SMP* submucosal plexus, *EPI* epithelium. Note that the intracellular recording electrode (*RECORD*) is impaling myenteric AH neurons [intrinsic primary afferent neurons (*IPAN*) at the *open circle*]. ATP and other agonists were applied to the mucosa and to the cell body of AH neurons via short-duration pressure ejection. Enterochromaffin cells (*EC Cell*) are present in about 1% of the total population

3 Peripheral Sensory Nerve Terminals

Sensory nerve terminals express purinoceptors and respond to ATP in many situations (Burnstock 2000, 2007). However, it has been shown that ATP sensitivity is not necessarily restricted to the terminals; increased axonal excitability to ATP and/or adenosine of unmyelinated fibres in rat vagus, sural and dorsal root nerves as well as human sural nerve has been described. During purinergic mechanosensory transduction, the ATP released from local epithelial cells acts on $P2X_3$, $P2X_{2/3}$ and $P2Y_1$ receptors on sensory nerve endings (see Sect. 5). In addition, released ATP is rapidly broken down by ectoenzymes to ADP (to act on $P2Y_1$, $P2Y_{12}$ and $P2Y_{13}$ receptors) or adenosine (to act on P1 receptors).

Since the seminal studies of Lewis in the 1920s, it has been well established that transmitters released following the passage of antidromic impulses down sensory nerve collaterals during "axon reflex" activity produce vasodilatation of skin vessels. The early work of Holton (1959) showing ATP release during antidromic stimulation of sensory collaterals, taken together with the evidence for glutamate in primary afferent sensory neurons, suggests that ATP and glutamate may be cotransmitters in these nerves. We know now that "axon reflex" activity is widespread in autonomic effector systems and forms an important physiological component of autonomic control (Maggi and Meli 1988; Rubino and Burnstock 1996). Calcitonin gene related peptide (CGRP) and substance P (SP) are well established as coexisting in sensory-motor nerves and, in some subpopulations, ATP is also likely to be a

Fig. 2 (continued) of epithelial cells. (**b**) Representative voltage trace from AH neurons during application of ATP to the mucosa; *dotted lines* in **b** and **c** indicate resting membrane potential. A brief application (100 ms; at the *filled triangle*) of ATP (2 mM) elicited a train of 12 action potentials that showed a slowing in frequency during the 1.1-s duration of the discharge. (**c**) Representative voltage recording from an intrinsic sensory neuron in the myenteric plexus. ATP was applied to the cell body and evoked a short latency depolarization – tetrodotoxin was present to block sodium-dependent action potentials. During superfusion with pyridoxal phosphate-6-azopheyl-2′,4′-disulphonic acid (60 μM), the ATP-evoked depolarization was blocked, whereas in the presence of suramin (100 μM), it was potentiated. (**d**) Effect of ATP and α,β-meATP in AH neurons from $P2X_3^{+/+}$ and $P2X_3^{-/-}$ mice. *Top panels*: Representative responses caused by ATP and α,β-meATP. ATP depolarized AH neurons from both types of mice. α,β-meATP caused depolarization of AH neurons in tissues from $P2X_3^{+/+}$ but not $P2X_3^{-/-}$ mice. *Bottom panel*: Pooled data from experiments illustrated in the *top panels*. (**e**) Morphology of intraganglionic laminar endings (IGLEs) revealed by $P2X_2$ receptor immunoreactivity in a group of three to four IGLEs at the surface of a myenteric ganglion in the duodenum. The axons that lead to the IGLEs also have $P2X_2$ receptor immunoreactivity (*arrowheads*). The IGLEs consist of clumps of axon dilatations, varying from small swellings (*arrows*) to large lamellae, one of which is indicated by an *asterisk*. *Scale bar* 50 μm. (**f**) $P2X_3$ receptor immunoreactivity in extrinsic vagal nerve fibres in the developing rat stomach with short branches at the ends at embryonic day 12. *Scale bar* 250 μm. (**g**) $P2X_3$ receptor immunoreactive neurons and IGLEs in myenteric plexus of rat stomach at postnatal day 60. *Scale bar* 30 μm. (**a**, **b** Reproduced from Bertrand and Bornstein 2002, with permission from the Society of Neuroscience; **c** reproduced from Bertrand 2003, with permission from Sage Publications; ~~(**d**)~~ reproduced from Bian et al. 2003, with permission from Blackwell Publishing; ~~(**e**)~~ reproduced from Castelucci et al. 2003, with permission from Springer~~-Verlag~~; ~~(**f**, **g**)~~ reproduced from Xiang and Burnstock 2004b, with permission from Springer~~-Verlag~~)

cotransmitter (Burnstock 1993). Concurrent release of ATP and SP from guinea pig trigeminal ganglionic neurons in vivo has been described (Matsuka et al. 2001).

3.1 Carotid Body

The ventilatory response to decreased oxygen tension in the arterial blood is initiated by excitation of specialized oxygen-sensitive chemoreceptor cells in the carotid body that release neurotransmitter to activate endings of the sinus nerve afferent fibres. ATP and adenosine were shown early on to excite nerve endings in the carotid bifurcation (Lahiri et al. 2007).

Large amounts of adenine nucleotides are localized in glomus cells, stored within specific granules together with catecholamines and proteins, and there is evidence of ATP release from carotid chemoreceptor cells. Corelease of ATP and ACh from type I glomus chemoreceptor cells is a likely mechanism for chemosensory signalling in the carotid body in vivo (Nurse 2005; Zapata 2007). The ATP released during hypoxic and mechanical stimulation was shown to act on $P2X_{2/3}$ receptors on nerve fibres arising from the petrosal ganglion (Reyes et al. 2007). Immunoreactivity for $P2X_2$ and $P2X_3$ receptor subunits has been localized on rat carotid body afferent terminals surrounding clusters of glomus cells. $P2X_2$ and $P2X_{2/3}$ receptor deficiency resulted in a dramatic reduction in the responses of the carotid sinus nerve to hypoxia in an in vitro mouse carotid body-sinus nerve preparation (Rong et al. 2003) (Fig. 3). ATP mimicked the afferent discharge and PPADS blocked the hypoxia-induced discharge. ATP induces a rise in intracellular Ca^{2+} concentration in rat carotid body cultured glomus cells. Evidence that this mechanism is involved in hypercapnia as well as in hypoxia came from CO_2/pH chemosensory signalling in co-cultures of rat carotid body and petrosal neurons (Zhang and Nurse 2004). In fresh tissue slices of rat carotid body, low glucose stimulated ATP secretion (Zhang et al. 2007a, ~~b~~). ATP, acting on $P2X_2$ receptors, contributed to modified chemoreceptor activity after *chronic* hypoxia, indicating a role for purinergic mechanisms in the adaptation of the carotid body in a chronic low-O_2 environment (He et al. 2006).

3.2 Lung

Pulmonary neuroepithelial bodies (NEBs) and more recently subepithelial receptor-like endings associated with smooth muscle (SMARs) have been shown to serve as sensory organs in the lung (Brouns et al. 2006). $P2X_3$ and $P2X_{2/3}$ receptors are expressed on a subpopulation of vagal sensory fibres that supply NEBs and SMARs ~~with~~ their origin in the nodose ganglia (Fig. 4a). Sensory afferent fibres within the respiratory tract, which are sensitive to ATP, probably largely via $P2X_{2/3}$ receptors, have been implicated in vagal reflex activity (Taylor-Clark and

Au6

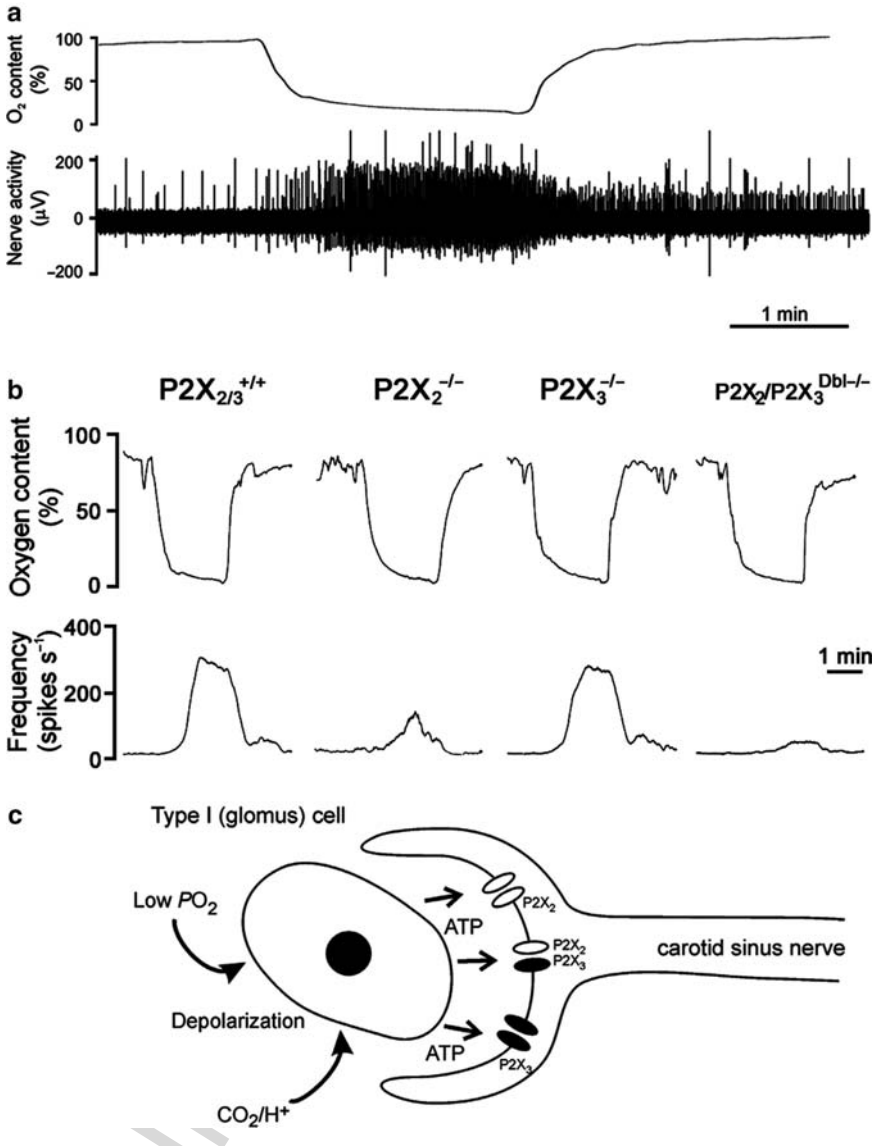


Fig. 3 Carotid body. (**a**) Representative recording of the afferent nerve responses to hypoxia in the isolated carotid body sinus nerve preparation taken from a wild-type mouse. Typical traces of changes in PO_2 and raw nerve activity. (**b**) Effects of ATP on carotid sinus nerve activity in wild-type mice and in $P2X_2$ ($P2X_2^{-/-}$), $P2X_3$ ($P2X_3^{-/-}$) and $P2X_2$ and $P2X_3$ ($P2X_2/P2X_3^{Db-/-}$)-deficient mice. (**c**) Hypothetical model of ATP involvement in the carotid body. P2X receptors containing the $P2X_2$ subunit play a pivotal role in transmitting information about arterial PO_2 and PCO_2 levels. A decrease in PO_2 or an increase in PCO_2/H^+ activates glomus cells, which release ATP as the main transmitter to stimulate afferent terminals of the sinus nerve via interaction with P2X receptors that contain the $P2X_2$ subunit, with or without the $P2X_3$ subunit. (**a** Reproduced from Rong et al. 2003, with permission from the Society of Neuroscience; ~~(**b**)~~ courtesy of Weifang Rong; **c** reproduced from Spyer et al. 2004, with permission from Blackwell Publishing)

Au14

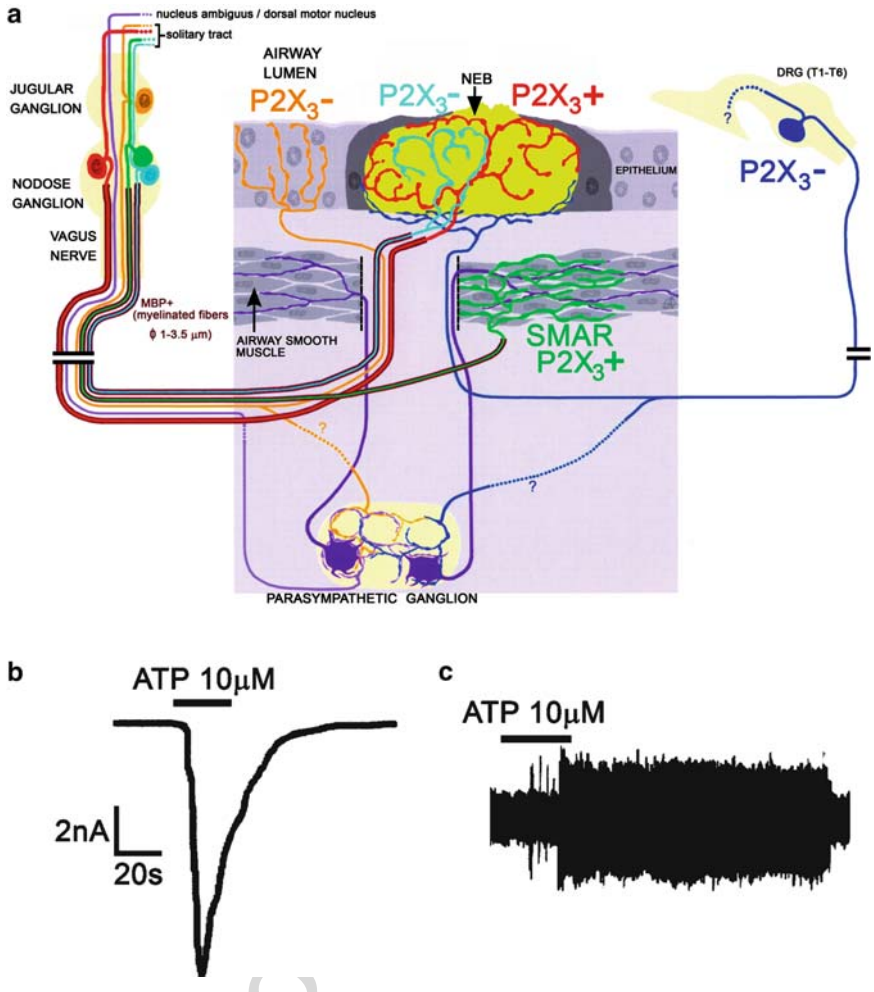


Fig. 4 Lung. (**a**) The main innervation of airway smooth muscle and of the sensory innervation of complex neuroendothelial body (NEB) receptors in rat airways. Nerve fibre populations are colour-coded. The *central* part of the scheme shows airway smooth muscle that receives laminar nerve terminals (SMAR; *green*) immunopositive for $P2X_3$ receptors that intercalate between the smooth muscle cells and nerve terminals from postganglionic parasympathetic neurons located in an airway ganglion (*bottom*; cholinergic neurons *purple*). The *top centre* part of the scheme represents a pulmonary NEB (*yellow*) and its extensive interactions with sensory nerve terminals. The *top left* part shows the myelinated vagal nodose afferent fibres immunopositive for $P2X_3$ receptors (*red*) and sensory fibres (*light blue*) that innervate the NEB but do not express $P2X_3$ receptors; C-fibre afferents that originate from the vagal jugular ganglion (*orange*) innervate the non-endocrine epithelium of large-diameter airways. The *top right* part represents dorsal root C-fibre afferents (*dark blue*) that innervate NEB but do not express $P2X_3$ receptors. ϕ diameter. (**b**) Representative inward ionic currents obtained with whole-cell patch recordings of nodose neurons retrogradely labelled from the lung. All neurons responded to ATP with a rapid inward current. (**c**) Representative extracellular recording of action potential discharge from

Undem 2006) (Fig. 4b, c), as well as in the cough and bradypneic reflexes (see Sect. 6.7). Quinacrine staining of NEBs indicates the presence of high concentrations of ATP in their secretory vesicles and it has been suggested that ATP is released in response to both mechanical stimulation during high-pressure ventilation and during hypoxia (Rich et al. 2005). NEBs are oxygen sensors especially in early development, before the carotid system has matured (Brouns et al. 2003).

Vagal C-fibres innervating the pulmonary system are derived from cell bodies situated in two distinct vagal sensory ganglia: the jugular (superior) ganglion neurons project fibres to the extrapulmonary airways (larynx, trachea, bronchus) and the lung parenchymal tissue, while the nodose (inferior) neurons innervate primarily structures within the lungs. Nerve terminals in the lungs from both jugular and nodose ganglia responded to capsaicin and bradykinin, but only the nodose C-fibres responded to α,β-meATP. In a study of bronchopulmonary afferent nerve activity of a mouse isolated perfused nerve-lung preparation it was found that C-fibres could be subdivided into two groups: fibres that conduct action potentials at less than 0.7 ms^{-1} and are responsive to capsaicin, bradykinin and ATP; and fibres that conduct action potentials on an average of 0.9 ms^{-1} and respond vigorously to ATP, but not to capsaicin or bradykinin (Kollarik et al. 2003). Both the TRPV1 receptor and P2X receptors mediate the sensory transduction of pulmonary reactive oxygen species, especially H_2O_2 and OH, by capsaicin-sensitive vagal lung afferent fibres.

The visceral pleura of the airways is often considered to be insensitive to painful stimuli and to lack sensory innervation. However, a recent paper has identified $P2X_3$ receptors on sensory fibres supplying the pleura, which appear to be myelinated and have a spinal origin (Pintelon et al. 2007).

3.3 Gut

ATP and α,β-meATP activate submucosal terminals of intrinsic sensory neurons in the guinea pig intestine (Bertrand and Bornstein 2002), supporting the hypothesis of Burnstock (2001a) that ATP released from mucosal epithelial cells has a dual action on $P2X_3$ and/or $P2X_{2/3}$ receptors in the subepithelial sensory nerve fibres. ATP acts on the terminals of low-threshold intrinsic enteric sensory neurons to initiate or modulate intestinal reflexes and acts on the terminals of high-threshold extrinsic sensory fibres to initiate pain (see Sects. 5.3, 6.1). Thirty-two percent of retrogradely labelled cells in the mouse DRG at levels T8–L1 and L6–S1, supplying sensory nerve fibres to the mouse distal colon, were immunoreactive for $P2X_3$ receptors (Robinson

Fig. 4 (continued) a nodose C-fibre ending with a receptive field within the right lung caused by tracheal infusion of ATP (10 μM). (**a** Modified from Adriaensen et al. 2006, and reproduced with permission from The American Physiological Society; (**b**) Reproduced from Undem et al. 2004, with permission from Blackwell Publishing; **c** reproduced from Taylor-Clark and Undem 2006, with permission from The American Physiological Society)

et al. 2004). Extrinsic and possibly intrinsic sensory nerves associated with mucosal epithelial cells appear to be sensitive to pH, probably via $P2X_2$ and $P2X_{2/3}$ receptors (Holzer 2007).

Intraganglionic laminar nerve endings (IGLEs) are specialized mechanosensory endings of vagal afferent nerves in the rat stomach, arising from the nodose ganglion; they express $P2X_2$ and $P2X_3$ receptors and are probably involved in physiological reflex activity, especially in early postnatal development (Castelucci et al. 2003; Xiang and Burnstock 2004b) (Fig. 2e–g). α,β-meATP caused concentration-dependent excitation of IGLEs of vagal tension receptors in the guinea pig oesophagus, but evidence was presented against chemical transmission being involved in the mechanotransduction mechanism (Zagorodnyuk et al. 2003). A subpopulation of nodose vagal afferent nociceptive nerves sensitive to $P2X_3$ receptor agonists was later identified and shown to be different from the non-nociceptive vagal nerve mechanoreceptors (Yu et al. 2005).

3.4 Urinary Bladder

In the absence of $P2X_3$ receptors in mouse knockouts, the bladder is hyperactive (Cockayne et al. 2000; Vlaskovska et al. 2001). It has been claimed that suburothelial myofibroblast cells isolated from human and guinea pig bladder that are distinct from epithelial cells provide an intermediate regulatory step between urothelial ATP release and afferent excitation involved in the sensation of bladder fullness (Wu et al. 2004). The majority of lumbosacral neurons (93%) supplying the bladder were sensitive to α,β-meATP, compared with 50% of thoracolumbar neurons (Dang et al. 2004). Almost all sensory neurons in lumbosacral DRG innervating the bladder coexpress P2X, ASIC, and TRPV1 receptors, but not those in the thoracolumbar DRG neurons supplying the bladder, indicating that pelvic and hypogastric afferent pathways to the bladder are structurally and functionally distinct.

