The concept of purinergic signalling (i.e. ATP as an extracellular signalling molecule and neurotransmitter) was introduced in a Pharmacological Reviews article in 1972 (Burnstock, 1972). Perhaps because of the emphasis on ATP as an intracellular energy source, it was regarded by many as unlikely that such a ubiquitous molecule would be an extracellular messenger. However, after receptor subtypes for ATP and its breakdown product, adenosine, were cloned and characterized in the early 1990s and purinergic synaptic, as well as neuromuscular, transmission was established, the concept is now well established and indeed the field is expanding rapidly (see Burnstock, 2007a). Nevertheless, we are still on the steep slope of the discovery curve and there are plenty of gaps in our knowledge, a number of controversial areas still to be resolved and exciting new areas to explore, some of which will be addressed in this short review.

**Mechanism(s) of ATP transport**

For many years it was thought that, apart from nerves, the only source of extracellular ATP acting on purinoceptors was damaged or dying cells. However, it is now recognized that ATP release from healthy cells is a physiological mechanism. ATP is released from many non-neuronal cell types during mechanical deformation in response to shear stress, stretch, or osmotic swelling, as well as hypoxia and stimulation by various agents. There is an active debate about the precise transport mechanism(s) involved in ATP release. 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 (ABC) transporters, connexin or pannexin hemichannels, and possibly plasmalemmal voltage-dependent anion or P2X7 receptor channels, as well as vesicular release. Perhaps surprisingly, evidence was presented that the release of ATP from vascular endothelial cells (Bodin & Burnstock, 2001) and from urothelial cells in the ureter (Knight et al. 2002) is largely vesicular, since monensin and brefeldin A, which interfere with vesicular formation and trafficking, inhibited distension-evoked ATP release, but not gadolinium, an inhibitor of stretch-activated channels, or glibenclamide, an inhibitor of members of the ABC protein family. Since then, exocytotic vesicular release of ATP from osteoblasts, fibroblasts and astrocytes has also been reported. There is increased release of ATP from endothelial cells during acute inflammation. Local probes for real-time measurement of ATP release in biological tissues have been developed recently (see Corriden et al. 2007).

Another question that has been raised over the years concerns the mechanism of transport of ATP into synaptic vesicles, but this might have been resolved recently with the identification of a vesicular nucleotide transporter (Sawada et al. 2008).

**Multiple receptors and single cells**

On the basis of cloning, studies of transduction mechanisms and pharmacology, receptor subtypes for
Figure 1
A, the molecular topology of the P2X\textsubscript{1-6} and the P2X\textsubscript{7} receptors. The key feature of the P2X\textsubscript{7} receptor distinguishing it from the other P2X receptors is its long carboxy-terminus tail. B, the 3 different forms of the P2X\textsubscript{7} receptor upon activation by ATP: closed channel, open channel, and pore formation. Brief ATP activation leads to opening of an ion channel permeable to small ions, such as Na\textsuperscript{+}, K\textsuperscript{+} and Ca\textsuperscript{2+}, whereas prolonged ATP exposure results in the pore formation, permeable to high molecular weight organic cations, e.g. N-methyl-D-glucamine (NMDG) and YO-PRO-1 dye. C, naturally occurring splice variants of the human P2X\textsubscript{7} gene are shown. Exons are
purines and pyrimidines have been identified: P1 adenosine receptors (A1, A2A, A2B and A3 subtypes), P2X ionotropic receptors (P2X1–7 subtypes forming both homomultimers and heteromultimers) and P2Y metabotropic receptors (P2Y1, P2Y2, P2Y4, P2Y6, P2Y11, P2Y12, P2Y13 and P2Y14), some of which are also sensitive to pyrimidines (Ralevic & Burnstock, 1998; Burnstock, 2007b). Many non-neuronal cells, as well as neurones, express multiple receptor subtypes (see Burnstock & Knight, 2004) and we face questions about how they interact in controlling cellular activity (see Volonté et al. 2006). Some receptors mediate fast signalling, such as secretion, neurotransmission or neuromodulation, while others mediate longer-term ‘trophic’ signalling, regulating cell proliferation, differentiation, motility and death. Some receptors appear to operate only in pathological conditions. Perhaps the best known example of cells with multiple receptors with different functional roles is microglia. P2Y12 and P2X7 receptors mediate chemotaxis and migration to sites of injury, while P2X1 and/or P2X2 receptors may mediate the change of phenotype from ‘resting’ microglia with elaborate processes to the ‘activated’ amoeboid form at the sites of injury. P2Y6 receptors are strongly expressed in activated microglia, where they mediate phagocytic activity (Koizumi et al. 2007). P2X4 and P2X7 receptors mediate neuropathic pain and P1 (adenosine) receptors mediate neuroprotection, although the underlying mechanisms are not yet understood.

