Purinergic Receptors and Pain

Geoffrey Burnstock*

Autonomic Neuroscience Centre, Royal Free and University College Medical School, Rowland Hill Street, London NW3 2PF, UK

Abstract: There is a brief summary of the early background literature about purinergic signalling and its involvement in pain, of ATP storage, release and ectoenzymatic breakdown and of the current classification of receptor subtypes for purines and pyrimidines. The review then focuses on purinergic mechanosensory transduction involved in visceral, cutaneous and musculoskeletal nociception and on the roles played by P2X1, P2Y2/3, P2X4, P2X7, and P2Y12 receptors in neuropathic and inflammatory pain. Current developments of compounds for the therapeutic treatment of both visceral and neuropathic pain are discussed.

Key Words: ATP, bladder, gut, inflammatory, joints, mechanosensory transduction, neuropathic.

A. INTRODUCTION

The concept of purinergic signalling was proposed in 1972 [1] following hints in the early literature, notably by Drury and Szent-Györgyi [2], Buchthal and Folkow [3] and Emmelin and Feldberg [4] about the potent extracellular actions of purines in the heart and peripheral ganglia and from Pamela Holton [5] about the release of adenosine 5'-triphosphate (ATP) during antidromal stimulation of sensory nerve collaterals, together with the evidence that ATP and is the transmitter in non-adrenergic, non-cholinergic nerves supplying the gut [6] and bladder [7]. It is now recognised that ATP is a cotransmitter in nerves in both peripheral and central nervous systems (see [8]) and that receptors for purines and pyrimidines are widely expressed on non-neuronal as well as nerve cells (see [9]).

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 [10, 11], ATP involvement in migraine [12] and ATP participation in pain pathways in the spinal cord [13, 14]. A significant advance was made when the P2X3 ionotropic ion channel purinergic receptor was cloned in 1995 and shown to be localized predominantly on small nociceptive sensory neurons in dorsal root ganglia (DRG) together with P2X2/3 heteromultimer receptors [15, 16]. Later, Burnstock [17] put forward a unifying purinergic hypothesis for the initiation of pain, suggesting that ATP released as a cotransmitter with noradrenaline and neuropeptide Y from sympathetic nerve terminal varicosities might be involved in sympathetic pain (causalgia and reflex sympathetic dystrophy); that ATP released from vascular endothelial cells of microvessels during reactive hyperaemia is associated with pain in migraine, angina and ischemia; and that ATP released from tumor cells (which contain very high levels), damaged during ablative activity, reaches P2X3 receptors on nociceptive sensory nerves. This has been followed by an increasing number of papers expanding on this concept (see [18-20]) and also on the involvement of adenosine [21]. Immunohistochemical studies showed that the nociceptive fibres expressing P2X3 receptors arose largely from the population of small neurons that labelled with the lectin IB4 [22, 23]. The decreased sensitivity to noxious stimuli associated with the loss of IB4-binding neurons expressing P2X3 receptors indicates that these sensory neurons are essential for the signaling of acute pain [24]. The central projections of these primary afferent neurons were shown to be in inner lamina II of the dorsal horn and peripheral projections 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 [25] (Fig. 1). While P2X3 and P2X2/3 receptors, expressed in sensory neurons, were the predominant P2 receptor subtypes first recognized to be involved in the initiation of nociception, it has become apparent that P2Y receptors are also present [26, 27] and that these are involved in modulation of pain transmission [28]. P2Y receptors appear to potentiate pain induced by chemical or physical stimuli via capsacinsensitive, transient receptor potential vanilloid 1 (TRPV1) channels, and it has been proposed that the functional interaction between P2Y2 receptors and TRPV1 channels in nociceptors could underlie ATP induced inflammatory pain [29]. ATP-induced hyperalgesia was abolished in mice lacking TRPV1 receptors.