3.5 Inner Ear

The inner ear encompasses three organs: the cochlea, responsible for hearing; the vestibule, sensitive to gravity and acceleration; and the endolymphatic sac, devoid of sensory function. A role for ATP as a cotransmitter generating intracellular Ca^{2+} currents in cochlea inner hair cells was first proposed in 1990 (Housley et al. 2006). Later, various P2X and P2Y receptor subtypes were shown to be expressed in other cell types in the cochlea, including outer hair cells, Henson cells and Deiters cells in the organ of Corti. Physiological studies suggested that ATP acts as a neurotransmitter, but probably not as part of the efferent system as previously supposed, but rather as a cotransmitter with glutamate in auditory afferent nerves activated by

glutamate released from hair cells and acting postsynaptically on the spiral ganglion neuron afferent dendrites (Housley et al. 2006). There are about 50,000 primary afferent neurons in the human cochlear and about half express $P2X_2$ (or $P2X_2$ variants) and probably $P2X_3$ receptors. ATP is released from K^+-depolarized organ of Corti in a Ca^{2+}-dependent manner and an increase in ATP levels in the endolymph has been demonstrated during sound exposure. The P2 receptor antagonist PPADS attenuated the effects of a moderately intense sound on cochlea mechanics. Nitric oxide enhances the ATP-induced intracellular Ca^{2+} increase in outer hair cells (Shen et al. 2006). $P2Y_2$ and/or $P2Y_4$ receptors mediate intercellular calcium wave propagation in supporting and epithelial cells in the organ of Corti (Piazza et al. 2007). Spiral ganglion neurons, located in the cochlear, convey to the brain stem the acoustic information arising from the mechanoelectrical transduction of the inner hair cells, express P2X receptors and are responsive to ATP (Dulon et al. 2006). P2X receptor signalling inhibits brain derived neurotrophic factor mediated spiral ganglion neuron development in the neonatal rat cochlea, when synaptic reorganization is occurring in the cochlea (Greenwood et al. 2007).

3.6 Eye

Amacrine cells and the pigment epithelial cells themselves have been shown to release ATP as well as retinal astrocytes and inner retinal amacrine-like neurons (Burnstock 2007). ATP is also released from antidromically stimulated sensory nerve endings in the ciliary body (Maul and Sears 1979).

3.7 Nasal Organ

There are three types of epithelial cells in the nasal mucosa: non-keratinized, stratified squamous epithelium, respiratory epithelium and olfactory epithelium. Primary olfactory neurons lie in the olfactory epithelium and function to detect odiferous substances, sending information to the olfactory cortex. $P2X_2$ receptors are localized on different subpopulations of primary olfactory neurons located both in the olfactory epithelium and in vomeronasal organs, and on sensory fibres arising from the trigeminal ganglion (Gayle and Burnstock 2005).

Odorant recognition is mediated by olfactory receptors predominantly situated on the microvilli of olfactory receptor neurons in the nasal organ. Nucleotides act via purinoceptors on olfactory neurons as well as sustentacular supporting cells (Hegg et al. 2003). ATP released from olfactory epithelium modulates odour sensitivity and nociception. The majority of nasal trigeminal neurons lacked $P2X_3$ receptor–mediated currents, but showed $P2X_2$-mediated responses when stimulated by ATP (Damann et al. 2006).

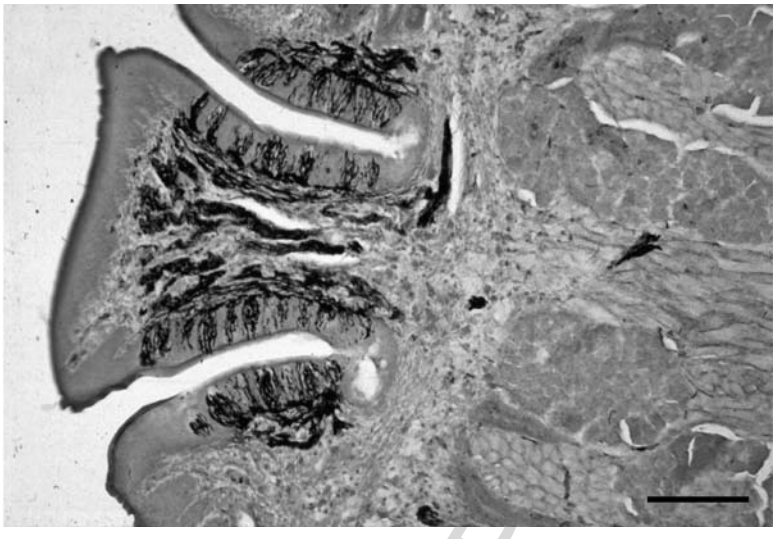


Fig. 5 Tongue. Distribution of $P2X_3$ receptor immunoreactivity in circumvallate papillae in rat tongue. *Scale bar* 200 μm. (Courtesy of Atossa Alavi)

3.8 Taste Buds

Taste bud cells and associated sensory nerve fibres express P2 receptors, including $P2X_2$ and $P2X_3$ receptor subunits (Bo et al. 1999) (Fig. 5) and $P2Y_1$ receptors (Kataoka et al. 2004). ATP is the key transmitter acting via $P2X_2$ and $P2X_3$ receptors on taste receptor cells detecting chemicals in the oral cavity (Finger et al. 2005). These authors showed that genetic elimination of $P2X_2$ and $P2X_3$ receptors abolished responses of the taste nerves, although the nerves remained responsive to touching, temperature and menthol and reduced responses to sweeteners, glutamate and bitter substances. They also showed that a bitter mixture containing denatonium and quinine stimulated release of ATP from the taste epithelium. Type A (but not type B and C) taste cells, defined electrophysiologically, which appear to be identical to type II cells, defined morphologically, have been shown to release ATP via connexin or pannexin hemichannels to activate $P2X_3$ receptors on sensory nerve endings (Romanov et al. 2007; Huang et al. 2007). Dystonin disruption, produced in mutant mice, resulted in a decrease in the number of vagal and glossopharyngeal sensory neurons, and in the number of taste buds as well as in the number of $P2X_3$ receptor labelled neurons and their peripheral endings in taste bud epithelium (Ichikawa et al. 2006). Other papers present data that suggest that $P2Y_2$ and $P2Y_4$ receptors also play a role in mediating taste cell responses to ATP and UTP (Bystrova et al. 2006). NTPDase2 has been shown to have a dominant presence on type 1 cells in mouse taste papillae (Bartel et al. 2006).

3.9 Skin, Muscle and Joints

It has been suggested that ATP receptors on keratinocytes might play a role in a variety of skin sensations (Denda et al. 2007). Ca^{2+} waves in human epidermal keratinocytes mediated by extracellular ATP, produce intracellular Ca^{2+} concentration elevation in DRG neurons, suggesting a dynamic cross talk between skin and sensory neurons mediated by extracellular ATP (Koizumi et al. 2004). ATP inhibits the heat response of the C-fibre polymodal receptor on a rat skin-nerve preparation at low concentrations, but facilitates it at high concentrations (Yajima et al. 2005).

P2 receptors on the endings of thin fibre muscle afferents play a role in evoking both the metabolic and the mechanoreceptor components of the exercise pressor reflex. PPADS attenuated the pressor response to contraction of the triceps muscle. ATP has been shown to be an effective stimulant of group IV receptors in mechanically sensitive muscle afferents (Kindig et al. 2007). Arterial injection of α,β-meATP in the blood supply of the triceps surae muscle evoked a pressor response that was a reflex localized to the cat hind limb and was reduced by P2X receptor blockade.

Sensory nerve fibres arising from the trigeminal ganglion supplying the temporomandibular joint have abundant receptors that respond to capsaicin, protons, heat and ATP; retrograde tracing revealed 25, 41 and 52% of neurons supplying this joint exhibited TRPV1 and $P2X_3$ receptors, respectively (Ichikawa et al. 2004).

3.10 Heart

An ATP-triggered vagal reflex has been described leading to suppression of sinus mode automaticity and atrioventricular nodal conduction (Pelleg and Hurt 1990). This is probably mediated by $P2X_{2/3}$ receptors located on vagal sensory nerve terminals in the left ventricle and lung (McQueen et al. 1998). This supports the hypothesis that ATP released from ischaemic myocytes is a mediator of atropine-sensitive bradyarrhythmias associated with left ventricular myocardial infarction (Xu et al. 2005).

4 Central Sensory Nerves

While the main areas of the central nervous system (CNS) concerned with control of autonomic function involving sensory nerves are the spinal cord, brain stem and hypothalamus (Burnstock 2007), the prefrontal cortex is implicated in the integration of sensory, limbic and autonomic information (Groenewegen and Uylings 2000). It seems likely that P1, P2X and P2Y receptors are involved in neurotransmission and

neuromodulation of sensory pathways in the somatic, visual, olfactory, auditory and gustatory cortex (North and Verkhratsky 2006).

4.1 Spinal Cord

Spinal circuits, spinal afferent influx as well as descending influences from brain stem and hypothalamus work together in the integrative activities of the preganglionic sympathetic neurons, which regulate the activity on many organs. There was early identification of dense areas of acid phosphatase and 5′-nucleotidase activity in the substantia gelatinosa of the spinal cords of rats and mice and the possible implication for purinergic transmission was raised (Burnstock 2007).

P2X receptors mediate sensory synaptic transmission between primary afferent fibres and spinal dorsal horn neurons (Li et al. 1998). ATP-evoked increases in intracellular calcium were demonstrated in both neurons and glia of the dorsal spinal cord. ATP was shown to inhibit slow depolarization via P2Y receptors in substantia gelatinosa neurons. A recent study has identified $P2Y_1$ and $P2Y_4$ receptor mRNA in subpopulations of dorsal horn neurons (Kobayashi et al. 2006). $P2X_3$ immunoreactivity is present on the axon terminals of DRG neurons that extend across the entire mediolateral extent of inner lamina II of the dorsal horn. The immunolabelled nerve profiles in lamina II for $P2X_3$ receptors are located largely on terminals with ultrastructural characteristics of sensory afferent terminals (Llewellyn-Smith and Burnstock 1998). In contrast, although $P2X_2$ immunoreactivity is most prominent in lamina II, it is also seen in deeper layers, and only rarely overlaps with $P2X_3$ immunoreactivity. A TNP-ATP-resistant P2X ionic current has been reported on the central terminals of capsaicin-insensitive Aδ-afferent fibres that play a role modulating sensory transmission to lamina V nerves. At central terminals of primary afferent neurons, ATP has been shown to act both presynaptically facilitating glutamate release (Nakatsuka and Gu 2006) and postsynaptically (Fyffe and Perl 1984). P2X receptors are also expressed on glycinergic presynaptic nerve terminals.

ATP has been shown to be released from dorsal spinal cord synaptosomes. Morphine and capsaicin release purines from capsaicin-sensitive primary afferent nerve terminals in the spinal cord. In addition to acting as a fast excitatory synaptic transmitter, ATP facilitates excitatory transmission by increasing glutamate release and enhancing inhibitory neurotransmission mediated by both GABA and glycine. A different P2X receptor subtype (perhaps $P2X_{1/5}$ or $P2X_{4/6}$) was involved in long-lasting modulation in lamina V (Nakatsuka et al. 2003). The authors concluded that differential modulation of sensory inputs into different sensory regions by P2X receptor subtypes represents an important mechanism of sensory processing in the spinal cord dorsal horn. Blockade of P2X receptors in the dorsal horn with PPADS attenuates the cardiovascular "exercise pressor reflex" to activation of muscle afferents, while stimulation of P2X receptors enhances the reflex response (Gao et al. 2005).

4.2 Nucleus Tractus Solitarius

The nucleus tractus solitarius (NTS) (particularly neurons in the caudal NTS) is a central relay station for relaying viscerosensory information to respiratory, cardiovascular and digestive neuronal networks. Extracellular purines have been claimed to be the primary mediators signalling emergency changes in the internal environment in the CNS. Stimulation of P2X receptors in the NTS evokes hypotension with decreases in both cardiac output and total peripheral resistance (Kitchen et al. 2001). Injection of adenosine into the NTS produced dose-related decreases in heart rate and systolic and diastolic blood pressures. NTS A_{2A} receptor activation elicits hind limb vasodilatation. ATP and β,γ-methylene-ATP (β,γ-meATP) produced dose-related potent vasodepressor and bradycardic effects, suggesting that P2 as well as P1 receptors were involved. Hindquarter vasodilatation during defence reactions is mediated by P2X receptors in the NTS (Korim et al. 2007). Patch-clamp studies of neurons dissociated from rat NTS revealed P2 receptor-mediated responses and microinjection of P2 receptor agonists into the subpostremal NTS in anaesthetized rats produced reduction of arterial blood pressure probably via a $P2X_1$ or a $P2X_3$ receptor subtype, since α,β-meATP was particularly potent. The actions of ATP and adenosine in the NTS may be functionally linked to selectively coordinate the regulation of regional vasomotor tone.

Microinjections into the caudal NTS of anaesthetized spontaneously breathing cats showed that α,β-meATP elicited a distinct pattern of cardiorespiratory response, namely dose-related decrease in tidal volume and respiratory minute volume; at higher doses a pronounced apnoea was produced. This suggested that a P2X receptor was present, perhaps involved in the processing of sensation from pulmonary receptors related to the Breuer–Hering and pulmonary C-fibre reflexes. Impaired arterial baroreflex regulation of heart rate after blockade of P2 receptors in the NTS has been reported. Microinjection of ATP into caudal NTS of awake rats produces respiratory responses (Antunes et al. 2005) and purinergic mechanisms are probably involved in the sympathoexcitatory component of the chemoreflex (Braga et al. 2007). It has been suggested that there is a sensory afferent selective role of P2 receptors in the NTS for mediating the cardiac component of the peripheral chemoreceptor reflex (Paton et al. 2002). Activation of NTS A_1 receptors differentially inhibits baroreflex pathways controlling regional sympathetic outputs (Scislo et al. 2007).

The immunohistochemical distribution of P2X receptor subtypes in the NTS of the rat and colocalization of $P2X_2$ and $P2X_3$ immunoreactivity has been described in the NTS. At the electron microscope level, $P2X_3$ receptor positive boutons have been shown to synapse on dendrites and cell bodies and have complex synaptic relationships with other axon terminals and dendrites (Llewellyn-Smith and Burnstock 1998). $P2X_2$ receptors have been localized presynaptically in vagal afferent fibres in rat NTS. A whole-cell patch-clamp study of neurons in the caudal NTS led to the conclusion that ATP activates presynaptic $P1(A_1)$ receptors after breakdown to adenosine, reducing evoked release of glutamate from the primary afferent nerve terminals. Purinergic and vanilloid receptor activation releases glutamate from

separate cranial afferent terminals in the NTS corresponding to myelinated and unmyelinated pathways in the NTS.

4.3 Ventrolateral Medulla

The ventrolateral medulla (VLM) contains a network of respiratory neurons that are responsible for the generation and shaping of respiratory rhythm; it also functions as a chemoreceptive area mediating the ventilating response to hypercapnia. Evidence has been presented that ATP acting on $P2X_2$ receptors expressed in VLM neurons influences these functions (Gourine et al. 2003). Recent studies suggest that P2X receptors on neurons in the raphe nucleus are also involved in respiratory regulation (Cao and Song 2007). It has also been shown in neonatal rats that respiratory rhythm generating networks in the pre-Bötzinger complex are very sensitive to $P2Y_1$ receptor activation and suggest a role for $P2Y_1$ receptors in respiratory motor control, particularly in the excitation of rhythm that occurs during hypoxia (Lorier et al. 2007).

Evidence has been presented to suggest that CO_2-evoked changes in respiration are mediated, at least in part, by P2X receptors in the retrofacial area of the VLM (Gourine 2005). CO_2–P2X-mediated actions were observed only in inspiratory neurons that have purinoceptors with pH sensitivity (characteristic of the $P2X_2$ receptor subtype) that could account for the actions of CO_2 in modifying ventilatory activity. During hypoxia, release of ATP in the VLM plays an important role in the hypoxic ventilatory response in rats. Adenosine acts as a neuromodulator of a variety of cardiorespiratory reflexes.

Intrathecal application of P2X receptor agonists and antagonists indicates that $P2X_3$ or $P2X_{2/3}$ receptors on the trigeminal primary afferent terminals in the medullary dorsal horn (trigeminal subnucleus caudalis) enhance trigeminal sensory transmission (Jennings et al. 2006).

4.4 Sensory Nuclei

$P1(A_1)$ adenosine receptor agonists presynaptically inhibit both GABAergic and glutamatergic synaptic transmission in periaqueductal grey neurons and adenosine suppresses excitatory glutamatergic inputs to rat hypoglossal motoneurons (Burnstock 2007). This is evidence for multiple P2X and P2Y subtypes in the rat medial vestibular nucleus.

P2X receptors are expressed in the medial nucleus of the trapezoid body of the auditory brain stem, where they act to facilitate transmitter release in the superior olivary complex (Watano et al. 2004). Although ATP potentiates release at both excitatory and inhibitory synapses, it does so via different P2X receptor subtypes expressed at different locations: $P2X_3$ receptors on cell bodies or axons of excitatory

pathways and $P2X_1$ receptors on the presynaptic terminals of inhibitory pathways. A_1 rather than P2X receptors have been implicated during high-frequency glutamatergic synaptic transmission in the calyx of Held (Wong et al. 2006). P2 receptors modulate excitability, but do not mediate pH sensitivity of respiratory chemoreceptors in the retrotrapezoid nucleus on the ventral surface of the brain stem (Mulkey et al. 2006).

4.5 Trigeminal Mesencephalic Nucleus

Although the trigeminal mesencephalic nucleus (MNV) is located in the CNS, it contains cell bodies of primary afferent neurons that relay proprioceptive information exclusively. The MNV is known to contain mRNA for $P2X_2$, $P2X_4$, $P2X_5$ and $P2X_6$ subtypes. With in situ hybridization studies, higher levels of mRNA for $P2X_5$ were found in this nucleus than in any other brain area. ATP-gated ion channels (P2X receptors) were described in rat trigeminal MNV proprioceptive neurons from whole-cell and outside-out patch-clamp recording, possibly mediated by $P2X_5$ receptor homomultimers and $P2X_{2/5}$ heteromultimers (Patel et al. 2001).

4.6 Locus Coeruleus

There were early reports of modulation of neuronal activities in the locus coeruleus (LC) by adenosine. The first report of the action (depolarization) of ATP on P2 receptors on neurons in LC was by Harms et al. (1992). α,β-Methylene ADP was later shown to increase the firing rate of rat LC neurons. P2Y receptors are also present on LC neurons (Frohlich et al. 1996). Intracellular recordings from slices of rat LC led to the suggestion that ATP may be released either as the sole transmitter from purinergic neurons terminating in the LC or as a cotransmitter with noradrenaline from recurrent axon collaterals or dendrites of the LC neurons themselves (Poelchen et al. 2001). Microinjection of ATP or α,β-meATP into LC (and periaqueductal grey matter) led to changes in bladder function and arterial blood pressure (Rocha et al. 2001).

4.7 Area Postrema

Injection of adenosine into the area postrema (AP) produced decreased heart rate and systolic and diastolic blood pressure. Dense areas of $P2X_2$ receptor immunoreactivity were demonstrated in the rat AP and excitatory effects of ATP in rat AP neurons have been demonstrated (Sorimachi et al. 2006).

4.8 Hypothalamus

ATP and α,β-meATP excite neurosecretory vasopressin cells in the supraoptic nucleus (SON), an effect blocked by suramin. Suramin also blocked excitation produced by vagus nerve stimulation. There is evidence for cotransmitter release of ATP with ~~noradrenaline~~ at synapses in the hypothalamus stimulating vasopressin and oxytocin release (Song and Sladek 2006). ATP and the α_1-adrenoceptor agonist phenylephrine evoke synergistic stimulation of vasopressin and oxytocin release from the hypothalamoneurohypophyseal systems and the authors speculate that this allows for a sustained elevation of vasopressin release in response to extended stimuli such as severe haemorrhage, chronic hypotension or congestive heart failure. Excitatory effects of ATP via P2X receptors in acutely dissociated ventromedial hypothalamic neurons have been described. A role for adenosine A_1 receptors in mediating cardiovascular changes evoked during stimulation of the hypothalamic defence area has been postulated.

Purinergic regulation of stimulus-secretion coupling in the neurohypophysis has been reported. Ultrastructural localization of both $P2X_2$ and $P2X_6$ receptor immunoreactivity at both pre- and postsynaptic sites in the rat hypothalamoneurohypophyseal system has been described (Loesch and Burnstock 2001). From a study of the expression of P2X receptor subtypes in the SON using RT-PCR, in situ hybridization, Ca^{2+} imaging and whole-cell patch-clamp techniques, it was concluded that $P2X_3$ and $P2X_4$ receptors were predominant, but that $P2X_7$ receptors were also present. A ~~recent~~ study has shown that $P2X_5$ receptors are expressed on neurons containing vasopressin and NOS in the rat hypothalamus (Xiang et al. 2006). P2Y as well as P2X receptors mediate increases in intracellular calcium in supraoptic neurons produced by ATP (Song et al. 2007).

It has been suggested that ATP, cosecreted with vasopressin and oxytocin, may play a key role in the regulation of stimulus-secretion coupling in the neurohypophysis by acting through $P2X_2$ receptors increasing AVP release, and after breakdown to adenosine, acting via $P1(A_1)$ receptors (inhibiting N-type Ca^{2+} channels) to decrease neuropeptide release. Evidence for the involvement of purinergic signalling in hypothalamus and brain stem nuclei in body temperature regulation has been presented (Gourine et al. 2002). Early studies of the roles of adenosine in the hypothalamus have been reviewed (Burnstock 2003). Adenosine deaminase containing neurons in the posterior hypothalamus innervate mesencephalic primary sensory neurons, perhaps indicating purinergic control of jaw movements.