Neuropathic and inflammatory pain

This is an exciting area of strong current interest. P2X3 receptors were cloned in 1995 (Chen et al. 1995; Lewis et al. 1995) and shown to be localized in the periphery on nociceptive sensory fibres arising from sensory ganglia, and on the central terminals in inner lamina 2 of the dorsal horn of the spinal cord (Bradbury et al. 1998). P2X3 receptors and P2X2,3 heteromultimer receptors were shown to mediate purinergic mechanosensory transduction that initiates pain in visceral organs, including bladder, ureter and gut (Burnstock, 2001a). P2X3 receptors in the central nervous system (CNS) have also been shown to be involved in neuropathic and inflammatory pain (see Jarvis, 2003). Selective antagonists to P2X3 and P2X1,3 receptors prevent allodynia produced by intrathecal administration of ATP in rats (Nakagawa et al. 2007). P2X3 and P2X1,3 receptor-dependent cytosolic phospholipase A2 activity in primary sensory neurons is claimed to be a key event in neuropathic pain (Tsuda et al. 2007; Wirkner et al. 2007). The cyclic AMP sensor Epac appears to play a critical role in P2X3 sensitization in inflammation. Short interfering RNA (siRNA) for the P2X3 receptor relieves chronic neuropathic pain (Dorn et al. 2004). P2X3 receptors mediate heat hyperalgesia in a rat model of trigeminal neuropathic pain (Shinoda et al. 2007).

Perhaps surprisingly, in a seminal paper, Tsuda et al. (2003) showed that, after nerve injury, up-regulated expression of P2X4 receptors on spinal microglia mediate allodynia and supporting evidence has followed (see Trang et al. 2006; Zhang et al. 2008). Further, a number of papers have shown that antagonists to P2X7 receptors expressed on microglia also significantly reduce neuropathic pain (Donnelly-Roberts & Jarvis, 2007; Fulgenzi et al. 2008), which has also been shown to be abolished in P2X7 knockout mice (Chessell et al. 2005). P2X12 receptor up-regulation in activated microglia has also been claimed recently as a gateway of p38 signalling in neuropathic pain (Kobayashi et al. 2008). However, unresolved issues concern the mechanism underlying these striking effects and the interactions of the different P2 receptor subtypes involved in neuropathic and inflammatory pain (see Khakh & North, 2006).

P2X7 receptors

One of the most controversial areas concerns the multiple identity and modes of action of P2X7 receptors (North, 2002; Sperlágh et al. 2006; Anderson & Nedergaard, 2006; Khakh & North, 2006). Of the P2X receptor family, it is unusual in that it has an extremely long C-terminal sequence (240 amino acids) (Fig. 1A). In addition to the opening of the usual cation channel with low concentrations of ATP, when occupied by high concentrations of ATP (or 2′,3′-O-(4-benzoylbzoyl)-ATP), large pores permeable to molecules up to 900 Da are opened (Fig. 1B), which
lead to apoptosis and cell death in most situations, although proliferative actions via P2X7 receptors have also been described. Occupation of P2X7 receptors leads to blebbing (the function of which is still not clear) and shedding of microvesicles associated with the production of pro-inflammatory cytokines, including interleukin-1β (IL-1β) and transforming growth factor β mRNA expression. The P2X7 receptor field is further complicated by the identification of a number of polymorphisms or spliced variants (Cheewatrakoolpong et al. 2005; Shemon et al. 2006; Fonfria et al. 2008) (Fig. 1C and D) and by a complex story regarding antagonists, some of which work in rats but not in humans and vice versa (Gever et al. 2006; Gunosewoyo et al. 2007).