B. ATP STORAGE, RELEASE AND BREAKDOWN

The cytoplasm of most neurons contains ~2–5mM ATP, and higher concentrations of ATP (up to 100 mM) are stored in synaptic vesicles. Synaptic vesicles also contain other nucleotides such as adenosine diphosphate (ADP), adenosine monophosphate (AMP), diadenosine tetra- and pentaphosphate, and guanosine triphosphate, but at lower concentrations (see [30, 31]).
ATP is released from sensory nerve collaterals [5], from exercising human forearm muscle [32], from non-adrenergic, non-cholinergic nerves [6] and from the perfused heart during coronary vasodilation in response to hypoxia [33]. However, until recently, apart from vesicular release from nerves (e.g. [34]), it was usually assumed that the only source of extracellular ATP acting on purinoceptors was damaged or dying cells, but it is now recognized that ATP release from healthy cells is a physiological mechanism (see [35, 36]). 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. The precise transport mechanism(s) involved in ATP release are currently being examined. There is compelling evidence for exocytotic vesicular release of ATP from nerves and also from endothelial cells [37], urothelial cells [38], osteoblasts [39], fibroblasts [40] and astrocytes [41]. There is increased release of ATP from endothelial cells during acute inflammation [42]. In addition, various ATP transport mechanisms in non-neuronal cells have been proposed, including ATP-binding cassette transporters, connexin or pannexin hemichannels, plasmalemmal voltage-dependent anion channels and P2X7 receptors, as well as vesicular release [36, 43]. ATP released from nerves, or by autocrine and paracrine mechanisms from nonneuronal cells, is involved in a wide spectrum of physiological and pathophysiological activities, including synaptic transmission and modulation, pain and touch perception. Local probes for real-time measurement of ATP release in biological tissues have been developed recently [44, 45].

After release, ATP and other nucleotides undergo rapid enzymatic degradation, which is functionally important since ATP metabolites act as physiological ligands for a wide array of purinergic receptors [46]. Availability of these ligands is controlled and modulated by ectonucleotidases. Ectonucleotidase families include the E-NTPDases (ecto-nucleoside triphosphate diphosphohydrolases), E-NPP (ecto-nucleotide pyrophosphatase/ phosphodiesterase), alkaline phosphatases and ecto-5’-nucleotidase. Individual enzymes differ in substrate specificity and product formation. E-NTPDases and E-
NPPs hydrolyse ATP and ADP to AMP that is further hydrolysed to adenosine by ecto-nucleotidase. Alkaline phosphatases equally hydrolyse nucleoside tri-, di- and monophosphates. Dinucleoside polyphosphates, NAD<sup>+</sup> and uridine diphosphate (UDP) sugars are substrates solely for E-NPPs. Besides the catabolic pathways, nucleotide interconverting enzymes exist for nucleotide rephosphorylation and extracellular synthesis of ATP (ecto-nucleoside diphosphate kinase, adenylosuccinate kinase). It is possible that, while adenosine is largely produced by ectoenzymatic breakdown of ATP, there may be subpopulations of neurons and/or astrocytes that release adenosine directly [47].

C. RECEPTOR SUBTYPES FOR PURINES AND PYRIMIDINES

A basis for distinguishing two types of purinoceptor, identified as P1 and P2 (for adenosine and ATP/ADP, respectively), was proposed in 1978 [48], but it was not until 1985 that a proposal suggesting a pharmacological basis for distinguishing two types of P2 receptor (P2X and P2Y) was made [49], Abbracchio and Burnstock [50], on the basis of studies of transduction mechanisms and the cloning of nucleotide receptors, proposed that purinoceptors should belong to two major families: a P2X family of ligand-gated ion channel receptors and a P2Y family of G protein-coupled receptors. Currently seven P2X subunits and eight P2Y receptor subtypes are recognized, including receptors that are sensitive to pyrimidines as well as purines [51, 52]. Receptors for diadenosine polyphosphates have been described on C6 glioma cells and presynaptic terminals in rat midbrain, although they have yet to be cloned.

P1 Receptors

Four subtypes of P1 receptors have been cloned, namely, A<sub>1</sub>, A<sub>2A</sub>, A<sub>2B</sub>, and A<sub>3</sub> (see [53]). All P1 adenosine receptors couple to G proteins and, in common with other G protein-coupled receptors, they have seven putative transmembrane (TM) domains; the NH<sub>2</sub> terminus of the protein lies on the extracellular side, and the COOH terminus lies on the cytoplasmic side of the membrane. It is the residues within the TM regions that are crucial for ligand binding and specificity. Specific agonists and antagonists are available for the P1 receptor subtypes [54].

P2X Receptors

P2X<sub>1–7</sub> receptor subunits show a subunit topology of intracellular NH<sub>2</sub> and COOH termini, two TM-spanning regions (TM1 and TM2), the first involved with channel gating and the second lining the ion pore, and a large extracellular loop, with 10 conserved cysteine residues [55, 56]. The stoichiometry of P2X<sub>1–7</sub> receptor subunits involves three subunits that form a stretched trimer (see [57]). P2X<sub>7</sub> receptors, in addition to small cation channels, upon prolonged exposure to high concentrations of agonist, large channels, or pores are activated that allow the passage of larger molecular weight molecules [58]. P2X<sub>7</sub> receptors are predominantly localized on immune cells and glia, where they mediate proinflammatory cytokine release, cell proliferation, and apoptosis. The P2X receptor family shows many pharmacological and operational differences [59].