ATP injected into the paraventricular nucleus stimulates release of AVP, resulting in antidiuretic action through renal AVP (V_2) receptors, and ATP (but not ADP, AMP or adenosine) injected into the SON also decreased urine outflow (Mori et al. 1994). Stimulation of the hypothalamic defence area produces autonomic responses that include papillary dilatation, pilorection, tachypnoea, tachycardia and a marked pressor response. Luteinizing hormone releasing hormone (LHRH) is released from the hypothalamus in pulses at hourly intervals, which is essential for the maintenance of normal reproductive function. Studies of an in vivo culture preparation of

LHRH neurons show that ATP stimulates LHRH release, probably via $P2X_2$ and $P2X_4$ receptor subtypes, and may be involved in synchronization of the Ca^{2+} oscillations that appear to underlie the pulsatile release of LHRH (Terasawa et al. 2005). The authors also speculate that glial cells expressing $P2Y_1$ and $P2Y_2$ receptors may also participate in this process. $P2X_{1–6}$ receptor subunits are present on paraventricular nucleus neurons projecting to the rostral ventrolateral medulla in the rat, suggesting a role for ATP on the paraventricular nucleus in the regulation of sympathetic nerve activity.

5 Purinergic Mechanosensory Transduction

A hypothesis was proposed that purinergic mechanosensory transduction occurred in visceral tubes and sacs, including ureter, bladder and gut, where ATP released from epithelial cells during distension acted on $P2X_3$ homomeric and $P2X_{2/3}$ heteromeric receptors on subepithelial sensory nerves initiating impulses in both local sensory pathways and pathways to pain centres in the CNS (Burnstock 1999) (Fig. 6b). Subsequent studies of bladder, ureter and gut have produced evidence in support of this hypothesis as presented in the following sections.

5.1 Urinary Bladder

Mice lacking the $P2X_3$ receptor exhibited reduced inflammatory pain and marked urinary bladder hyporeflexia with reduced voiding frequency and increased voiding volume, suggesting that $P2X_3$ receptors are involved in mechanosensory transduction underlying both physiological voiding reflexes and inflammatory pain (Cockayne et al. 2000). A later study from this group, using $P2X_2$ knockout mice and $P2X_2/P2X_3$ double knockout mice, revealed a role for the $P2X_2$ subtype too in mediating the sensory effect of ATP (Cockayne et al. 2005). In a systematic study of purinergic mechanosensory transduction in the mouse urinary bladder, ATP was shown to be released from urothelial cells during distension and discharge initiated in pelvic sensory nerves, was mimicked by ATP and α,β-meATP and was attenuated by $P2X_3$ antagonists as well as in $P2X_3$ knockout mice (Fig. 6a); $P2X_3$ receptors were localized on suburothelial sensory nerve fibres (Vlaskovska et al. 2001). Single-unit analysis of sensory fibres in the mouse urinary bladder revealed both low- and high-threshold fibres sensitive to ATP contributing to physiological (non-nociceptive) and nociceptive mechanosensory transduction, respectively. The amilorode-sensitive mechanosensitive channels, including epithelial Na^+ channels, expressed in the rat bladder epithelium might be involved in the mechanosensory transduction mechanisms by controlling stretch-evoked ATP release (Du et al. 2007). TRPV1 receptors participate in normal bladder function and are essential for normal mechanically evoked purinergic signalling by ATP released from the urothelium. Purinergic agonists increase the excitability of afferent fibres to distension.

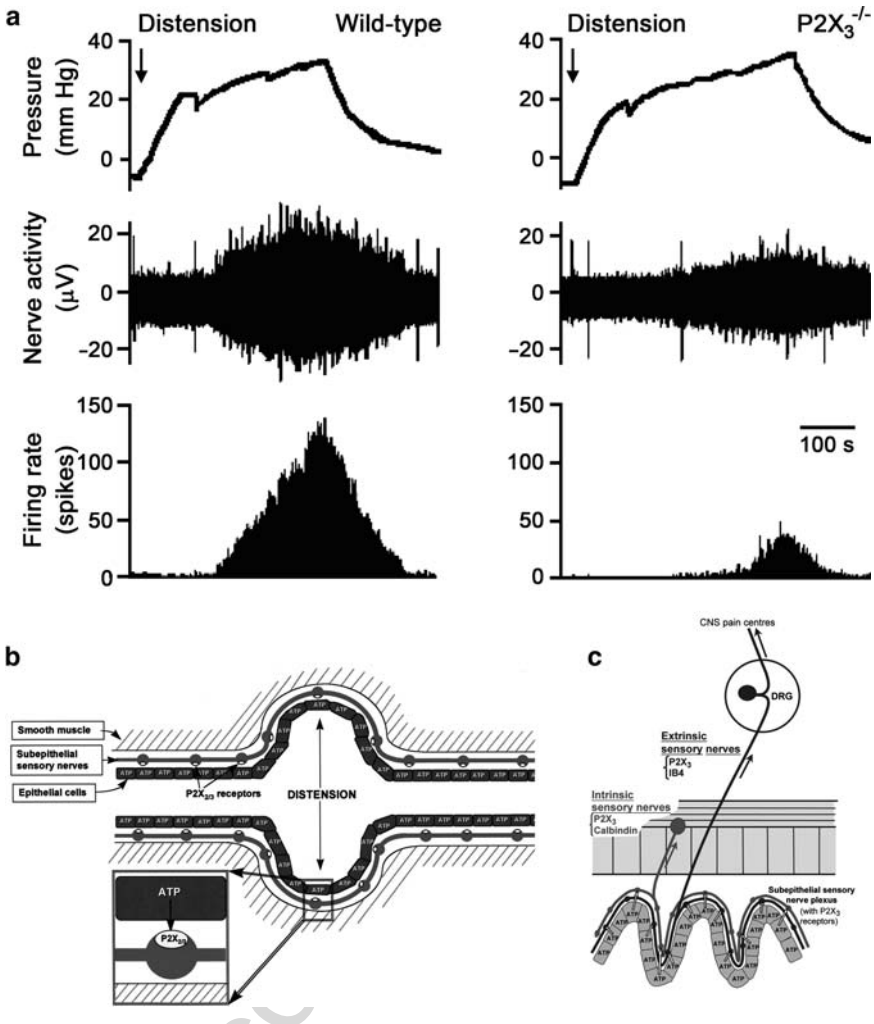


Fig. 6 Urinary bladder. (**a**) Comparison of the firing rate in sensory nerves during distension of the bladder in wild-type mice (*left*) and $P2X_3$ receptor deficient mice ($P2X_3^{-/-}$) (*right*). (**b**) Hypothesis for purinergic mechanosensory transduction in tubes (e.g. ureter, vagina, salivary and bile ducts, and gut) and sacs (e.g. urinary and gall bladders and lung). It is proposed that distension leads to release of ATP from epithelium lining the tube or sac, which then acts on $P2X_3$ and $P2X_{2/3}$ receptors on subepithelial sensory nerves to convey sensory/nociceptive information to the CNS. (**c**) Purinergic mechanosensory transduction in the gut. It is proposed that ATP released from mucosal epithelial cells during moderate distension acts preferentially on $P2X_3$ and/or $P2X_{2/3}$ receptors on low-threshold subepithelial intrinsic sensory nerve fibres (labelled with calbindin) to modulate peristaltic reflexes. ATP released during extreme (colic) distension also acts on $P2X_3$ and/or $P2X_{2/3}$ receptors on high-threshold extrinsic sensory nerve fibres (labelled with isolectin B4) that send messages via the DRG to pain centres in the CNS. (**a** Courtesy of Weifang Rong; ~~(**b**)~~ Reproduced from Burnstock 1999, with permission from Blackwell Publishing; **c** reproduced from Burnstock 2001a, with permission from John Wiley and Sons)

Bladder sensory DRG neurons, projecting via pelvic nerves, express predominantly $P2X_{2/3}$ heteromultimer receptors. Stretch induces release of both ACh and ATP from urothelial cells of the human bladder.

ATP given intravesically stimulates the micturition reflex in awake, freely moving rats, probably by stimulating suburothelial C-fibres (Pandita and Andersson 2002). The findings of studies of resiniferatoxin desensitization of capsaicin-sensitive afferents on detrusor overactivity induced by intravesical ATP in conscious rats support the view that ATP has a role in mechanosensory transduction and that ATP-induced facilitation of the micturition reflex is mediated, at least partly, by nerves other than capsaicin-sensitive afferents (Brady et al. 2004). ATP has also been shown to induce a dose-dependent hypereflexia in conscious and anaesthetized mice, largely via capsaicin-sensitive C-fibres; these effects were dose-dependently inhibited by PPADS and TNP-ATP (Hu et al. 2004). $P2X_1$ and $P2X_3$ receptors play a fundamental role in the micturition reflex in female urethane-anaesthetized rats; $P2X_3$ receptor blockade by phenol red raised the pressure and volume thresholds for the reflex, while $P2X_1$ receptor blockade diminished motor activity associated with voiding (King et al. 2004).

It has been claimed that suburothelial myofibroblast cells isolated from human and guinea pig bladder that are distinct from epithelial cells provide an intermediate regulatory step between urothelial ATP release and afferent excitation involved in the sensation of bladder fullness (Wu et al. 2004). The roles of ATP released from urothelial cells and suburothelial myofibroblasts on various bladder functions have been considered at length in several reviews (e.g. Birder 2006) and evidence has been presented that urothelial-released ATP may alter afferent nerve excitability (de Groat 2006).

5.2 Ureter

The ureteric colic induced by the passage of a kidney stone causes severe pain. Distension of the ureter resulted in substantial ATP release from the urothelium in a pressure-dependent manner (Knight et al. 2002). Cell damage was shown not to occur during distension with scanning electron microscopy, and after removal of the urothelium there was no ATP release during distension. Evidence was presented that the release of ATP from urothelial cells was vesicular. Immunostaining of $P2X_3$ receptors in sensory nerves in the subepithelial region was reported. Multifibre recordings from ureter afferent nerves were made using a guinea pig preparation perfused in vitro (Rong and Burnstock 2004). Distension of the ureter resulted in a rapid, followed by maintained, increase in afferent nerve discharge. The rapid increase was mimicked by intraluminal application of ATP or α,β-meATP, and TNP-ATP attenuated these nerve responses to distension; the maintained increase was partly due to adenosine.

5.3 Gut

A hypothesis was proposed suggesting that purinergic mechanosensory transduction in the gut initiated both physiological reflex modulation of peristalsis via intrinsic sensory fibres and nociception via extrinsic sensory fibres (Burnstock 2001a) (Fig. 6c). Evidence in support of this hypothesis was obtained from a rat pelvic sensory nerve colorectal preparation (Wynn et al. 2003). Distension of the colorectum led to pressure-dependent increase in release of ATP from mucosal epithelial cells and also evoked pelvic nerve excitation. This excitation was mimicked by application of ATP and α,β-meATP and was attenuated by the selective $P2X_3$ and $P2X_{2/3}$ antagonist TNP-ATP and by PPADS. The sensory discharge was potentiated by ARL-67156, an ATPase inhibitor. Single-fibre analysis showed that high-threshold fibres were particularly affected by α,β-meATP. Lumbar splanchnic and sacral pelvic nerves convey different mechanosensory information from the colon to the spinal cord. Forty percent of lumbar splanchnic nerve afferents responded to α,β-meATP compared with only 7% of pelvic nerve afferents (Brierley et al. 2005). The $P2X_3$ receptor subtype predominates in AH-type neurons and probably participates in mechanosensory transduction (Raybould et al. 2004).

Purinergic mechanosensory transduction has also been implicated in reflex control of secretion, whereby ATP released from mucosal epithelial cells acts on $P2Y_1$ receptors on enterochromaffin cells to release 5-hydroxytryptamine, which leads to regulation of secretion either directly or via intrinsic reflex activity (Cooke et al. 2003; Xue et al. 2007).

5.4 Uterus

It has been hypothesized that tissue stress or damage in the uterine cervix during late pregnancy and parturition leads to ATP release and sensory signalling via P2X receptors (Papka et al. 2005). In support of this proposal, these authors have shown $P2X_3$ receptor immunoreactivity in axons in the cervix, in small and medium-sized neurons in L6-S1 DRG and in lamina II of the L6–S1 spinal cord segments and increases in $P2X_3$ receptor expression between pregnancy day 10 and parturition (day 22/23) in the rat cervix, although not in DRG or spinal cord.

5.5 Tooth Pulp

$P2X_3$ and $P2X_{2/3}$ receptors on sensory afferents in tooth pulp appear to mediate nociception (Alavi et al. 2001; Renton et al. 2003), perhaps from ATP released by mechanical distension or inflammation of odontoblasts. Mustard oil application to the tooth pulp in anaesthetized rats produced long-lasting central sensitization,

reflected by increases in neuronal mechanoreceptive field size; TNP-ATP reversibly attenuated the mustard oil sensitization for more than 15 min (Hu et al. 2002). $P2X_3$ receptor expression is transiently upregulated and anterogradely transported in trigeminal sensory neurons after orthodontic tooth movement (Cao et al. 2006).

5.6 Tongue

$P2X_3$ receptors are abundantly present on sensory nerve terminals in the tongue (see Sect. 3.8), and ATP and α,β-meATP have been shown to excite trigeminal lingual nerve terminals in an in vitro preparation of intra-arterially perfused rat mimicking nociceptive responses to noxious mechanical stimulation and high temperature (Rong et al. 2000). A purinergic mechanosensory transduction mechanism for the initiation of pain has been considered.

5.7 Skin and Joints

Skin cell damage causes action-potential firing and inward currents in sensory nerve fibres, which was eliminated by enzymatic degradation of ATP or blockade of P2X receptors, indicating release of cytosolic ATP (Cook and McCleskey 2002).

ATP has been shown to be a stimulant of articular nociceptors in the knee joint via $P2X_3$ receptors (Dowd et al. 1998) and also to some extent in lumbar intervertebral disc, but not as prominently as in the skin (Aoki et al. 2003). $P2Y_2$ receptor mRNA is expressed in both cultured normal and osteoarthritic chondrocytes taken from human knee joints and ATP was shown to be released by mechanical stimulation (Millward-Sadler et al. 2004).

6 Purinergic Sensory Pathology

6.1 Pain

There is much current interest in the involvement of purinergic signalling in pain and recent reviews are available (Burnstock 2006, 2007; McGaraughty and Jarvis 2006; Shieh et al. 2006; Inoue 2007).

There were early hints that ATP might be involved in pain, including the demonstration of pain produced by injection of ATP into human skin blisters and ATP participation in pain pathways in the spinal cord (see Sect. 1). $P2X_3$ ionotropic receptors were cloned in 1995 and shown to be localized predominantly on small nociceptive sensory neurons in DRG together with $P2X_{2/3}$ heteromultimer receptors.

Later, Burnstock (1996b) put forward a unifying purinergic hypothesis for the initiation of pain by ATP on nociceptive afferent nerves. It was suggested that ATP released as a cotransmitter with ~~noradrenaline~~ noradrenaline and neuropeptide Y from sympathetic nerve terminal varicosities might be involved in causalgia and reflex sympathetic dystrophy (see also Ren et al. 2006); that ATP released from vascular endothelial cells of microvessels during reactive hyperaemia is associated with pain in migraine, angina and ischaemia; and that ATP released from tumour cells (which contain very high levels), damaged during abrasive activity, reaches $P2X_3$ receptors on nociceptive sensory nerves. This was followed by an increasing number of papers expanding on this concept. Immunohistochemical studies have shown that the nociceptive fibres expressing $P2X_3$ receptors arose largely from the population of small neurons that were labelled with the lectin IB_4. IB_4-positive fibres expressing $P2X_3$ and $P2X_{2/3}$ receptors are C-fibres, but the smaller population of CGRP-positive fibres expressing $P2X_3$ and $P2X_{2/3}$ receptors appear to be Aδ-fibres. The central projections of these neurons were shown to be in inner lamina II of the dorsal horn and peripheral projections were demonstrated to skin, tooth pulp, tongue and subepithelial regions of visceral organs. A schematic illustrating the initiation of nociception on primary afferent fibres in the periphery and purinergic relay pathways in the spinal cord was presented by Burnstock and Wood (1996) (Fig. 7). The decreased sensitivity to noxious stimuli associated with the loss of IB_4-binding neurons expressing $P2X_3$ receptors indicates that these sensory neurons are essential for the signalling of acute pain. However, persistent pain during inflammation may also involve sensitization and/or spread of $P2X_3$ or $P2X_{2/3}$ receptors. In a study of the behavioural effects of intraplantar injections of ATP in freely moving rats, evidence was presented that ATP was more effective in exciting nociceptors in inflamed compared with normal skin (Hamilton et al. 2001). Cannabinoids appear to inhibit nociceptive responses produced by P2X receptors (Krishtal et al. 2006). Locally released ATP can sensitize large mechanosensitive afferent endings via P2 receptors, leading to increased nociceptive responses to pressure or touch; it has been suggested that such a mechanism, together with central changes in the dorsal horn, may contribute to touch-evoked pain. Enhanced expression of glial cell line derived neurotrophic factor (GDNF) in the skin can change the mechanical sensitivity of IB_4-positive nociceptive afferents expressing $P2X_3$ and $P2X_{2/3}$ receptors. Treatment with oxidized ATP, a selective inhibitor of $P2X_7$ receptors, reduced the hyperalgesia produced by complete Freund's adjuvant and carrageenan-induced inflammation in rats. Data have been presented to support a pathogenic role for keratinocyte-derived ATP in irritant dermatitis. Pain related to the musculoskeletal system (myofascial pain) is very common and ATP has been claimed to excite or sensitize myofascial nociceptors (Makowska et al. 2006).

The search is on for selective $P2X_3$ and $P2X_{2/3}$ receptor antagonists that are orally bioavailable and do not degrade in vivo for the treatment of pain (Burnstock 2006; Gever et al. 2006). Suramin, PPADS and reactive blue 2 have been used as non-selective antagonists at $P2X_3$ and $P2X_{2/3}$ receptors on nociceptive sensory nerve endings. PPADS has the advantage that it associates and dissociates approximately 100–10,000 times more slowly than other known antagonists. The

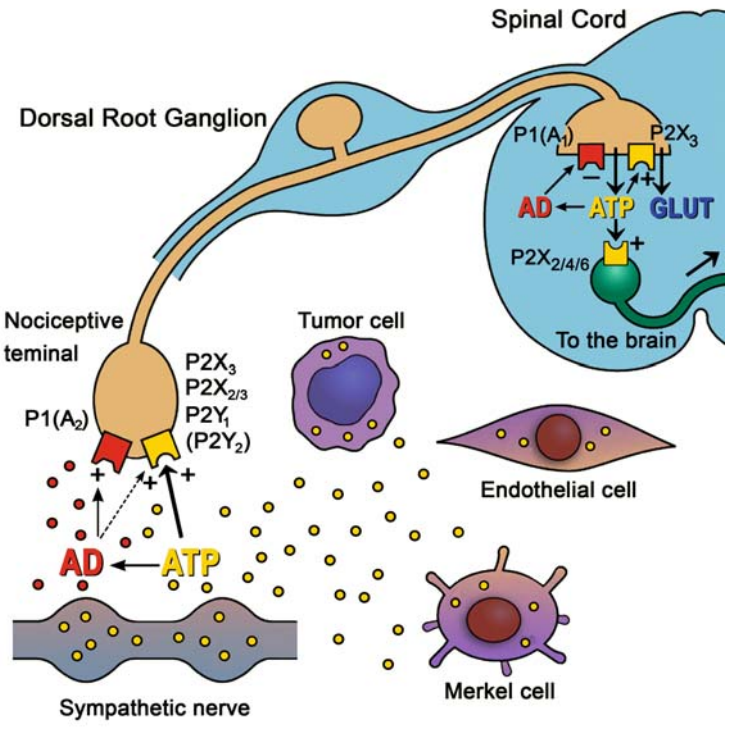


Fig. 7 Hypothetical schematic of the roles of purine nucleotides and nucleosides in pain pathways. At sensory nerve terminals in the periphery, $P2X_3$ and $P2X_{2/3}$ receptors have been identified as the principal P2X purinoceptors present, although recent studies have also shown expression of $P2Y_1$ and possibly $P2Y_2$ receptors on a subpopulation of $P2X_3$ receptor immunopositive fibres. Other known P2X purinoceptor subtypes (1–7) are also expressed at low levels in dorsal root ganglia. Although less potent than ATP, adenosine also appears to act on sensory terminals, probably directly via $P1(A_2)$ purinoceptors; however, it also acts synergistically (*broken black line*) to potentiate $P2X_{2/3}$ receptor activation, which also may be true for 5-hydroxytryptamine, capsaicin and protons. At synapses in sensory pathways in the CNS, ATP appears to act postsynaptically via $P2X_2$, $P2X_4$ and/or $P2X_6$ purinoceptor subtypes, perhaps as heteromultimers, and after breakdown to adenosine it acts as a prejunctional inhibitor of transmission via $P1(A_2)$ purinoceptors. $P2X_3$ receptors on the central projections of primary afferent neurons in lamina II of the dorsal horn mediate facilitation of glutamate and probably also ATP release. Sources of ATP acting on $P2X_3$ and $P2X_{2/3}$ receptors on sensory terminals include sympathetic nerves as well as endothelial, Merkel and tumour cells. *Yellow dots* molecules of ATP, *red dots* molecules of adenosine. (Modified from Burnstock and Wood 1996, and reproduced with permission from the American Physiological Society)

TNP-substituted nucleotide TNP-ATP is a very potent antagonist at both $P2X_3$ and $P2X_{2/3}$ receptors. A-317491 (synthesized by Abbott Laboratories) and compound RO3 (synthesized by Roche Palo Alto) are both effective $P2X_3$ and $P2X_{2/3}$ antagonists, the latter being orally bioavailable and stable in vivo. Antagonism of $P2X_1$ 926 927 928 929

and $P2X_3$ receptors by phenol red has been reported and tetramethylpyrazine, a traditional Chinese medicine, used as an analgesic for dysmenorrhoea, was claimed to block $P2X_3$ receptor signalling. Antisense oligonucleotides have been used to downregulate the $P2X_3$ receptor, and in models of neuropathic (partial sciatic nerve ligation) and inflammatory (complete Freund's adjuvant) pain, inhibition of the development of mechanical hyperalgesia as well as significant reversal of established hyperalgesia were observed within 2 days of treatment (Stone and Vulchanova 2003). Combined antisense and RNA interference mediated treatment for specific inhibition of the recombinant rat $P2X_3$ receptor appears to be promising for pain therapy (Hemmings-Mieszczak et al. 2003). $P2X_3$ double-stranded short interfering RNA (siRNA) relieves chronic neuropathic pain and opens up new avenues for therapeutic pain strategies in man (Dorn et al. 2004).