Other questions need to be resolved. For example, is pannexin1 part of the pore-forming unit of the P2X7 receptor death complex and release of IL-1β (Locovei et al. 2007; Pelegrin & Surprenant, 2007)? Is mitogen-activated protein kinase (MAPK) involved in P2X7 receptor-mediated changes in cellular permeability? Is Cl− influx involved in P2X7 receptor-mediated apoptotic cell death? Do P2X7 receptors play a pivotal role in immuno-inflammatory responses (Chen & Brosnan, 2006)? What is the significance of P2X7 receptor expression on the nuclear membrane (Atkinson et al. 2002)? Is glutamate release from astrocytes mediated by P2X7 receptors? Is the P2X7 receptor located on presynaptic and/or postsynaptic membranes in the brain or largely expressed on glial cells? There is still much to be resolved, but it is important because of potential therapeutic developments related to the P2X7 receptor in cancer, inflammatory pain and kidney disease (see Burnstock, 2006).

**Evolution of purinergic signalling**

There are many studies of the effect of nucleotides and nucleosides in most invertebrate phyla as well as in lower vertebrates, suggesting that P1 (adenosine), P2X and P2Y receptors were all present early in evolution (see Burnstock, 1996). More recently receptors have been cloned in both Dictostelium discoideum (a social amoeba) and Schistosoma mansoni (a parasitic trematode) that resemble P2X receptors in mammals (Agboh et al. 2004; Fountain et al. 2007). Also there have been recent papers suggesting that purinergic signalling is involved in regeneration of plants (Demidchik & Maathuis, 2007; Roux & Steinebrunner, 2007). It is important that more molecular studies are carried out to identify purinoceptors in invertebrates, to establish the primitive nature of the purinergic signalling system and to give insights into the advantages of this system leading to its retention in mammals.

**Development and regeneration**

The importance of purinergic signalling in embryological and postnatal development is emerging (see Burnstock, 2001b; Zimmermann, 2006), where transient expression of purinoceptor subtypes suggests that they may be involved in proliferation, differentiation and cell death of specific sequential cellular steps in the developmental process. However, hopefully this is only the beginning of studies in this interesting area with the need for further work, particularly those relating purinergic messengers to the influence of growth factors and genetic programming.

There are also an increasing number of studies of the role of purines and pyrimidines in regeneration and wound healing (see Abbracchio & Burnstock, 1998; Burnstock, 2007a) and studies of the involvement of purinergic signalling in stem cell activities are just beginning (e.g. Mishra et al. 2006; Heo & Han, 2006; Coppi et al. 2007), but more are needed.

**Behavioural studies**

At one physiological level, it is now clear that purinergic synaptic neurotransmission and neuromodulation is widespread in the CNS and that there is complex expression of different purinoceptor subtypes on both neurons and glial cells in different regions of the brain (see Burnstock, 2007a). However, there are relatively few studies of the behavioural roles of purines and pyrimidines. There are some reports of their roles in learning and memory, feeding and locomotive behaviour and some hints about their involvement in neurodegenerative diseases and psychiatric disorders, but much more research is needed in this area. Perhaps the main limiting factor for such studies is the absence at present of selective and stable purinoceptor subtype agonists and antagonists that can cross the blood–brain barrier.

**Therapeutic developments**

There has been considerable enthusiastic exploration in recent years for the use of purinergic agents for the treatment of a number of diseases, including: stroke, thrombosis, bladder incontinence, pain, cystic fibrosis, dry eye, cancer, diabetes and osteoporosis (see Burnstock, 2006). Clopidogrel, a breakdown product of which blocks P2Y12 receptor-mediated platelet aggregation, is now widely used for the treatment of stroke and thrombosis. However, for other diseases, we still await the synthesis by medicinal chemists of small molecule drugs that are stable in vivo and are orally bioavailable. For example, Roche Bioscience (Palo Alto) have recently produced a P2X3 receptor antagonist (RO3) that seems to satisfy these criteria and is in clinical trial for pain and bladder incontinence (Gever et al. 2006).
References