P2Y receptors are also present on nociceptive sensory nerves and these are involved in modulation of pain transmission (Gerevich et al. 2007). With use of a mouse skin-sensory nerve preparation, evidence was presented that $P2Y_2$ receptors in the terminals of capsaicin-sensitive cutaneous sensory neurons mediate nociceptive transmission and further that P2Y signalling may contribute to mechanotransduction in low-threshold Aβ-fibres (Stucky et al. 2004). P2Y receptors appear to potentiate pain induced by chemical or physical stimuli via capsaicin-sensitive TRPV1 channels and it has been proposed that the functional interaction between $P2Y_2$ receptors and TRPV1 channels in nociceptors could underlie ATP-induced inflammatory pain (Ma and Quirion 2007). ATP-induced hyperalgesia was abolished in mice lacking TRPV1 receptors. A hypothesis that purinergic mechanosensory transduction occurs in visceral organs initiating nociception was discussed in Sect. 5.

Changes in central purinergic pathways that occur in chronic neuropathic pain have attracted considerable attention in recent years and have been well reviewed. There is purinoceptor involvement in nociceptive pathways in the spinal cord. For example, intrathecally administered P2 receptor antagonists, suramin and PPADS, produced antinociceptive effects in rats. ATP-activated P2X receptors in lamina II of the rat spinal cord play a role in transmitting or modulating nociceptive information. α,β-meATP-induced thermal hyperalgesia may be mediated by spinal $P2X_3$ receptors, perhaps by evoking glutamate release. Spinal endogenous ATP may play a role in capsaicin-induced neurogenic pain via $P2X_3$ or $P2X_{2/3}$ receptors and formalin-induced inflammatory pain via different P2X and/or P2Y receptors. Of the six lamina regions in the dorsal horn of the spinal cord, inner lamina II and lamina I are the major sensory regions involved in nociceptive transmission, as well as lamina V. Central terminals of nociceptive afferents coexpress ionotropic glutamate and $P2X_3$ receptors. Glial cells contribute to the α,β-meATP-induced long-term potentiation in the dorsal horn, which might be part of a cellular mechanism for the induction of persistent pain (Ikeda et al. 2007). An inhibitory role of supraspinal $P2X_{2/3}$ receptors on nociception in rats has been described (Fukui et al. 2006).

There are three potential sources of ATP release during sensory transmission in the spinal cord. ATP may be released from the central terminals of primary afferent neurons. ATP may be also released from astrocytes and/or postsynaptic dorsal horn

neurons. The presence of $P2X_3$ mRNA-labelled neurons in the DRG increased 3 days after peripheral injury. $P2X_3$ receptors on DRG neurons increase their activity after inflammation and contribute to the hypersensitivity to mechanical stimulation. Evidence has been presented for increased release of ATP from DRG neurons on the side of the injury after induction of painful peripheral neuropathy by sciatic nerve entrapment; however, sensitization of $P2X_3$ receptors rather than a change in ATP release appears to be responsible for the neuropathic pain behaviour. For neuropathic pain, the tactile allodynia that follows peripheral nerve injury is reduced by A-134974, a novel adenosine kinase inhibitor acting at spinal sites. PPADS, TNP-ATP and apyrase attenuate central sensitization in nociceptive neurons in medullary dorsal horn, which suggests that release of ATP plays a key role in the central sensitization induced by injury or inflammation of peripheral tissues. Upregulated homomeric $P2X_3$ and heteromeric $P2X_{2/3}$ receptors augmented thermal hyperalgesia and mechanical allodynia, respectively, at the spinal level in the acute stage of chronic constriction injury; at the chronic stage (after 40 days), thermal hyperalgesia disappeared, but mechanical allodynia persisted. A-317491, a potent and selective antagonist of $P2X_3$ and $P2X_{2/3}$ receptors, reduces chronic inflammatory and neuropathic pain in the rat, but not acute, inflammatory or visceral pain. When A-317491 and also Compound A (US patent reference 2005/0209260A1) were administered spinally to animals after chronic nerve constriction injury, there was a reduction in sensory fibre responses, unmasking a central role for these P2X receptors and suggesting a potential role of their antagonists in the modulation of neuropathic pain (Sharp et al. 2006). Endogenous ATP acting on P2X receptors appears to be necessary for the induction of the postoperative pain characterized by mechanical allodynia. Suramin inhibits spinal cord microglia activation and long-term hyperalgesia induced by inflammation produced by formalin injection. Endogenous opioid mechanisms partially mediate spinal $P2X_3$/$P2X_{2/3}$ receptor-related antinociception in rat models of inflammatory and chemogenic pain, but not neuropathic pain (Chen et al. 2006).

Analgesic effects with intrathecal administration of P2Y receptor agonists UTP and UDP in a normal and neuropathic pain rat model have been reported, suggesting that $P2Y_2$ (and/or $P2Y_4$) and $P2Y_6$ receptors produce inhibitory effects in spinal pain transmission. It has been suggested that, while $P2X_3$ receptor activation leads to increased firing of DRG neurons and subsequently to increased release of sensory transmitter from their central processes, $P2Y_1$ receptor activation may decrease the release of sensory transmitter onto spinal cord neurons and may thereby partly counterbalance the algogenic effect of ATP. $P2Y_1$ receptor expression is upregulated in rat DRG neurons following transection of sciatic nerves and has been implicated in the mechanisms underlying neuropathic pain.

$P2X_7$ receptor activation of cultured astrocytes from rat brain increases the release of cysteinyl leukotrienes, which are potent lipid mediators of inflammation, further supporting a role for extracellular ATP as an integral component of the inflammatory brain pain response.

The roles of $P2X_4$ and $P2X_7$ receptors on microglia (immune cells) in neuropathic and inflammatory pain has attracted strong interest in the past few years

(Färber and Kettenmann 2006; Trang et al. 2006; Hughes et al. 2007). $P2X_4$ and $P2X_7$ knockout mice share a common pain-reduced phenotype, but apparently via different mechanisms (Chessell et al. 2006). Recently developed selective $P2X_7$ receptor antagonists, compound 15d (Nelson et al. 2006), A-740003 (Honore et al. 2006) and A-438079 (McGaraughty et al. 2007), reduce chronic inflammatory and neuropathic pain. After spinal cord injury, an increased number of lumbar microglia expressing the $P2X_4$ receptor in the spinal cord of rats with allodynia and hyperalgesia have been reported. Pharmacological blockade of $P2X_4$ receptors or intraspinal administration of $P2X_4$ antisense oligodeoxynucleotide reversed tactile allodynia caused by peripheral nerve injury without affecting acute pain behaviours in naïve animals (Tsuda et al. 2003).

Purinergic mechanisms are beginning to be explored in relation to cancer pain. It was suggested that the unusually high levels of ATP contained in tumour cells may be released by mechanical rupture to activate $P2X_3$ receptors on nearby nociceptive sensory nerve fibres. There is increased expression of $P2X_3$ receptors on CGRP immunoreactive epidermal sensory nerve fibres in a bone cancer pain model (Gilchrist et al. 2005) and in other cancers that involve mechanically sensitive tumours. For example, in bone tumours, destruction reduces the mechanical strength of the bone and antagonists that block the mechanically gated channels and/or ATP receptors in the richly innervated periosteum might reduce movement-associated pain. The hyperalgesia associated with tumours appears to be linked to increase in expression of $P2X_3$ receptors in nociceptive sensory neurons expressing CGRP by analogy with that described for increased $P2X_3$ receptor expression in a model of inflammatory colitis. Increased expression of $P2X_3$ receptors was also reported associated with thermal and mechanical hyperalgesia in a rat model of squamous cell carcinoma of the lower gingival (Nagamine et al. 2006).

6.2 Migraine

ATP has been implicated in the pathogenesis of pain during migraine via stimulation of primary afferent nerve terminals located in the cerebral microvasculature (Burnstock 1981, 1989; Fumagalli et al. 2006). $P2X_3$ receptors are expressed on primary afferent nerve terminals supplying cerebral vessels arising from trigeminal, nodose and spinal ganglia. Thus, $P2X_3$ receptor antagonists may be candidates for antimigraine drug development (Waeber and Moskowitz 2003). CGRP is expressed in human trigeminal neurons and is released during migraine attacks; a recent study shows that the algogenic action of CGRP is linked to sensitization of trigeminal $P2X_3$ nociceptive receptors, suggesting that trigeminal $P2X_3$ receptors may be a potential target for the early phase of migraine attack. There is also evidence that migraine is a chronic sympathetic nervous system disorder, with which there is an increase in release of sympathetic cotransmitters, including ATP, which may contribute to the initial vasospasm. ATP may contribute to pain in migraine by

sensitizing nociceptors against acidosis via $P2Y_2$ receptor supported release of endogenous prostaglandin (Zimmermann et al. 2002). It has been suggested that there is an interaction of P2Y receptors on trigeminal sensory terminals with $P2X_3$ receptors after sensitization of trigeminal neurons with algogenic stimuli (e.g. NGF, ~~brain-derived neurotrophic factor~~, or bradykinin) and that this may help identify new targets for the development of novel antimigraine drugs. It was shown recently that the majority of trigeminal primary afferent neurons innervating the dura mater express $P2X_2$ and/or $P2X_3$ receptors, suggesting that purines may be involved in nociceptive processing in migraine (Goadsby 2005).

6.3 Diseases of Special Senses

6.3.1 Eye

Purinergic signalling is widespread in the eye and novel therapeutic strategies are being developed for glaucoma, dry eye and retinal detachment. ATP, acting via both P2X and P2Y receptors, modulates retinal neurotransmission, affecting retinal blood flow and intraocular pressure. The ATP analogue β,γ-meATP is more effective in reducing intraocular pressure (40%) than are muscarinic agonists such as pilocarpine (25%) and β-adrenoceptor blockers (30%), raising the potential for the use of purinergic agents in glaucoma (Pintor et al. 2003). It was shown ~~recently~~ that rapid elevation of intraocular pressure leads to release of ATP that results in retinal ganglion cell injury and consequent visual defects (Resta et al. 2007).

6.3.2 Ear

ATP may regulate hearing sensitivity and thus may be useful in the treatment of Ménière's disease, tinnitus and sensorineural deafness (Housley et al. 2006). Sustained loud noise alters the response of outer hair cells in the inner ear to ATP and produces an upregulation of $P2X_2$ receptors, particularly at the site of outer hair cell sound transduction (Chen et al. 1995a~~, b~~), although with a longer time course of noise exposure up to 24 days, downregulation of P2X and P2Y receptor subtypes has been reported (Szücs et al. 2006). $P2X_2$ expression is also increased in spiral ganglion neurons, indicating that extracellular ATP acts as a modulator of auditory neurotransmission that is adaptive and dependent on the noise level (Wang et al. 2003). Excessive noise can irreversibly damage hair cell stereocilia, leading to deafness. Data have been presented showing that release of ATP from damaged hair cells is required for Ca^{2+} wave propagation through the support cells of the organ of Corti, involving P2Y receptors, and this may constitute the fundamental mechanism to signal the occurrence of hair cell damage (Gale et al. 2004). Noise-induced

upregulation of NTPDase3 in the rat cochlear has been reported and its potential neuroprotective effect discussed (Vlajkovic et al. 2006).

6.3.3 Nasal Organs

Purinergic receptors have been described in the nasal mucosa, including the expression of $P2X_3$ receptors on olfactory neurons. Enhanced sensitivity to odours in the presence of P2 receptor antagonists suggests that low-level endogenous ATP normally reduces odour responsiveness. It appears that the induction of heat-shock proteins by noxious odour damage can be prevented by the in vivo administration of P2 receptor antagonists (Hegg and Lucero 2006). The predominantly suppressive effect of ATP in odour responses could play a role in the reduced odour sensitivity that occurs during acute exposure to noxious fumes and may be a novel neuroprotective mechanism. Purinergic receptors appear to play an integral role in signalling acute damage in the olfactory epithelium by airborne pollutants. Damaged cells release ATP, thereby activating purinergic receptors on neighbouring sustentacular cells, olfactory receptor neurons and basal cells.

6.4 Bladder Diseases

Purinergic signalling plays a role in afferent sensation from the bladder (see Sect. 3). Purinergic agonists acting on $P2X_3$ receptors in the bladder can sensitize bladder afferent nerves and these effects mimic the sensitizing effect of cystitis induced by cyclophosphamide (Nazif et al. 2007). Thus, $P2X_3$ receptors are a potential target for pharmacological manipulation in the treatment of both pain and detrusor instability. Subsensitivity of $P2X_3$ and $P2X_{2/3}$ receptors, but not vanilloid receptors, has been shown in L6–S1 DRG in the rat model of cyclophosphamide cystitis (Borvendeg et al. 2003). Release of ATP from urothelial cells with hypoosmotic mechanical stimulation was increased by over 600% in inflamed bladder from cyclophosphamide-treated animals; botulinum toxin inhibited this release (Smith et al. 2005). Botulinum neurotoxin type A is effective in the treatment of intractable detrusor overactivity; decreased levels of sensory receptors $P2X_3$ and/or TRPVI may contribute to its clinical effect (Apostolidis et al. 2005; Atiemo et al. 2005).

It is believed that the predominant sensory afferents involved in detecting bladder volume changes are the Aδ pelvic nerve afferents which convey information about the state of bladder fullness to spinal and supraspinal centres coordinating the micturition reflex (Andersson and Wein 2004). In contrast, the normally silent pelvic afferent C-fibres are thought to assume a prominent role under pathophysiological conditions, where they become hyperexcitable and convey information about noxious, inflammatory or painful stimuli, and evoke reflex contractions mainly through a localized spinal reflex. In the absence of $P2X_3$ receptors in mice knockouts, the bladder exhibits hyporeflexia, characterized by

decreased voiding frequency and increased bladder capacity, but normal bladder pressures (Cockayne et al. 2000). The recently developed $P2X_3$ and $P2X_{2/3}$ antagonist RO3, which is orally bioavailable and metabolically stable, is being explored as a therapeutic agent for urinary tract dysfunction (Ford et al. 2006). The $P2X_3$ receptor is largely expressed in the IB_4 small nociceptive capsaicin-sensitive nerves in the DRG, so it is interesting that IB_4-conjugated saporin, a cytotoxin that destroys neurons binding IB_4, when administered intrathecally at the level of L6–S1 spinal cord, reduced bladder overactivity induced by ATP infusion. Voiding dysfunction involves $P2X_3$ receptors in conscious chronic spinal cord injured rats, which raises the possibility that $P2X_3$ receptor antagonists might be useful for the treatment of neurogenic bladder dysfunction. Chronic spinal cord injury results in a dramatic increase in muscarinic receptor-evoked release of ATP from primary afferents in the lumbosacral spinal cord and from the bladder (Salas et al. 2007).

Stretch-activated ATP release from bladder epithelial cells from patients with interstitial cystitis is significantly greater than from healthy cells and also in animal models of interstitial cystitis (Birder et al. 2004). The $P2X_3$ receptor subunit was upregulated during stretch of cultured urothelial cells from patients with interstitial cystitis. $P2X_2$ and $P2X_3$ receptor expression has been demonstrated on human bladder urothelial cells (as well as on afferent nerve terminals); the expression was greater in cells from interstitial cystitis bladder (Tempest et al. 2004).

Reduction of $P2X_3$ and $P2X_5$ receptors in human detrusor from adults with urge incontinence has been claimed (Moore et al. 2001). Overdistension of the bladder is caused by urinary retention, but it has also been used as a method for treating unstable bladder or interstitial cystitis, possibly damaging sensory nerve fibres. However, micturition problems often reoccur after overdistension treatment.

Recent reviews of management of detrusor dysfunction highlight the growing potential of therapeutic strategies related to purinergic sensory signalling (Ford et al. 2006; Ruggieri 2006).

6.5 Gut Disorders

The excitability of visceral afferent nerves is enhanced following injury, ischaemia and during inflammation, for example in irritable bowel syndrome (IBS). Under these conditions, substances are released from various sources that often act synergistically to cause sensitization of afferent nerves to mechanical or chemical stimuli. Receptors to these substances (including ATP) represent potential targets for drug treatment aimed at attenuating the inappropriate visceral sensation and subsequent reflex activities that underlie abnormal bowel function and visceral pain (Holzer 2004). α,β-meATP was shown to stimulate mechanosensitive mucosal and tension receptors in mouse stomach and oesophagus, leading to activity in vagal afferent nerves. The sensitizing effects of $P2X_3$ receptor agonists on mechanosensory function are induced in oesophagitis. $P2X_3$ purinergic signalling enhancement in an animal model of colonic inflammation has been described, owing, at least in

part, to the appearance of $P2X_3$ receptor expression in a greater number of CGRP-labelled small nociceptive neurons in the DRG (Wynn et al. 2004). $P2X_3$ receptor expression is increased in the enteric plexuses in human IBS, suggesting a potential role in dysmotility and pain and the possibility that P2X receptors are potential targets for the drug treatment of IBS has been raised (Galligan 2004). It has also been suggested that agonists acting on P2X receptors on intrinsic enteric neurons may enhance gastrointestinal propulsion and secretion and that these drugs might be useful for treating constipation-predominant IBS, while P2X antagonists might be useful for treating diarrhoea-predominant IBS. The peripheral sensitization of $P2X_3$ receptors on vagal and spinal afferents in the stomach may contribute to dyspeptic symptoms and the development of visceral hyperalgesia (Dang et al. 2005). Enhanced activity in purinergic pathways occurs in postoperative ileus, but is reversed by orphanin FQ.

6.6 Arthritis

It was recognized early that the nervous system may contribute to the functional changes associated with rheumatoid arthritis. A role for purinergic signalling in rheumatic diseases has been considered (Green et al. 1991; Dowd et al. 1998; Seino et al. 2006). Quinacrine (Atabrine), a drug that binds strongly to ATP, has been used for the treatment of rheumatoid arthritis patients for many years. One of its mechanisms of action is to decrease levels of prostaglandin E_2 and cyclooxygenase-2, which are known to be produced following occupation of P2Y receptors by ATP. The articular fluid removed from arthritic joints contains high levels of ATP. Purinergic regulation of bradykinin-induced plasma extravasation and adjuvant-induced arthritis has been reported. ATP and UTP activate calcium-mobilizing $P2Y_2$ or $P2Y_4$ receptors and act synergistically with interleukin-1 to stimulate prostaglandin E_2 release from human rheumatoid synovial cells (Loredo and Benton 1998). Spinal P1 receptor activation has been claimed to inhibit inflammation and joint destruction in rat adjuvant-induced arthritis (Chan et al. 2007). When monoarthritis was induced by injection of complete Freund's adjuvant into the unilateral temporomandibular joint of the rat, the pain produced was associated with an increase in $P2X_3$ receptor positive small neurons in the trigeminal ganglion (Shinoda et al. 2005). Activation of P2X receptors in the rat temporomandibular joint induces nociception and blockage by PPADS decreases carrageenan-induced inflammatory hyperalgesia (Oliveira et al. 2005).

Evidence is accumulating to suggest that blockers of $P2X_7$ receptors may have a future as anti-inflammatory drugs (Ferrari et al. 2006). Oxidized ATP inhibits inflammatory pain in arthritic rats by inhibition of the $P2X_7$ receptor for ATP localized in nerve terminals (Dell'Antonio et al. 2002). The $P2X_7$ receptor antagonist AZD9056 has been reported to be in phase II clinical trials for rheumatoid arthritis (Okuse 2007).

6.7 Respiratory Diseases

Vagal afferent purinergic signalling may be involved in the hyperactivity associated with asthma and chronic obstructive pulmonary disease (Adriaensen and Timmermans 2004). The need to support the failing lung (acute respiratory distress syndrome) with mechanical ventilation is potentially life-saving but, unfortunately, alveolar overdistension and pulmonary shear stress may cause lung injury (ventilator-induced lung injury), increasing bronchoalveolar lavage leading to lung oedema. It has been suggested that ventilator-induced lung injury may involve stretch-associated release of ATP from neuroepithelial cell bodies and activation of sensory nerves and reflex responses (Rich et al. 2003). P2X receptors are involved in the reactive oxygen species evoked bradypneic reflex in anaesthetized rats (Ruan et al. 2006). Acid-sensitive vagal sensory pathways involved in the cough reflex may involve $P2X_2$ receptors (Kamei et al. 2005; Kollarik et al. 2007). P2X and $GABA_A$ receptors play an important role in CO_2 chemoreception and are involved in mediation of the ventilatory response to hypercapnia (Gourine 2005).

6.8 Central Disorders

Purinergic signalling appears to play a significant role in the regulation of body temperature during fever by central hypothalamic and brain stem nuclei (Gourine et al. 2004). Mice lacking the $P2X_3$ receptor subunit exhibit enhanced avoidance of both hot and cold thermal extremes (Shimizu et al. 2005). Evaluation of the roles of purinergic signalling in processing of the sympathoexcitatory component of the chemoreflex at the NTS level may illuminate the mechanisms underlying the sympathetic overactivity observed in pathophysiological conditions such as hypertension, obstructive sleep apnoea, and heart failure.

Although ethanol is probably the oldest and most widely used psychoactive drug, the cellular mechanisms by which it affects the nervous system have been poorly understood, although some insights in relation to purinergic P2 receptor signalling have emerged in recent years. Ethanol inhibits P2X receptor mediated responses of DRG neurons by an allosteric mechanism (Li et al. 1998). Ethanol differentially affects ATP-gated $P2X_3$ and $P2X_4$ receptor subtypes expressed in *Xenopus* oocytes (Davies et al. 2005).

7 Development of Purinergic Sensory Signalling

There are a limited number of studies of the roles of purinergic sensory signalling in both embryonic and postnatal development and in regeneration (Burnstock 2001b, 2007; Zimmermann 2006). An immunohistochemical study revealed intense labelling

of $P2X_3$ receptors in the embryonic and postnatal (postnatal days 7 and 14; Fig. 8a), but not adult, rat brain. The staining was restricted to the hindbrain at embryonic day 16, in particular the mesencephalic trigeminal nucleus, the superior and inferior olive, the intermediate reticular zone, the spinal trigeminal tract and the prepositus hypoglossal nucleus. $P2X_3$ receptors first appeared in the hindbrain neural tube and sensory ganglia in embryonic day 11–11.5 embryos; at embryonic day 14.5 they appeared in the optic tract, NTS mesencephalic trigeminal nucleus, but $P2X_3$ immunoreactivity was downregulated in early postnatal brain stem. The $P2X_3$ receptor was coexpressed with the $P2X_2$ receptor in neurons in NTS and sensory ganglia (Cheung and Burnstock 2002). α,β-meATP is ineffective on glycinergic presynaptic nerve terminals projecting to rat substantia gelatinosa neurons at postnatal days 10–12, and is strongly active at postnatal days 28–30, perhaps contributing to the fine control of the pain signal in spinal cord dorsal horn neurons. In rat superficial dorsal horn, excitatory synapses mediated by both glutamate and ATP are functional from the first postnatal days. Distinct subtypes of P2X receptors have been shown to be functionally expressed at pre- and postsynaptic sites in lamina V neurons in rat dorsal spinal cord and it was suggested that purinergic signalling in deep dorsal horn neurons is more important during postnatal development (Shiokawa et al. 2006).

$P2X_3$ receptors are expressed in the trigeminal ganglia of zebrafish from a very early stage of development, most likely in neural-crest-derived trigeminal cells rather than in placode-derived cells (Norton et al. 2000) (Fig. 8c). $P2X_3$ receptors were also expressed in the spinal sensory Rohan–Beard cells and in the putative lateral line ganglion in the early development of zebrafish. ATP-gated currents activated via $P2X_2$ and $P2X_3$ receptors in cultured embryonic rat DRG neurons show heterogeneity of time courses comparable to that seen in different adult subpopulations of dissociated adult DRG neurons (Labrakakis et al. 2000). Activation of P2X receptors on cultured embryonic DRG neurons results in the release of SP. Immunostaining of $P2X_3$ receptors was found in most neurons in embryonic mouse trigeminal ganglia and DRG, in contrast to adult ganglia, which express $P2X_3$ receptors only on small-diameter neurons (Ruan et al. 2004) (Fig. 8b). Nearly all sensory neurons in mouse DRG, trigeminal and nodose ganglia expressed $P2X_3$ receptors at embryonic day 14, but after birth there was a gradual decline to about 50% of neurons showing positive staining. IB_4-positive neurons in sensory ganglia did not appear until birth; the numbers increased to about 50% by postnatal day 14, when they were mostly colocalized with $P2X_3$ receptors. Responses to ATP have been described in ciliary neurons acutely dissociated from embryonic chick ciliary ganglia taken at day 14. ATP augments peptide release from neurons in embryonic DRG through activation of P2Y receptors. IB_4-binding DRG neurons (that express $P2X_3$ receptors) switch from NGF to GDNF dependence in early postnatal life.

While there are many studies of purinergic signalling in the retina of adult mammals, there are only a few reports about embryonic retina (Burnstock 2001b, 2007). Spontaneous waves of excitation in the developing mammalian retina are believed to play an important role in activity-dependent visual development of retinogeniculate connectivity. The earliest age at which spontaneous waves were

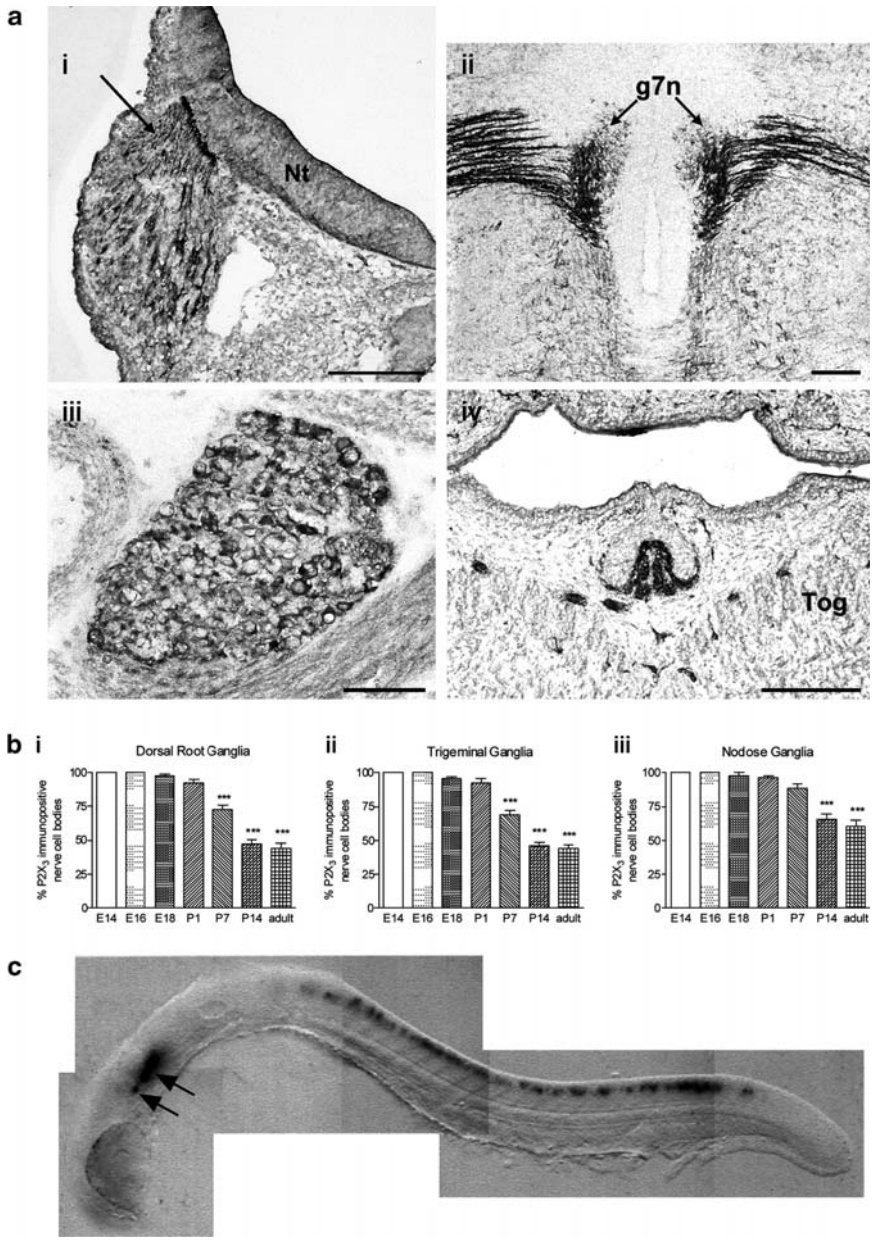


Fig. 8 Development of sensory nerves. (**a**) $P2X_3$ immunoreactivity in embryonic rat embryos. *i* $P2X_3$ immunoreactivity in an embryonic day 12.5 rat embryo. Transverse sections at the first branchial arch levels showing $P2X_3$ immunoreactivity (*arrow*) in the trigeminal ganglion. Note the expression of $P2X_3$ in the primitive spinal trigeminal tract between the trigeminal ganglion and the neural tube (*Nt*). *ii* $P2X_3$ immunoreactivity in an embryonic day 14.5 rat embryo. Coronal section at the pontine level showing the genu of the facial nerve (*g7n*) stained strongly with $P2X_3$ receptor

detected in rabbit retina was embryonic day 22 and the involvement of purinergic receptor activation in these waves was suggested. Suramin blocked the wave. Adenosine has also been implicated in chick retinal development; A_1 receptors may have different functions in the embryonic retina as compared with mature chick retina. Studies of embryonic chick neural retina have shown that the ATP-induced rise in intracellular Ca^{2+} is mediated by $P2Y_2$ or $P2Y_4$ receptors and that there is a dramatic decline of the ATP-induced rise in intracellular Ca^{2+} just before synaptogenesis. Suramin and reactive blue 2 almost completely block these responses. Injection of reactive blue 2 into early embryonic chicks produced severe effects in embryogenesis. ATP increased [^{3}H]thymidine incorporation in retinal cultures from embryonic day 3 and suramin and PPADS inhibited these activities. It was suggested that the change in Ca^{2+} signalling mediated by $P2Y_2$ or $P2Y_4$ receptors during development may underlie the differentiation of neuroepithelial cells or undifferentiated progenitor cells into neurons. ATP acting on P2 receptors is involved in the regulation of retinal progenitor cell proliferation at early embryonic stages, perhaps in collaboration with growth factors. ATP, probably via $P2Y_1$ receptors, stimulates proliferation of both bipolar and Müller cells in early developing chick retina at embryonic days 6–8. RT-PCR studies of $P2X_7$ mRNA in postnatal rats (postnatal days 23–210) showed positive identification in the retina. Changes in $P2Y_4$ receptor expression during development of rat cochlea outer sulcus cells have been described recently (Lee et al. 2007).

The perinatal development of nerves expressing $P2X_3$ receptors in the myenteric plexus of the rat stomach has been examined (Xiang and Burnstock 2004b). $P2X_3$ receptor immunoreactive nerves in the embryonic rat stomach are of both extrinsic and intrinsic origin. The extrinsic sensory nerve fibres first express $P2X_3$ receptors as early as embryonic day 12 and extend rapidly on to the whole stomach by embryonic day 14. In contrast, the intrinsic enteric neuron cell bodies showing $P2X_3$ immunoreactivity did not appear until birth (postnatal day 1), reached peak numbers by postnatal day 14, then decreased in maturing animals. IGLEs and intramuscular arrays expressing $P2X_3$ receptors were first seen postnatally at postnatal day 1 and postnatal day 7, respectively (Xiang and Burnstock 2004b). $P2X_3$ receptor immunoreactive neurons in the gastric myenteric plexus expressed calbindin only in the early postnatal days, while 14–21% of neurons from postnatal

Fig. 8 (continued) antibody. *iii* $P2X_3$ immunoreactivity in a neural-crest-derived nodose ganglion of an embryonic day 18.5 rat embryo. *iv* $P2X_3$ immunoreactivity in an embryonic day 18.5 rat embryo. Transverse section showing strong $P2X_3$ receptor staining in the taste bud of the tongue (*Tog*). *Scale bar* in *i* 200 μm, in *ii–iv* 100 μm. (**b**) Percentage of $P2X_3$-immunoreactive nerve cell bodies in sensory ganglia of mouse in embryonic and postnatal development. Note statistical significance indicated by *asterisks* relates to postnatal ages 7 days, 14 days and adult as compared with embryonic days 14, 16 and 18. ***$p < 0.001$. **c** Early expression of $P2X_3$ receptors in putative central and peripheral neural cells in a 24-h zebrafish embryo in which expression in the putative trigeminal ganglia cells has condensed to two spots (*arrows*) and in which expression in dorsal Rohon–Beard neurons is prominent. (**a** Reproduced from Cheung and Burnstock 2002, with permission from Wiley–Liss; ~~(b)~~ reproduced from Ruan et al. (2004), with permission from Springer~~–Verlag;~~ ~~(c)~~ reproduced from Norton et al. 2000, with permission from Elsevier)

day 1 to postnatal day 60 increasingly expressed calretinin. About 20% of $P2X_3$ positive neurons coexpressed NOS throughout perinatal development.

Vagal sensory nerve terminals in rat lung express $P2X_3$ receptors from the first moment that they make contact with NEBs a few days before birth (Brouns et al. 2003). This is consistent with the important function of NEBs as oxygen sensors perinatally before the carotid body O_2-sensory system is fully developed at about 2 weeks after birth.

During embryonic development of the rat inner ear, $P2X_2$ receptor mRNA expression was present in the precursors of the cells bordering the cochlear endolymphatic compartment at embryonic day 12, as well as in spinal and vestibular ganglia (Housley et al. 2006). Both inner and outer hair cells did not exhibit $P2X_2$ receptor mRNA until after postnatal day 10 through postnatal day 12, concomitant with the onset of hearing. These data are consistent with roles for the $P2X_2$ receptor both in the process of labyrinthine development and in the regulation of auditory and vestibular sensory transduction. $P2X_1$ receptors provide the signal transduction pathway for development of afferent and efferent innervation of the sensory hair cells and purinergic influence on cochlea morphogenesis. $P2X_3$ receptor expression has been characterized in the mouse cochlea from embryonic day 16 using confocal immunofluorescence. From embryonic day 18 to postnatal day 6, spiral ganglion neuron cell bodies and peripheral neurites projecting to the inner and outer hair cells were labelled for $P2X_3$ receptor protein, but diminished around postnatal day 6, and were no longer detected at the onset of hearing (around postnatal day 11). These data suggest a role for $P2X_3$ receptor-mediated purinergic signalling in cochlea synaptic reorganization and establishment of neurotransmission that occurs just prior to the onset of hearing function (Huang et al. 2006).

Merkel cells appear in the epidermis of the planum nasale of rat fetuses from the 16th day of intrauterine development and sensory nerve fibres form close association with them by day 20. This is of interest since it is known that Merkel cells contain high levels of peptide-bound ATP and are in close association with sensory fibres expressing $P2X_3$ receptors (Burnstock and Wood 1996).

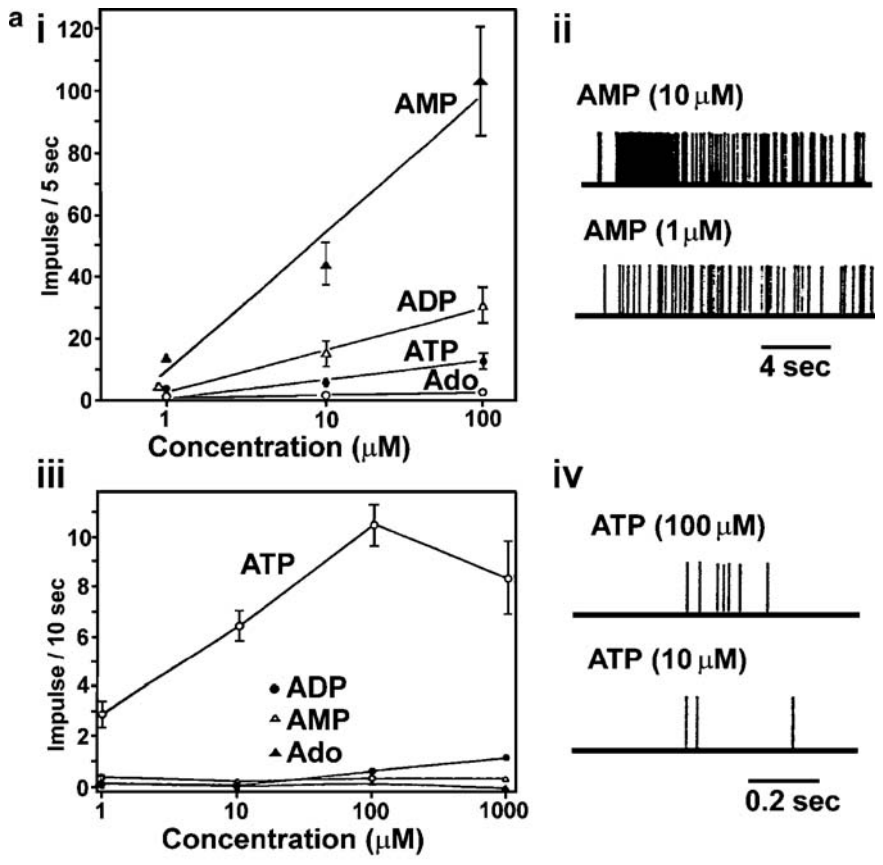
Studies of purinergic signalling in stem cells are beginning; the preliminary reports are encouraging and hopefully this will develop into a major new area of purinergic research (see, e.g., Mishra et al. 2006; Lin et al. 2007).

8 Evolution of Purinergic Sensory Mechanisms

Nucleosides and nucleotides are part of a primitive signalling system with potent actions in both invertebrates and lower vertebrates (Burnstock 1996a, 2007). For example, in the leech, ATP and ADP potently activated “noxious” and touch neurons. AMP was found to be the most potent chemoattractant of octopus, initiating a locomotor response; the suckers in the arms carry sensory organs with chemoreceptors that direct the arms towards a meal. There is considerable information about the effects of ATP and adenosine in crustaceans in the early literature,

particularly by Carr and colleagues, which has been reviewed. The olfactory organs of the spiny lobsters *Panulirus argus* and *Panulirus interruptus* have different populations of purinergic chemoreceptors that are excited by AMP, ADP or ATP (Fig. 9a), via receptors that show similarities to P2 receptors described in vertebrates. These receptors reside on chemosensitive neurons that are contained within aesthetasc sensilla on the lateral filaments of the antennules. 5′-AMP odorant receptor sites have been localized ultrastructurally, utilizing 5′-AMP-biotin, along the entire dendritic region, including the transitional zone between inner and outer dendritic segments, the region that also contains 5′-ectonuclotidase and phosphatase. Since these receptors are more sensitive to the slowly degradable analogues of ATP, α,β-meATP and β,γ-meATP, they appear to be comparable to mammalian $P2X_1$ and $P2X_3$ receptors. Ectonucleotidases dephosphorylate adenine nucleotides to yield a nucleoside, which is internalized by an uptake system. Activation of olfactory and gustatory P2 receptors in lobsters induces a feeding behavioural response. ATP is an ideal stimulus for such animals that feed on wounded or recently killed animals, since ATP occurs at high concentrations in fresh animal flesh but decays rapidly as cells die. Since predators such as lobsters often inhabit crevices and only emerge to feed at night, foraging is directed principally by chemical stimuli, rather than visual or mechanical stimuli. ATP is detected in prey organisms, such as mussels and oysters, which contain high concentrations of nucleotides that are released when the animal dies. Olfactory purinoceptors have also been identified in the shrimp and blue crab. In lobsters and other decapod crustaceans, the sites of olfaction and gustation are anatomically distinct, the former in the antennules, the latter on the walking legs, maxillipeds and mouthparts. The sensilla on the walking legs of the spiny lobster have also been shown to possess ATP- and AMP-sensitive cells as well as enzymes that dephosphorylate purine nucleotides.

ATP released from mammalian erythrocytes stimulates the gorging responses in a variety of blood-feeding insects such as mosquitoes, black fly, horsefly, stable fly, tsetse fly and haematophagous ticks. Electrophysiological methods have been used to demonstrate that the apical sensilla of the labrum of mosquito express the ATP receptors involved in blood feeding (Fig. 9b). Novobiocin, which blocks ATP access to its binding site, inhibits the gorging response. The ED_{50} of ATP for tsetse fly females is 13 nM, while for males it is 140 nM; this level of sensitivity for detecting ATP is the highest recorded for an insect. Other chemosensory P2 receptors have been identified that are involved in the recognition of a blood meal in haematophagous insects. These represent a heterogeneous group. Many blood-feeding insects recognize ATP and related compounds as phagostimulants. In mosquitoes and tsetse flies, ATP is found to be more potent than ADP at stimulating feeding, while AMP is a very poor phagostimulant, indicating an ATP-selective P2 receptor. A similar ATP-selective receptor mediates the phagostimulatory response of insect larvae, suggesting that this response is not limited to the adult form. α,β-meATP and β,γ-meATP are less potent than ATP as phagostimulants in the tsetse fly, raising the possibility that a P2Y receptor maybe involved. A similar order of potency was found for the bug *Rhodnius*, while the potency order



b

Nucleotide	ED_{50} [x] μmol/l	Phagostimulant index=(ED_{50}ATPx100)/ ED_{50}test nucleotide)
Control	No feeding	—
Adenosine	No feeding	—
AMP	463.0	3
ADP	96.5	13
ATP	12.2	100
UTP	No feeding	—
2d ADP	248.5	5
2d ATP	24.9	49
2'3'dd ATP	1.2	1017

Fig. 9 Invertebrate sensory mechanisms. (**a**) Comparisons of response characteristics of AMP-sensitive and ATP-sensitive sensory nerves in the antennule of the spiny lobster. *i* response of AMP-best cells to the compounds indicated. *ii* series of action potentials produced by an AMP-best

was ADP > ATP > β,γ-meATP > AMP for the mosquito. ADP was also found to be the most potent phagostimulant of the horsefly. ADP-selective receptors, namely, $P2Y_1$, $P2Y_{12}$ and $P2Y_{13}$, have been identified in mammals. It is fascinating that apyrase (ATP diphosphohydrolase) has been reported to have exceptionally high activity in the salivary glands or saliva of blood-sucking insects, including the bug *Rhodnius*, tsetse fly, mosquito and sandfly. In all cases, since ADP induces platelet aggregation, breakdown of ADP by apyrase leads to enhanced haemorrhage and more effective blood sucking.

Taste chemosensilla sensitive to nucleotides have been identified in some non-haematophagous insects. ATP was first reported to be a feeding stimulant in a flea and tick. In the omnivorous common blowfly, ATP does not have a direct stimulatory action, but rather modulates the responses of the labilla sensilla; it reduces the responses to NaCl and fructose, but enhances responses to sucrose and glucose. Adenosine stimulates feeding in the African army worm; this larva of an owlmoth exclusively feeds on grasses. There are multiple nucleotide receptor sites in the labellar taste receptor cells of the flesh fly: ATP, ADP and AMP stimulate the sugar receptor cells, while the salt receptor cells only responded to GDP and to a lesser extent IDP and UDP. ATP receptors cloned in the platyhelminth *Schistosoma mansoni* and the protozoan *Dictyostelium* show surprisingly close similarity to mammalian P2X receptors (Agboh et al. 2004; Ludlow and Ennion 2006; Fountain et al. 2007).

9 Concluding Comments

This review has covered a wide spectrum of information about the roles of purinergic signalling in the physiological and pathophysiological processes of sensory nerves and mechanosensory transduction.

The last 10 years has been a period of rapid progress in identifying the numerous types of purinergic receptors and in understanding their relationships, pharmacological properties and intracellular transduction mechanisms. This progress has facilitated new appreciation of the wide spectrum of neural activities involving purinergic signalling, including the roles of ATP, ADP and adenosine in sensory signalling in both the peripheral nervous system and the CNS.

Fig. 9 (continued) cell to the concentration of AMP indicated. *iii* response of ATP-best cells to the compounds indicated. *iv* series of action potentials produced by an ATP-best cell to the concentrations of ATP indicated. Note the differences in time scale in *ii* and *iv*. (**b**) Values for the phagostimulant (gorging) response of the mosquito *Aedes aegypti* produced by different nucleotides dissolved in the control (150 mmol l^{-1} NaCl with 10 mmol l^{-1} $NaHCO_3$). There were also no feeding responses to GTP and ITP. *2d ADP* 2′-deoxy ADP, *2d ATP* 2′-deoxy ATP, *2′3′dd ATP* 2′3′-dideoxy ATP. (**a** Reproduced from Trapido-Rosenthal et al. 1989, with permission from Taylor and Francis; **b** reproduced from Werner-Reiss et al. 1999, with permission from Elsevier)

The chemistry of ATP in the extracellular environment is dynamic and complex, and more must be learned about the extracellular biochemistry and enzymes that regulate the synthesis and degradation of ATP outside the cell. The activity of ectonucleotidases in subcellular domains and how these enzymes change during development, disease and physiological state are still to be resolved. The development of selective inhibitors for the different subtypes of ectonucleotidases would be a valuable step forward.

While it is now clear that many different cell types release ATP, often acting on P2 receptors on sensory nerve terminals, we still await a clear understanding of the mechanisms that underlie ATP transport. Until recently, it was usually assumed that the source of extracellular ATP acting on purinoceptors was damaged or dying cells, but it is now recognized that the ATP release from healthy cells by mechanical distortion, hypoxia and various agents is a physiological mechanism (Bodin and Burnstock 2001; Lazarowski et al. 2003; Schwiebert et al. 2003). There is an active debate, however, about the precise transport mechanism(s) involved. There is compelling evidence for exocytotic vesicular release of ATP from nerves, but for ATP release from non-neuronal cells, various transport mechanisms have been proposed, including ATP binding cassette transporters, connexin or pannexin hemichannels or possibly plasmalemmal voltage-dependent anion channels, as well as vesicular release. Perhaps surprisingly, evidence was presented that the release of ATP from urothelial cells during purinergic mechanosensory transduction in the bladder and ureter (as well as from endothelial cells) is vesicular, since monensin and brefeldin A, which interfere with vesicular formation and trafficking, inhibited distension-evoked ATP release, but gadolinium, a stretch-activated channel inhibitor, and glibenclamide, an inhibitor of two members of the ATP binding cassette protein family, did not (Knight et al. 2002). Hopefully, when the ATP transport mechanisms become clearer, agents will be developed that will be able to enhance or inhibit ATP release, another useful way forward as a therapeutic strategy.

There are an increasing number of explorations of the therapeutic potential of purinergic signalling in various diseases of the nervous system and hopefully this will expand even further. Advances still depend on the serious endeavours of medicinal chemists to produce receptor subtype selective, small, orally bioavailable agonists and antagonists that survive degradation in vivo. However, other approaches are promising, including the development of agents that control the expression of receptors that inhibit ATP breakdown by selective inhibition of the known ectonucleotidases and agents that can be used to regulate ATP transport.

Knockout mice are available for a number of P1, P2X and P2Y receptor subtypes, but there are gaps that need to be filled and transgenic models that overexpress receptors, as well as antisense oligonucleotides, are also needed. The siRNA technique is only just beginning to be explored for purinergic signalling.

To conclude, while studies of purinergic sensory neurosignalling are moving forward rapidly and we are clearly on the steep slope of the growth curve, the field is still in its infancy and much new knowledge will hopefully emerge in the coming years.

References

Abbracchio MP, Burnstock G, Boeynaems J-M, Barnard EA, Boyer JL, Kennedy C, Knight GE, Fumagalli M, Gachet C, Jacobson KA, Weisman GA (2006) International Union of Pharmacology. Update and subclassification of the P2Y G protein-coupled nucleotide receptors: from molecular mechanisms and pathophysiology to therapy. Pharmacol Rev 58:281–341

Adriaensen D, Timmermans JP (2004) Purinergic signalling in the lung: important in asthma and COPD? Curr Opin Pharmacol 4:207–214

Adriaensen D, Brouns I, Pintelon I, De Proost I, Timmermans JP (2006) Evidence for a role of neuroepithelial bodies as complex airway sensors: comparison with smooth muscle-associated airway receptors. J Appl Physiol 101:960–970

Agboh KC, Webb TE, Evans RJ, Ennion SJ (2004) Functional characterization of a P2X receptor from *Schistosoma mansoni*. J Biol Chem 279:41650–41657

Alavi AM, Dubyak GR, Burnstock G (2001) Immunohistochemical evidence for ATP receptors in human dental pulp. J Dental Res 80:476–483

Alcayaga C, Varas R, Valdes V, Cerpa V, Arroyo J, Iturriaga R, Alcayaga J (2007) ATP- and ACh-induced responses in isolated cat petrosal ganglion neurons. Brain Res 1131:60–67

Andersson KE, Wein AJ (2004) Pharmacology of the lower urinary tract: basis for current and future treatments of urinary incontinence. Pharmacol Rev 56:581–631

Antunes VR, Bonagamba LG, Machado BH (2005) Hemodynamic and respiratory responses to microinjection of ATP into the intermediate and caudal NTS of awake rats. Brain Res 1032:85–93

Aoki Y, Ohtori S, Takahashi K, Ino H, Ozawa T, Douya H, Chiba T, Moriya H (2003) $P2X_3$-immunoreactive primary sensory neurons innervating lumbar intervertebral disc in rats. Brain Res 989:214–220

Apostolidis A, Popat R, Yiangou Y, Cockayne D, Ford AP, Davis JB, Dasgupta P, Fowler CJ, Anand P (2005) Decreased sensory receptors $P2X_3$ and TRPV1 in suburothelial nerve fibers following intradetrusor injections of botulinum toxin for human detrusor overactivity. J Urol 174:977–982

Arthur DB, Akassoglou K, Insel PA (2005) $P2Y_2$ receptor activates nerve growth factor/TrkA signaling to enhance neuronal differentiation. Proc Natl Acad Sci USA 102:19138–19143

Atiemo H, Wynes J, Chuo J, Nipkow L, Sklar GN, Chai TC (2005) Effect of botulinum toxin on detrusor overactivity induced by intravesical adenosine triphosphate and capsaicin in a rat model. Urology 65:622–626

Bartel DL, Sullivan SL, Lavoie EG, Sévigny J, Finger TE (2006) Nucleoside triphosphate diphosphohydrolase-2 is the ecto-ATPase of type I cells in taste buds. J Comp Neurol 497:1–12

Bertrand PP (2003) ATP and sensory transduction in the enteric nervous system. Neuroscientist 9:243–260

Bertrand PP, Bornstein JC (2002) ATP as a putative sensory mediator: activation of intrinsic sensory neurons of the myenteric plexus via P2X receptors. J Neurosci 22:4767–4775

Bian X, Ren J, DeVries M, Schnegelsberg B, Cockayne DA, Ford AP, Galligan JJ (2003) Peristalsis is impaired in the small intestine of mice lacking the $P2X_3$ subunit. J Physiol 551:309–322

Birder LA (2006) Urinary bladder urothelium: molecular sensors of chemical/thermal/mechanical stimuli. Vasc Pharmacol 45:221–226

Birder LA, Ruan HZ, Chopra B, Xiang Z, Barrick S, Buffington CA, Roppolo JR, Ford AP, de Groat WC, Burnstock G (2004) Alterations in P2X and P2Y purinergic receptor expression in urinary bladder from normal cats and cats with interstitial cystitis. Am J Physiol Renal Physiol 287:F1084–F1091

Blackshaw LA, Brookes SJ, Grundy D, Schemann M (2007) Sensory transmission in the gastrointestinal tract. Neurogastroenterol Motil 19:1–19

Bleehen T, Keele CA (1977) Observations on the algogenic actions of adenosine compounds on human blister base preparation. Pain 3:367–377

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

Bodin P, Burnstock G (2001) Purinergic signalling: ATP release. Neurochem Res 26:959–969

Borvendeg SJ, Al Khrasani M, Rubini P, Fischer W, Allgaier C, Wirkner K, Himmel HM, Gillen C, Illes P (2003) Subsensitivity of P2X but not vanilloid 1 receptors in dorsal root ganglia of rats caused by cyclophosphamide cystitis. Eur J Pharmacol 474:71–75

Bradbury EJ, Burnstock G, McMahon SB (1998) The expression of $P2X_3$ purinoceptors in sensory neurons: effects of axotomy and glial-derived neurotrophic factor. Mol Cell Neurosci 12:256–268

Brady CM, Apostolidis A, Yiangou Y, Baecker PA, Ford AP, Freeman A, Jacques TS, Fowler CJ, Anand P (2004) $P2X_3$-immunoreactive nerve fibres in neurogenic detrusor overactivity and the effect of intravesical resiniferatoxin. Eur Urol 46:247–253

Braga VA, Soriano RN, Braccialli AL, de Paula PM, Bonagamba LG, Paton JF, Machado BH (2007) Involvement of L-glutamate and ATP in the neurotransmission of the sympathoexcitatory component of the chemoreflex in the commissural nucleus tractus solitarii of awake rats and in the working heart-brainstem preparation. J Physiol 581:1129–1145

Brierley SM, Carter R, Jones W III, Xu L, Robinson DR, Hicks GA, Gebhart GF, Blackshaw LA (2005) Differential chemosensory function and receptor expression of splanchnic and pelvic colonic afferents in mice. J Physiol 567:267–281

Brouns I, Van Genechten J, Burnstock G, Timmermans J-P, Adriaensen D (2003) Ontogenesis of $P2X_3$ receptor-expressing nerve fibres in the rat lung, with special reference to neuroepithelial bodies. Biomed Res 14:80–86

Brouns I, Pintelon I, De Proost I, Alewaters R, Timmermans JP, Adriaensen D (2006) Neurochemical characterisation of sensory receptors in airway smooth muscle: comparison with pulmonary neuroepithelial bodies. Histochem Cell Biol 125:351–367

Burnstock G (1981) Pathophysiology of migraine: a new hypothesis. Lancet 317:1397–1399

Burnstock G (1989) The role of adenosine triphosphate in migraine. Biomed Pharmacother 43:727–736

Burnstock G (1993) Introduction: changing face of autonomic and sensory nerves in the circulation. In: Edvinsson L, Uddman R (eds) Vascular innervation and receptor mechanisms: new perspectives. Academic, San Diego, pp 1–22

Burnstock G (1996a) Purinoceptors: ontogeny and phylogeny. Drug Dev Res 39:204–242

Burnstock G (1996b) A unifying purinergic hypothesis for the initiation of pain. Lancet 347:1604–1605

Burnstock G (1999) Release of vasoactive substances from endothelial cells by shear stress and purinergic mechanosensory transduction. J Anat 194:335–342

Burnstock G (2000) P2X receptors in sensory neurones. Br J Anaesth 84:476–488

Burnstock G (2001a) Purine-mediated signalling in pain and visceral perception. Trends Pharmacol Sci 22:182–188

Burnstock G (2001b) Purinergic signalling in development. In: Abbracchio MP, Williams M (eds) Purinergic and pyrimidinergic signalling I – molecular, nervous and urinogenitary system function. Handbook of experimental pharmacology, vol 151/I. Springer, Berlin, pp 89–127

Burnstock G (2003) Purinergic receptors in the nervous system. In: Schwiebert EM (ed) Purinergic receptors and signalling. Current topics in membranes, vol 54. Academic, San Diego, pp 307–368

Burnstock G (2006) Purinergic P2 receptors as targets for novel analgesics. Pharmacol Therap 110:433–454

Burnstock G (2007) Physiology and pathophysiology of purinergic neurotransmission. Physiol Rev 87:659–797

Burnstock G, Knight GE (2004) Cellular distribution and functions of P2 receptor subtypes in different systems. Int Rev Cytol 240:31–304

Burnstock G, Wood JN (1996) Purinergic receptors: their role in nociception and primary afferent neurotransmission. Curr Opin Neurobiol 6:526–532

Bystrova MF, Yatzenko YE, Fedorov IV, Rogachevskaja OA, Kolesnikov SS (2006) P2Y isoforms operative in mouse taste cells. Cell Tissue Res 323:377–382

Cao Y, Song G (2007) Purinergic modulation of respiration via medullary raphe nuclei in rats. Respir Physiol Neurobiol 155:114–120

Cao Y, Lai W-L, Chen Y-X (2006) Differential regulation of $P2X_3$ protein expression in the rat trigeminal ganglion after experimental tooth movement. West China J Stomatol 24:389–392

Castelucci P, Robbins HL, Furness JB (2003) $P2X_2$ purine receptor immunoreactivity of intraganglionic laminar endings in the mouse gastrointestinal tract. Cell Tissue Res 312:167–174

Ceruti C, Fumagalli M, Verderio C, Abbracchio MP (2006) Nucleotides as neurotransmitters of pain in migraine: a role for P2Y receptors in primary cultures from mouse trigeminal ganglia. In: Proceedings of the American Society for Neuroscience, Atlanta, GA, 14-18 October 2006

Chan ESL, Fernandez P, Cronstein BN (2007) Adenosine in inflammatory joint diseases. Purinergic Signal 3:145–152

Chen C, Nenov A, Bobbin RP (1995a) Noise exposure alters the response of outer hair cells to ATP. Hear Res 88:215–221

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

Chen CL, Broom DC, Liu Y, de Nooij JC, Li Z, Cen C, Samad OA, Jessell TM, Woolf CJ, Ma Q (2006) Runx1 determines nociceptive sensory neuron phenotype and is required for thermal and neuropathic pain. Neuron 49:365–377

Chessell IP, Hatcher JP, Hughes JP, Ulmann L, Green P, Mander PK, Reeve AJ, Rassendren F (2006) The role of $P2X_7$ and $P2X_4$ in pain processing; common or divergent pathways? Purinergic Signal 2:46–47

Cheung K-K, Burnstock G (2002) Localisation of $P2X_3$ and co-expression with $P2X_2$ receptors during rat embryonic neurogenesis. J Comp Neurol 443:368–382

Cockayne DA, Hamilton SG, Zhu Q-M, Dunn PM, Zhong Y, Novakovic S, Malmberg AB, Cain G, Berson A, Kassotakis L, Hedley L, Lachnit WG, Burnstock G, McMahon SB, Ford APDW (2000) Urinary bladder hyporeflexia and reduced pain-related behaviour in $P2X_3$-deficient mice. Nature 407:1011–1015

Cockayne DA, Dunn PM, Zhong Y, Hamilton SG, Cain GR, Knight GE, Ruan H-Z, Ping Y, Nunn P, Bei M, McMahon SB, Burnstock G, Ford APDW (2005) $P2X_2$ knockout mice and $P2X_2/P2X_3$ double knockout mice reveal a role for the $P2X_2$ receptor subunit in mediating multiple sensory effects of ATP. J Physiol 567:621–639

Collier HO, James GWL, Schneider C (1966) Antagonism by aspirin and fenamates of bronchoconstriction and nociception induced by adenosine-5′-triphosphate. Nature 212:411–412

Cook SP, McCleskey EW (2002) Cell damage excites nociceptors through release of cytosolic ATP. Pain 95:41–47

Cooke HJ, Wunderlich J, Christofi FL (2003) "The force be with you": ATP in gut mechanosensory transduction. News Physiol Sci 18:43–49

Damann N, Rothermel M, Klupp BG, Mettenleiter TC, Hatt H, Wetzel CH (2006) Chemosensory properties of murine nasal and cutaneous trigeminal neurons identified by viral tracing. BMC Neurosci 7:46

Dang K, Bielefeldt K, Gebhart GF (2004) Distinct P2X receptors on thoracolumbar and lumbosacral dorsal root ganglion neurons innervating the rat urinary bladder. Abstract viewer/itinerary planner. Program no. 2856.1 2004. Society for Neuroscience, Washington

Dang K, Bielfeldt K, Lamb K, Gebhart GF (2005) Gastric ulcers evoke hyperexcitability and enhance P2X receptor function in rat gastric sensory neurons. J Neurophysiol 93:3112–3119

Davies DL, Kochegarov AA, Kuo ST, Kulkarni AA, Woodward JJ, King BF, Alkana RL (2005) Ethanol differentially affects ATP-gated $P2X_3$ and $P2X_4$ receptor subtypes expressed in Xenopus oocytes. Neuropharmacology 49:243–253

de Groat WC (2006) Integrative control of the lower urinary tract: preclinical perspective. Br J Pharmacol 147:S25–S40

Dell'Antonio G, Quattrini A, Cin ED, Fulgenzi A, Ferrero ME (2002) Relief of inflammatory pain in rats by local use of the selective $P2X_7$ ATP receptor inhibitor, oxidized ATP. Arthritis Rheumatism 46:3378–3385

Denda M, Nakatani M, Ikeyama K, Tsutsumi M, Denda S (2007) Epidermal keratinocytes as the forefront of the sensory system. Exp Dermatol 16:157–161

Dorn G, Patel S, Wotherspoon G, Hemmings-Mieszczak M, Barclay J, Natt FJ, Martin P, Bevan S, Fox A, Ganju P, Wishart W, Hall J (2004) siRNA relieves chronic neuropathic pain. Nucleic Acids Res 32:e49

Dowd E, McQueen DS, Chessell IP, Humphrey PPA (1998) P2X receptor-mediated excitation of nociceptive afferents in the normal and arthritic rat knee joint. Br J Pharmacol 125:341–346

Du S, Araki I, Mikami Y, Zakoji H, Beppu M, Yoshiyama M, Takeda M (2007) Amiloride-sensitive ion channels in urinary bladder epithelium involved in mechanosensory transduction by modulating stretch-evoked adenosine triphosphate release. Urology 69:590–595

Dulon D, Jagger DJ, Lin X, Davis RL (2006) Neuromodulation in the spiral ganglion: shaping signals from the organ of corti to the CNS. J Membr Biol 209:167–175

Dunn PM, Zhong Y, Burnstock G (2001) P2X receptors in peripheral neurones. Prog Neurobiol 65:107–134

Färber K, Kettenmann H (2006) Purinergic signaling and microglia. Pflugers Arch Eur J Physiol 452:615–621

Ferrari D, Pizzirani C, Adinolfi E, Lemoli RM, Curti A, Idzko M, Panther E, Di Virgilio F (2006) The $P2X_7$ receptor: a key player in IL-1 processing and release. J Immunol 176:3877–3883

Finger TE, Danilova V, Barrows J, Bartel DL, Vigers AJ, Stone L, Hellekant G, Kinnamon SC (2005) ATP signaling is crucial for communication from taste buds to gustatory nerves. Science 310:1495–1499

Ford AP, Gever JR, Nunn PA, Zhong Y, Cefalu JS, Dillon MP, Cockayne DA (2006) Purinoceptors as therapeutic targets for lower urinary tract dysfunction. Br J Pharmacol 147:S132–S143

Fountain SJ, Parkinson K, Young MT, Cao L, Thompson CR, North RA (2007) An intracellular P2X receptor required for osmoregulation in *Dictyostelium discoideum*. Nature 448:200–203

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

Fukui M, Nakagawa T, Minami M, Satoh M, Kaneko S (2006) Inhibitory role of supraspinal $P2X_3$/$P2X_{2/3}$ subtypes on nociception in rats. Mol Pain 2:19–25

Fumagalli M, Ceruti S, Verderio C, Abbracchio MP (2006) ATP as a neurotransmitter of pain in migraine: a functional role for P2Y receptors in primary cultures from mouse trigeminal sensory ganglia. Purinergic Signal 2:120–121

Furness JB, Kunze WA, Bertrand PP, Clerc N, Bornstein JC (1998) Intrinsic primary afferent neurons of the intestine. Prog Neurobiol 54:1–18

Fyffe REW, Perl ER (1984) Is ATP a central synaptic mediator for certain primary afferent fibres from mammalian skin? Proc Natl Acad Sci USA 81:6890–6893

Gale JE, Piazza V, Ciubotaru CD, Mammano F (2004) A mechanism for sensing noise damage in the inner ear. Curr Biol 14:526–529

Galligan JJ (2004) Enteric P2X receptors as potential targets for drug treatment of the irritable bowel syndrome. Br J Pharmacol 141:1294–1302

Gao Z, Kehoe V, Sinoway LI, Li J (2005) Spinal P2X receptor modulates reflex pressor response to activation of muscle afferents. Am J Physiol Heart Circ Physiol 288:H2238–H2243

Gao N, Hu HZ, Zhu MX, Fang X, Liu S, Gao C, Wood JD (2006) The $P2Y_1$ purinergic receptor expressed by enteric neurones in guinea-pig intestine. Neurogastroenterol Motil 18:316–323

Gayle S, Burnstock G (2005) Immunolocalisation of P2X and P2Y nucleotide receptors in the rat nasal mucosa. Cell Tissue Res 319:27–36

Gerevich Z, Zadori Z, Müller C, Wirkner K, Schröder W, Rubini P, Illes P (2007) Metabotropic P2Y receptors inhibit P2X3 receptor-channels via G protein-dependent facilitation of their desensitization. Br J Pharmacol 151:226–236

Gever J, Cockayne DA, Dillon MP, Burnstock G, Ford APDW (2006) Pharmacology of P2X channels. Pflugers Arch Eur J Physiol 452:513–537

Gilchrist LS, Cain DM, Harding-Rose C, Kov AN, Wendelschafer-Crabb G, Kennedy WR, Simone DA (2005) Re-organization of $P2X_3$ receptor localization on epidermal nerve fibers in a murine model of cancer pain. Brain Res 1044:197–205

Goadsby PJ (2005) Migraine, allodynia, sensitisation and all of that. Eur Neurol 53:10–16

Gourine AV (2005) On the peripheral and central chemoreception and control of breathing: an emerging role of ATP. J Physiol 568:715–724

Gourine AV, Melenchuk EV, Poputnikov DM, Gourine VN, Spyer KM (2002) Involvement of purinergic signalling in central mechanisms of body temperature regulation in rats. Br J Pharmacol 135:2047–2055

Gourine AV, Atkinson L, Deuchars J, Spyer KM (2003) Purinergic signalling in the medullary mechanisms of respiratory control in the rat: respiratory neurones express the $P2X_2$ receptor subunit. J Physiol 552:197–211

Gourine AV, Dale N, Gourine VN, Spyer KM (2004) Fever in systemic inflammation: roles of purines. Front Biosci 9:1011–1022

Green PG, Basbaum AI, Helms C, Levine JD (1991) Purinergic regulation of bradykinin-induced plasma extravasation and adjuvant-induced arthritis in the rat. Proc Natl Acad Sci USA 88:4162–4165

Greenwood D, Jagger DJ, Huang LC, Hoya N, Thorne PR, Wildman SS, King BF, Pak K, Ryan AF, Housley GD (2007) P2X receptor signaling inhibits BDNF-mediated spiral ganglion neuron development in the neonatal rat cochlea. Development 134:1407–1417

Groenewegen HJ, Uylings HB (2000) The prefrontal cortex and the integration of sensory, limbic and autonomic information. Prog Brain Res 126:3–28

Gu YZ, Yin GF, Guan BC, Li ZW (2006) Characteristics of P2X purinoceptors in the membrane of rat trigeminal ganglion neurons. Sheng Li Xue Bao 58:164–170

Hamilton SG, McMahon SB, Lewin GR (2001) Selective activation of nociceptors by P2X receptor agonists in normal and inflamed rat skin. J Physiol 534:437–445

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

He L, Chen J, Dinger B, Stensaas L, Fidone S (2006) Effect of chronic hypoxia on purinergic synaptic transmission in rat carotid body. J Appl Physiol 100:157–162

Hegg CC, Lucero MT (2006) Purinergic receptor antagonists inhibit odorant-induced heat shock protein 25 induction in mouse olfactory epithelium. Glia 53:182–190

Hegg CC, Greenwood D, Huang W, Han P, Lucero MT (2003) Activation of purinergic receptor subtypes modulates odor sensitivity. J Neurosci 23:8291–8301

Hemmings-Mieszczak M, Dorn G, Natt FJ, Hall J, Wishart WL (2003) Independent combinatorial effect of antisense oligonucleotides and RNAi-mediated specific inhibition of the recombinant rat $P2X_3$ receptor. Nucleic Acids Res 31:2117–2126

Holton P (1959) The liberation of adenosine triphosphate on antidromic stimulation of sensory nerves. J Physiol (Lond) 145:494–504

Holzer P (2004) Gastrointestinal pain in functional bowel disorders: sensory neurons as novel drug targets. Expert Opin Ther Targets 8:107–123

Holzer P (2007) Taste receptors in the gastrointestinal tract. V. Acid sensing in the gastrointestinal tract. Am J Physiol Gastrointest Liver Physiol 292:G699–G705

Honore P, Donnelly-Roberts D, Namovic MT, Hsieh G, Zhu CZ, Mikusa JP, Hernandez G, Zhong C, Gauvin DM, Chandran P, Harris R, Medrano AP, Carroll W, Marsh K, Sullivan JP, Faltynek CR, Jarvis MF (2006) A-740003 [*N*-(1-{[(cyanoimino)(5-quinolinylamino) methyl]amino}-2, 2-dimethylpropyl)-2-(3,4-dimethoxyphenyl)acetamide], a novel and selective $P2X_7$ receptor

antagonist, dose-dependently reduces neuropathic pain in the rat. J Pharmacol Exp Ther 319:1376–1385

Housley GD, Marcotti W, Navaratnam D, Yamoah EN (2006) Hair cells – beyond the transducer. J Membr Biol 209:89–118

Hu B, Chiang CY, Hu JW, Dostrovsky JO, Sessle BJ (2002) P2X receptors in trigeminal subnucleus caudalis modulate central sensitization in trigeminal subnucleus oralis. J Neurophysiol 88:1614–1624

Hu ST, Gever J, Nunn PA, Ford AP, Zhu Q-M (2004) Cystometric studies with ATP, PPADS and TNP-ATP in conscious and anaesthetised C57BL/6 mice. J Urol 171:461–462

Huang LC, Ryan AF, Cockayne DA, Housley GD (2006) Developmentally regulated expression of the $P2X_3$ receptor in the mouse cochlea. Histochem Cell Biol 125:681–692

Huang YJ, Maruyama Y, Dvoryanchikov G, Pereira E, Chaudhari N, Roper SD (2007) The role of pannexin 1 hemichannels in ATP release and cell-cell communication in mouse taste buds. Proc Natl Acad Sci USA 104:6436–6441

Hughes JP, Hatcher JP, Chessell IP (2007) The role of $P2X_7$ in pain and inflammation. Purinergic Signal 3:163–169

Ichikawa H, Fukunaga T, Jin HW, Fujita M, Takano-Yamamoto T, Sugimoto T (2004) VR1-, VRL-1- and $P2X_3$ receptor-immunoreactive innervation of the rat temporomandibular joint. Brain Res 1008:131–136

Ichikawa H, De Repentigny Y, Kothary R, Sugimoto T (2006) The survival of vagal and glossopharyngeal sensory neurons is dependent upon dystonin. Neuroscience 137:531–536

Ikeda H, Tsuda M, Inoue K, Murase K (2007) Long-term potentiation of neuronal excitation by neuron-glia interactions in the rat spinal dorsal horn. Eur J Neurosci 25:1297–1306

Inoue K (2007) P2 receptors and chronic pain. Purinergic Signal 3:135–144

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

Jennings EA, Christie MJ, Sessle BJ (2006) ATP potentiates neurotransmission in the rat trigeminal subnucleus caudalis. Neuroreport 17:1507–1510

Kamei J, Takahashi Y, Yoshikawa Y, Saitoh A (2005) Involvement of P2X receptor subtypes in ATP-induced enhancement of the cough reflex sensitivity. Eur J Pharmacol 528:158–161

Kataoka S, Toyono T, Seta Y, Ogura T, Toyoshima K (2004) Expression of $P2Y_1$ receptors in rat taste buds. Histochem Cell Biol 121:419–426

Kindig AE, Hayes SG, Kaufman MP (2007) Purinergic 2 receptor blockade prevents the responses of group IV afferents to post-contraction circulatory occlusion. J Physiol 578:301–308

King BF, Knowles I, Burnstock G, Ramage A (2004) Investigation of the effects of P2 purinoceptor ligands on the micturition reflex in female urethane-anaesthetised rats. Br J Pharmacol 142:519–530

Kitchen AM, Collins HL, DiCarlo SE, Scislo TJ, O'Leary DS (2001) Mechanisms mediating NTS P2x receptor-evoked hypotension: cardiac output vs. total peripheral resistance. Am J Physiol Heart Circ Physiol 281:H2198–H2203

Knight GE, Bodin P, de Groat WC, Burnstock G (2002) ATP is released from guinea pig ureter epithelium on distension. Am J Physiol Renal Physiol 282:F281–F288

Kobayashi K, Fukuoka T, Yamanaka H, Dai Y, Obata K, Tokunaga A, Noguchi K (2006) Neurons and glial cells differentially express P2Y receptor mRNAs in the rat dorsal root ganglion and spinal cord. J Comp Neurol 498:443–454

Koizumi S, Fujishita K, Inoue K, Shigemoto-Mogami Y, Tsuda M, Inoue K (2004) Ca^{2+} waves in keratinocytes are transmitted to sensory neurons: the involvement of extracellular ATP and $P2Y_2$ receptor activation. Biochem J 380:329–338

Kollarik M, Dinh QT, Fischer A, Undem BJ (2003) Capsaicin-sensitive and -insensitive vagal bronchopulmonary C-fibres in the mouse. J Physiol 551:869–879

Kollarik M, Ru F, Undem BJ (2007) Acid-sensitive vagal sensory pathways and cough. Pulm Pharmacol Ther 20:402–411

Korim WS, Ferreira-Neto ML, Cravo SLD (2007) Role of NTS P2x receptors in cardiovascular adjustments during alerting defense reactions. FASEB J 21:5750.16

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

Krishtal O, Lozovaya N, Fedorenko A, Savelyev I, Chizhmakov I (2006) The agonists for nociceptors are ubiquitous, but the modulators are specific: P2X receptors in the sensory neurons are modulated by cannabinoids. Pflugers Arch Eur J Physiol 453:353–360

Labrakakis C, Gerstner E, MacDermott AB (2000) Adenosine triphosphate-evoked currents in cultured dorsal root ganglion neurons obtained from rat embryos: desensitization kinetics and modulation of glutamate release. Neuroscience 101:1117–1126

Lahiri S, Mitchell CH, Reigada D, Roy A, Cherniack NS (2007) Purines, the carotid body and respiration. Respir Physiol Neurobiol 157:123–129

Lazarowski ER, Boucher RC, Harden TK (2003) Mechanisms of release of nucleotides and integration of their action as P2X- and P2Y-receptor activating molecules. Mol Pharmacol 64:785–795

Lee JH, Heo JH, Kim CH, Chang SO, Kim CS, Oh SH (2007) Changes in $P2Y_4$ receptor expression in rat cochlear outer sulcus cells during development. Hear Res 228:201–211

Lewis C, Neidhart S, Holy C, North RA, Buell G, Surprenant A (1995) Coexpression of $P2X_2$ and $P2X_3$ receptor subunits can account for ATP-gated currents in sensory neurons. Nature 377:432–435

Li P, Calejesan AA, Zhou M (1998) ATP P_{2X} receptors and sensory synaptic transmission between primary afferent fibers and spinal dorsal horn neurons in rats. J Neurophysiol 80:3356–3360

Lin JH, Takano T, Arcuino G, Wang X, Hu F, Darzynkiewicz Z, Nunes M, Goldman SA, Nedergaard M (2007) Purinergic signaling regulates neural progenitor cell expansion and neurogenesis. Dev Biol 302:356–366

Llewellyn-Smith IJ, Burnstock G (1998) Ultrastructural localization of $P2X_3$ receptors in rat sensory neurons. Neuroreport 9:2245–2250

Loesch A, Burnstock G (2001) Immunoreactivity to $P2X_6$ receptors in the rat hypothalamo-neurohypophysial system: an ultrastructural study with ExtrAvidin and colloidal gold-silver immunolabelling. Neuroscience 106:621–631

Loredo GA, Benton HP (1998) ATP and UTP activate calcium-mobilizing P2U-like receptors and act synergistically with interleukin-1 to stimulate prostaglandin E_2 release from human rheumatoid synovial cells. Arthritis Rheumatism 41:246–255

Lorier AR, Huxtable AG, Robinson DM, Lipski J, Housley GD, Funk GD (2007) $P2Y_1$ receptor modulation of the pre-Bötzinger complex inspiratory rhythm generating network in vitro. J Neurosci 27:993–1005

Ludlow M, Ennion S (2006) A putative *Dictyostelium discoideum* P2X receptor. Purinergic Signal 2:81–82

Luo J, Yin GF, Gu YZ, Liu Y, Dai JP, Li C, Li ZW (2006) Characterization of three types of ATP-activated current in relation to P2X subunits in rat trigeminal ganglion neurons. Brain Res 1115:9–15

Ma W, Quirion R (2007) Inflammatory mediators modulating the transient receptor potential vanilloid 1 receptor: therapeutic targets to treat inflammatory and neuropathic pain. Expert Opin Ther Targets 11:307–320

Maggi CA, Meli A (1988) The sensory-efferent function of capsaicin-sensitive sensory neurons. Gen Pharmacol 19:1–43

Makowska A, Panfil C, Ellrich J (2006) ATP induces sustained facilitation of craniofacial nociception through P2X receptors on neck muscle nociceptors in mice. Cephalalgia 26:697–706

Matsuka Y, Neubert JK, Maidment NT, Spigelman I (2001) Concurrent release of ATP and substance P within guinea pig trigeminal ganglia in vivo. Brain Res 915:248–255

Matsuka Y, Edmonds B, Mitrirattanakul S, Schweizer FE, Spigelman I (2007) Two types of neurotransmitter release patterns in isolectin B4-positive and negative trigeminal ganglion neurons. Neuroscience 144:665–674

Maul E, Sears M (1979) ATP is released into the rabbit eye by antidromic stimulation of the trigeminal nerve. Invest Ophthalmol Vis Sci 18:256–262

McGaraughty S, Jarvis MF (2006) Purinergic control of neuropathic pain. Drug Dev Res 67:376–388

McGaraughty S, Chu KL, Namovic MT, Donnelly-Roberts DL, Harris RR, Zhang XF, Shieh CC, Wismer CT, Zhu CZ, Gauvin DM, Fabiyi AC, Honore P, Gregg RJ, Kort ME, Nelson DW, Carroll WA, Marsh K, Faltynek CR, Jarvis MF (2007) $P2X_7$-related modulation of pathological nociception in rats. Neuroscience 146:1817–1828

McQueen DS, Bond SM, Moores C, Chessell I, Humphrey PP, Dowd E (1998) Activation of P2X receptors for adenosine triphosphate evokes cardiorespiratory reflexes in anaesthetized rats. J Physiol 507:843–855

Millward-Sadler SJ, Wright MO, Flatman PW, Salter DM (2004) ATP in the mechanotransduction pathway of normal human chondrocytes. Biorheology 41:567–575

Mishra SK, Braun N, Shukla V, Füllgrabe M, Schomerus C, Korf HW, Gachet C, Ikehara Y, Sévigny J, Robson SC, Zimmermann H (2006) Extracellular nucleotide signaling in adult neural stem cells: synergism with growth factor-mediated cellular proliferation. Development 133:675–684

Monro RL, Bertrand PP, Bornstein JC (2004) ATP participates in three excitatory postsynaptic potentials in the submucous plexus of the guinea pig ileum. J Physiol 556:51–584

Moore KH, Ray FR, Barden JA (2001) Loss of purinergic $P2X_3$ and $P2X_5$ receptor innervation in human detrusor from adults with urge incontinence. J Neurosci 21:C166:1–6

Mori M, Tsushima H Matsuda T (1994) Antidiuretic effects of ATP induced by microinjection into the hypothalamic supraoptic nucleus in water-loaded and ethanol-anesthetized rats. Jpn J Pharmacol 66:445–450

Mulkey DK, Mistry AM, Guyenet PG, Bayliss DA (2006) Purinergic P2 receptors modulate excitability but do not mediate *p*H sensitivity of RTN respiratory chemoreceptors. J Neurosci 26:7230–7233

Nagamine K, Ozaki N, Shinoda M, Asai H, Nishiguchi H, Mitsudo K, Tohnai I, Ueda M, Sugiura Y (2006) Mechanical allodynia and thermal hyperalgesia induced by experimental squamous cell carcinoma of the lower gingiva in rats. J Pain 7:659–670

Nakatsuka T, Gu JG (2006) P2X purinoceptors and sensory transmission. Pflugers Arch Eur J Physiol 452:598–607

Nakatsuka T, Tsuzuki K, Ling JX, Sonobe H, Gu JG (2003) Distinct roles of P2X receptors in modulating glutamate release at different primary sensory synapses in rat spinal cord. J Neurophysiol 89:3243–3252

Nazif O, Teichman JM, Gebhart GF (2007) Neural upregulation in interstitial cystitis. Urology 69:24–33

Neal M, Cunningham J (1994) Modulation by endogenous ATP of the light-evoked release of ACh from retinal cholinergic neurones. Br J Pharmacol 113:1085–1087

Nelson DW, Gregg RJ, Kort ME, Perez-Medrano A, Voight EA, Wang Y, Grayson G, Namovic MT, Donnelly-Roberts DL, Niforatos W, Honore P, Jarvis MF, Faltynek CR, Carroll WA (2006) Structure-activity relationship studies on a series of novel, substituted 1-benzyl-5-phenyltetrazole $P2X_7$ antagonists. J Med Chem 49:3659–3666

North RA (2002) Molecular physiology of P2X receptors. Physiol Rev 82:1013–1067

North RA, Verkhratsky A (2006) Purinergic transmission in the central nervous system. Pflugers Arch Eur J Physiol 452:479–485

Norton WHJ, Rohr KB, Burnstock G (2000) Embryonic expression of a $P2X_3$ receptor encoding gene in zebrafish. Mech Dev 99:149–152

Nurse CA (2005) Neurotransmission and neuromodulation in the chemosensory carotid body. Auton Neurosci 120:1–9

Okuse K (2007) Pain signalling pathways: from cytokines to ion channels. Int J Biochem Cell Biol 39:490–496

Oliveira MC, Parada CA, Veiga MC, Rodrigues LR, Barros SP, Tambeli CH (2005) Evidence for the involvement of endogenous ATP and P2X receptors in TMJ pain. Eur J Pain 9:87–93

Pandita RK, Andersson KE (2002) Intravesical adenosine triphosphate stimulates the micturition reflex in awake, freely moving rats. J Urol 168:1230–1234

Papka RE, Hafemeister J, Storey-Workley M (2005) P2X receptors in the rat uterine cervix, lumbosacral dorsal root ganglia, and spinal cord during pregnancy. Cell Tissue Res 321:35–44

Patel MK, Khakh BS, Henderson G (2001) Properties of native P2X receptors in rat trigeminal mesencephalic nucleus neurones: lack of correlation with known, heterologously expressed P2X receptors. Neuropharmacology 40:96–105

Paton JF, De Paula PM, Spyer KM, Machado BH, Boscan P (2002) Sensory afferent selective role of P2 receptors in the nucleus tractus solitarii for mediating the cardiac component of the peripheral chemoreceptor reflex in rats. J Physiol 543:995–1005

Pelleg A, Hurt CM (1990) Evidence for ATP-triggered vagal reflex in the canine heart in vivo. Ann N Y Acad Sci 603:441–442

Piazza V, Ciubotaru CD, Gale JE, Mammano F (2007) Purinergic signalling and intercellular Ca^{2+} wave propagation in the organ of Corti. Cell Calcium 41:77–86

Pintelon I, Brouns I, De Proost I, Van Meir F, Timmermans JP, Adriaensen D (2007) Sensory receptors in the visceral pleura: neurochemical coding and live staining in whole mounts. Am J Respir Cell Mol Biol 36:541–551

Pintor J (2000) Purinergic signalling in the eye. In: Burnstock G, Sillito AM (eds) Nervous control of the eye. Harwood, Amsterdam, pp 171–210

Pintor J, Peral A, Pelaez T, Martin S, Hoyle CH (2003) Presence of diadenosine polyphosphates in the aqueous humor: their effect on intraocular pressure. J Pharmacol Exp Therap 304:342–348

Poelchen W, Sieler D, Wirkner K, Illes P (2001) Co-transmitter function of ATP in central catecholaminergic neurons of the rat. Neuroscience 102:593–602

Puthussery T, Fletcher EL (2006) $P2X_2$ receptors on ganglion and amacrine cells in cone pathways of the rat retina. J Comp Neurol 496:595–609

Puthussery T, Fletcher EL (2007) Neuronal expression of $P2X_3$ purinoceptors in the rat retina. Neuroscience 146:403–414

Puthussery T, Yee P, Vingrys AJ, Fletcher EL (2006) Evidence for the involvement of purinergic $P2X_7$ receptors in outer retinal processing. Eur J Neurosci 24:7–19

Raybould HE, Cooke HJ, Christofi FL (2004) Sensory mechanisms: transmitters, modulators and reflexes. Neurogastroenterol Motil 16:60–63

Ren Y, Zou X, Fang L, Lin Q (2006) Involvement of peripheral purinoceptors in sympathetic modulation of capsaicin-induced sensitization of primary afferent fibers. J Neurophysiol 96:2207–2216

Renton T, Yiangou Y, Baecker PA, Ford AP, Anand P (2003) Capsaicin receptor VR1 and ATP purinoceptor $P2X_3$ in painful and nonpainful human tooth pulp. J Orofac Pain 17:245–250

Resta V, Novelli E, Vozzi G, Scarpa C, Caleo M, Ahluwalia A, Solini A, Santini E, Parisi V, Di Virgilio F, Galli-Resta L (2007) Acute retinal ganglion cell injury caused by intraocular pressure spikes is mediated by endogenous extracellular ATP. Eur J Neurosci 25:2741–2754

Reyes EP, Fernández R, Larraín C, Zapata P (2007) Effects of combined cholinergic-purinergic block upon cat carotid body chemoreceptors in vitro. Respir Physiol Neurobiol 156:17–22

Rich PB, Douillet CD, Mahler SA, Husain SA, Boucher RC (2003) Adenosine triphosphate is released during injurious mechanical ventilation and contributes to lung edema. J Trauma 55:290–297

Robinson DR, McNaughton PA, Evans ML, Hicks GA (2004) Characterization of the primary spinal afferent innervation of the mouse colon using retrograde labelling. Neurogastroenterol Motil 16:113–124

Rocha I, Burnstock G, Spyer KM (2001) Effect on urinary bladder function and arterial blood pressure of the activation of putative purine receptors in brainstem areas. Auton Neurosci 88:6–15

Romanov RA, Rogachevskaja OA, Bystrova MF, Jiang P, Margolskee RF, Kolesnikov SS (2007) Afferent neurotransmission mediated by hemichannels in mammalian taste cells. EMBO J 26:657–667

Rong W, Burnstock G (2004) Activation of ureter nociceptors by exogenous and endogenous ATP in guinea pig. Neuropharmacology 47:1093–1101

Rong W, Burnstock G, Spyer KM (2000) P2X purinoceptor-mediated excitation of trigeminal lingual nerve terminals in an in vitro intra-arterially perfused rat tongue preparation. J Physiol 524:891–902

Rong W, Gourine A, Cockayne DA, Xiang Z, Ford APDW, Spyer KM, Burnstock G (2003) Pivotal role of nucleotide $P2X_2$ receptor subunit mediating ventilatory responses to hypoxia: knockout mouse studies. J Neurosci 23:11315–11321

Ruan H-Z, Burnstock G (2003) Localisation of $P2Y_1$ and $P2Y_4$ receptors in dorsal root, nodose and trigeminal ganglia of the rat. Histochem Cell Biol 120:415–426

Ruan H-Z, Moules E, Burnstock G (2004) Changes in P2X purinoceptors in sensory ganglia of the mouse during embryonic and postnatal development. Histochem Cell Biol 122:539–551

Ruan H-Z, Birder LA, de Groat WC, Tai C, Roppolo J, Buffington A, Burnstock G (2005) Localization of P2X and P2Y receptors in dorsal root ganglia of the cat. J Histochem Cytochem 53:1273–1282

Ruan T, Lin YS, Lin KS, Kou YR (2006) Mediator mechanisms involved in TRPV1 and P2X receptor-mediated, ROS-evoked bradypneic reflex in anesthetized rats. J Appl Physiol 101:644–654

Rubino A, Burnstock G (1996) Capsaicin-sensitive sensory-motor neurotransmission in the peripheral control of cardiovascular function. Cardiovasc Res 31:467–479

Ruggieri MR Sr (2006) Mechanisms of disease: role of purinergic signaling in the pathophysiology of bladder dysfunction. Nat Clin Pract Urol 3:206–215

Salas NA, Somogyi GT, Gangitano DA, Boone TB, Smith CP (2007) Receptor activated bladder and spinal ATP release in neurally intact and chronic spinal cord injured rats. Neurochem Int 50:45–350

Salter MW, Henry JL (1985) Effects of adenosine 5′-monophosphate and adenosine 5′-triphosphate on functionally identified units in the cat spinal dorsal horn. Evidence for a differential effect of adenosine 5′-triphosphate on nociceptive vs non-nociceptive units. Neuroscience 15:15–825

Schwiebert EM, Zsembery A, Geibel JP (2003) Cellular mechanisms and physiology of nucleotide and nucleoside release from cells: current knowledge, novel assays to detect purinergic agonists, and future directions. Curr Top Membr 54:31–58

Scislo TJ, Ichinose T, O'Leary DS (2007) Activation of NTS A_1 adenosine receptors differentially resets baroreflex control of adrenal (ASNA) and renal (RSNA) sympathetic nerve activity. FASEB J 21:582.15

Seino D, Tokunaga A, Tachibana T, Yoshiya S, Dai Y, Obata K, Yamanaka H, Kobayashi K, Noguchi K (2006) The role of ERK signaling and the P2X receptor on mechanical pain evoked by movement of inflamed knee joint. Pain 123:193–203

Sharp CJ, Reeve AJ, Collins SD, Martindale JC, Summerfield SG, Sargent BS, Bate ST, Chessell IP (2006) Investigation into the role of $P2X_3$/$P2X_{2/3}$ receptors in neuropathic pain following chronic constriction injury in the rat: an electrophysiological study. Br J Pharmacol 148:845–852

Shen J, Harada N, Nakazawa H, Kaneko T, Izumikawa M, Yamashita T (2006) Role of nitric oxide on ATP-induced Ca^{2+} signaling in outer hair cells of the guinea pig cochlea. Brain Res 1081:101–112

Shieh C-C, Jarvis MF, Lee C-H, Perner RJ (2006) P2X receptor ligands and pain. Expert Opin Ther Patents 16:1113–1127

Shimizu I, Iida T, Guan Y, Zhao C, Raja SN, Jarvis MF, Cockayne DA, Caterina MJ (2005) Enhanced thermal avoidance in mice lacking the ATP receptor $P2X_3$. Pain 116:96–108

Shinoda M, Ozaki N, Asai H, Nagamine K, Sugiura Y (2005) Changes in $P2X_3$ receptor expression in the trigeminal ganglion following monoarthritis of the temporomandibular joint in rats. Pain 116:42–51

Shiokawa H, Nakatsuka T, Furue H, Tsuda M, Katafuchi T, Inoue K, Yoshimura M (2006) Direct excitation of deep dorsal horn neurones in the rat spinal cord by the activation of postsynaptic P2X receptors. J Physiol 573:753–763

Smith CP, Vemulakonda VM, Kiss S, Boone TB, Somogyi GT (2005) Enhanced ATP release from rat bladder urothelium during chronic bladder inflammation: effect of botulinum toxin A. Neurochem Int 47:291–297

Song Z, Sladek CD (2006) Site of ATP and phenylephrine synergistic stimulation of vasopressin release from the hypothalamo-neurohypophyseal system. J Neuroendocrinol 18:266–272

Song Z, Vijayaraghavan S, Sladek CD (2007) ATP increases intracellular calcium in supraoptic neurons by activation of both P2X and P2Y purinergic receptors. Am J Physiol Regul Integr Comp Physiol 292:R423–R431

Sorimachi M, Wakamoria M, Akaikeb N (2006) Excitatory effect of ATP on rat area postrema neurons. Purinergic Signal 2:545–557

Spyer KM, Dale N, Gourine AV (2004) ATP is a key mediator of central and peripheral chemosensory transduction. Exp Physiol 89:53–59

Staikopoulos V, Sessle BJ, Furness JB, Jennings EA (2007) Localization of $P2X_2$ and $P2X_3$ receptors in rat trigeminal ganglion neurons. Neuroscience 144:208–216

Stone LS, Vulchanova L (2003) The pain of antisense: in vivo application of antisense oligonucleotides for functional genomics in pain and analgesia. Ady Drug Deliv Rev 55:1081–1112

Stucky CL, Medler KA, Molliver DC (2004) The P2Y agonist UTP activates cutaneous afferent fibers. Pain 109:36–44

Szücs A, Szappanos H, Batta TJ, Tóth A, Szigeti GP, Panyi G, Csernoch L, Sziklai I (2006) Changes in purinoceptor distribution and intracellular calcium levels following noise exposure in the outer hair cells of the guinea pig. J Membr Biol 213:135–141

Taylor-Clark T, Undem BJ (2006) Transduction mechanisms in airway sensory nerves. J Appl Physiol 101:950–959

Tempest HV, Dixon AK, Turner WH, Elneil S, Sellers LA, Ferguson DR (2004) P2X and P2X receptor expression in human bladder urothelium and changes in interstitial cystitis. BJU Int 93:1344–1348

Terasawa E, Keen KL, Grendell RL, Golos TG (2005) Possible role of 5′-adenosine triphosphate in synchronization of Ca^{2+} oscillations in primate luteinizing hormone-releasing hormone neurons. Mol Endocrinol 19:2736–2747

Trang T, Beggs S, Salter MW (2006) Purinoceptors in microglia and neuropathic pain. Pflugers Arch Eur J Physiol 452:645–652

Trapido-Rosenthal HG, Carr WE, Gleeson RA (1989) Biochemistry of purinergic olfaction. The importance of nucleotide dephosphorylation. In: Brand JG, Teeter H, Cagan RH, Kare MR (eds) Receptor events and transduction in taste and olfaction. Chemical senses, vol 1. Dekker, New York, pp 243–262

Tsuda M, Inoue K (2006) P2X receptors in sensory neurons. Curr Top Membr 57:277–310

Tsuda M, Shigemoto-Mogami Y, Koizumi S, Mizokoshi A, Kohsaka S, Salter MW, Inoue K (2003) $P2X_4$ receptors induced in spinal microglia gate tactile allodynia after nerve injury. Nature 424:778–783

Undem BJ, Chuaychoo B, Lee MG, Weinreich D, Myers AC, Kollarik M (2004) Subtypes of vagal afferent C-fibres in guinea-pig lungs. J Physiol 556:905–917

Vlajkovic SM, Vinayagamoorthy A, Thorne PR, Robson SC, Wang CJ, Housley GD (2006) Noise-induced up-regulation of NTPDase3 expression in the rat cochlea: implications for auditory transmission and cochlear protection. Brain Res 1104:55–63

Vlaskovska M, Kasakov L, Rong W, Bodin P, Bardini M, Cockayne DA, Ford APDW, Burnstock G (2001) $P2X_3$ knockout mice reveal a major sensory role for urothelially released ATP. J Neurosci 21:5670–5677

Waeber C, Moskowitz MA (2003) Therapeutic implications of central and peripheral neurologic mechanisms in migraine. Neurology 61:S9–S20

Wang JC, Raybould NP, Luo L, Ryan AF, Cannell MB, Thorne PR, Housley GD (2003) Noise induces up-regulation of $P2X_2$ receptor subunit of ATP-gated ion channels in the rat cochlea. Neuroreport 14:817–823

Wang LC, Xiong W, Zheng J, Zhou Y, Zheng H, Zhang C, Zheng LH, Zhu XL, Xiong ZQ, Wang LY, Cheng HP, Zhou Z (2006) The timing of endocytosis after activation of a G-protein-coupled receptor in a sensory neuron. Biophys J 90:3590–3598

Watano T, Calvert JA, Vial C, Forsythe ID, Evans RJ (2004) P2X receptor subtype-specific modulation of excitatory and inhibitory synaptic inputs in the rat brainstem. J Physiol 558:745–757

Werner-Reiss U, Galun R, Crnjar R, Liscia A (1999) Sensitivity of the mosquito *Aedes aegypti* (Culicidae) labral apical chemoreceptors to phagostimulants. J Insect Physiol 45:629–636

Wheeler-Schilling TH, Marquordt K, Kohler K, Guenther E, Jabs R (2001) Identification of purinergic receptors in retinal ganglion cells. Brain Res Mol Brain Res 92:177–180

Wong AY, Billups B, Johnston J, Evans RJ, Forsythe ID (2006) Endogenous activation of adenosine A1 receptors, but not P2X receptors, during high-frequency synaptic transmission at the calyx of Held. J Neurophysiol 95:3336–3342

Wu C, Sui GP, Fry CH (2004) Purinergic regulation of guinea pig suburothelial myofibroblasts. J Physiol 559:231–243

Wynn G, Rong W, Xiang Z, Burnstock G (2003) Purinergic mechanisms contribute to mechanosensory transduction in the rat colorectum. Gastroenterology 125:1398–1409

Wynn G, Bei M, Ruan H-Z, Burnstock G (2004) Purinergic component of mechanosensory transduction is increased in a rat model of colitis. Am J Physiol Gastrointest Liver Physiol 287:G647–G657

Xiang Z, Burnstock G (2004a) $P2X_2$ and $P2X_3$ purinoceptors in the rat enteric nervous system. Histochem Cell Biol 121:169–179

Xiang Z, Burnstock G (2004b) Development of nerves expressing $P2X_3$ receptors in the myenteric plexus of rat stomach. Histochem Cell Biol 122:111–119

Xiang Z, Burnstock G (2006) Distribution of $P2Y_6$ and $P2Y_{12}$ receptors: their colocalisation with calbindin, calretinin and nitric oxide synthase in the guinea pig enteric nervous system. Histochem Cell Biol 125:327–336

Xiang Z, He C, Burnstock G (2006) $P2X_5$ receptors are expressed on neurons containing arginine vasopressin and neuronal nitric oxide synthase in the rat hypothalamus. Brain Res 1099:56–63

Xu J, Kussmaul W, Kurnik PB, Al-Ahdav M, Pelleg A (2005) Electrophysiological-anatomic correlates of ATP-triggered vagal reflex in the dog. V. Role of purinergic receptors. Am J Physiol Regul Integr Comp Physiol 288:R651–R655

Xue J, Askwith C, Javed NH, Cooke HJ (2007) Autonomic nervous system and secretion across the intestinal mucosal surface. Auton Neurosci 133:55–63

Yajima H, Sato J, Giron R, Nakamura R, Mizumura K (2005) Inhibitory, facilitatory, and excitatory effects of ATP and purinergic receptor agonists on the activity of rat cutaneous nociceptors in vitro. Neurosci Res 51:405–416

Yu S, Undem BJ, Kollarik M (2005) Vagal afferent nerves with nociceptive properties in guinea-pig oesophagus. J Physiol 563:831–842

Zagorodnyuk VP, Chen BN, Costa M, Brookes SJ (2003) Mechanotransduction by intraganglionic laminar endings of vagal tension receptors in the guinea-pig oesophagus. J Physiol 553:575–587

Zapata P (2007) Is ATP a suitable co-transmitter in carotid body arterial chemoreceptors? Respir Physiol Neurobiol 157:106–115

Zarei MM, Toro B, McCleskey EW (2004) Purinergic synapses formed between rat sensory neurons in primary culture. Neuroscience 126:195–201

Zhang M, Nurse CA (2004) CO_2/*p*H chemosensory signaling in co-cultures of rat carotid body receptors and petrosal neurons: role of ATP and ACh. J Neurophysiol 92:3433–3445

Zhang M, Zhong H, Vollmer C, Nurse CA (2000) Co-release of ATP and ACh mediates hypoxic signalling at rat carotid body chemoreceptors. J Physiol (Lond) 525:143–158

Zhang X, Zhang M, Laties AM, Mitchell CH (2005) Stimulation of $P2X_7$ receptors elevates Ca^{2+} and kills retinal ganglion cells. Invest Ophthalmol Vis Sci 46:2183–2191

Zhang X, Zhang M, Laties AM, Mitchell CH (2006) Balance of purines may determine life or death of retinal ganglion cells as A_3 adenosine receptors prevent loss following $P2X_7$ receptor stimulation. J Neurochem 98:566–575

Zhang M, Buttigieg J, Nurse CA (2007a) Neurotransmitter mechanisms mediating low-glucose signalling in cocultures and fresh tissue slices of rat carotid body. J Physiol 578:735–750

Zhang X, Chen Y, Wang C, Huang LY (2007b) Neuronal somatic ATP release triggers neuron-satellite glial cell communication in dorsal root ganglia. Proc Natl Acad Sci USA 104:9864–9869

Zimmermann H (2006) Nucleotide signaling in nervous system development. Pflugers Arch Eur J Physiol 452:573–588

Zimmermann K, Reeh PW, Averbeck B (2002) ATP can enhance the proton-induced CGRP release through P2Y receptors and secondary PGE_2 release in isolated rat dura mater. Pain 97:259–265

Au13

Author Query Form

Chapter No.: 10

Query Refs.	Details Required	Author's response
AU1	Please clarify whether Burnstock (1996) refers to Burnstock (1996a) or Burnstock (1996b) or both.	1996b
AU2	Reference Chen et al. (1995) has been changed to Chen et al. (1995a,b). Please delete inappropriate label 'a' or 'b'.	1995b
AU3	Please check this sentence as it is incomplete.	done
AU4	Reference Zhang et al. (2007) has been changed to Zhang et al. (2007a,b). Please delete inappropriate label 'a' or 'b'.	2007b
AU5	Please check what you want to say here. As written, you are comparing ATP release by IB4-positive neurons with ATP release by IB4-negative neurons that release neuropeptides (and not with all IB4-negative neurons).	changed
AU6	Please advise if you mean, 'which have their origin in the nodose ganglia'.	changes
AU7	Please provide the details of reference Rich et al. (2005) in the reference list.	should be 2003
AU8	Reference Brouns et al. (2003a) has been changed to Brouns et al. (2003). Please check.	OK
AU9	Please provide full reference details so this can be added to the reference list.	See below
AU10	Please check this change. In Springer publications, '-ology' should only be used to mean 'the study of. . ..'	OK
AU11	Please check this change. In Springer publications, ''-ology 'should only be used to mean 2 the study of. . ..'	OK

AU12	Please check this change. In Springer publications, '-ology' should only be used to mean 2 the study of. . .'	OK
AU13	Reference Zhang et al. (2000) has not been cited in text. Please cite.	Remove
AU14	Please confirm that as written 'deficient' only applies to the last of this list.	no - to all of them - changed
AU15	Please approve the part figure label	OK

AU9

US 2005/0209260 A1 (Hoffmann-La Roche Pharmaceuticals)

Broka CA, Carter DS, Dillon MP, Hawley RC, Jahangir A, Lin CJJ, Parish DW (Sep 22, 2005). Diaminopyrimidines as P2X3 and P2X2/3 antagonists.