P2Y Receptors

Metabotropic P2Y receptors (P2Y<sub>1</sub>, P2Y<sub>2</sub>, P2Y<sub>4</sub>, P2Y<sub>6</sub>, P2Y<sub>11</sub>, P2Y<sub>12</sub>, P2Y<sub>13</sub> and P2Y<sub>14</sub>) are characterized by a subunit topology of an extracellular NH<sub>2</sub> terminus and intracellular COOH terminus and seven TM-spanning regions (see [60]). An additional ‘hybrid’ uracil nucleotide-responsive receptor activated by cysteinyl-leukotrienes has been reported [61]. There is structural diversity of intracellular loops and the COOH terminus among P2Y subtypes, so influencing the degree of coupling with G<sub>q/11</sub>-linked P2Y receptor subtypes. (see [62]). Dopamine D<sub>1</sub> and adenosine A<sub>1</sub> receptors have also been shown to form functional homomultimers with P2X<sub>4</sub> receptors [63], while P2X<sub>7</sub> receptors will not form a functional homomultimer without extensive glycosylation.

Heteromultimeric Receptors

The pharmacology of purinergic signalling is complicated because P2X receptor subunits can combine to form either homomultimers or heteromultimers (see [52, 55, 62]). Heteromultimers are clearly established for P2X<sub>2/3</sub> receptors in nodose ganglia, P2X<sub>6</sub> receptors in central nervous system (CNS) neurons, P2X<sub>1/5</sub> receptors in some blood vessels and P2X<sub>2/6</sub> receptors in the brain stem. P2X<sub>7</sub> receptors have recently been claimed to form heteromultimers with P2X<sub>4</sub> receptors [63], while P2X<sub>7</sub> receptors will not form a functional homomultimer without extensive glycosylation.

P2Y receptor subtypes can also form heterodimeric complexes [64] or with other receptors. For example, adenosine A<sub>2</sub> receptors have been shown to form a heteromeric complex with P2Y<sub>1</sub> receptors (see [65]). Dopamine D<sub>1</sub> and adenosine A<sub>1</sub> receptors have also been shown to form functionally interactive heteromeric complexes.

D. ATP AND SENSORY NERVES

Sensory Ganglia

There have been many reports characterizing the P2X receptors in sensory neurons, including those from dorsal root, trigeminal, nodose, and petrosal ganglia. DRG and tri-
geminal ganglia contain primary somatosensory neurons, receiving nociceptive, mechanical and proprioceptive inputs [8]. All P2X subtypes are found on sensory neurons, although the P2X3 receptor has the highest level of expression (both in terms of mRNA and protein) (see [66]). P2X2/3 heteromultimers are particularly prominent in the nodose ganglion. P2X3 and P2X2/3 receptors are expressed on IB4-binding subpopulations of small nociceptive neurons [23]. RT-PCR showed that P2Y1, P2Y2, P2Y4 and P2Y6 receptor mRNA is also expressed on neurons of DRG, nodose and trigeminal ganglia and receptor protein for the P2Y1 receptor is localized on over 80% of mostly small neurons [26]. Double immunolabelling showed that 73–84% of P2X3 receptor positive neurons also stained for the P2Y1 receptor, while 25–35% also stained for the P2Y4 receptor. The sensitivity to ATP of satellite cells is increased 500-fold after axotomy or inflammation, which is likely to contribute to chronic pain [67].

P2X2 receptors have been identified in retinal ganglion cells, particularly within cone pathways. Functional studies have also identified P2X2/3 heteromultimeric receptors in cultured rat retinal ganglion cells. RT-PCR at the single-cell level revealed expression of P2X2, P2X3, P2X4 and P2X5 receptor mRNA in approximately one-third of the bipolar cells. P2X7 receptors have also been identified on both inner and outer retinal ganglion cell layers [68], which may be

<table>
<thead>
<tr>
<th>Antagonist</th>
<th>P2X3</th>
<th>P2X2/3</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Suramin and analogues NF449, NF110</td>
<td>✓</td>
<td>✓</td>
<td>8</td>
</tr>
<tr>
<td>PPADS and derivatives MRS2159 &amp; MRS2257</td>
<td>✓ ✓</td>
<td>✓</td>
<td>8</td>
</tr>
<tr>
<td>Reactive blue 2 and derivatives</td>
<td>✓</td>
<td>✓</td>
<td>8</td>
</tr>
<tr>
<td>TNP-ATP</td>
<td>✓ ✓</td>
<td>✓ ✓</td>
<td>189</td>
</tr>
<tr>
<td>A-317491 (selective)</td>
<td>✓ ✓</td>
<td>✓ ✓</td>
<td>190, 193</td>
</tr>
<tr>
<td>Phenol red</td>
<td>✓ ✓</td>
<td>-</td>
<td>8</td>
</tr>
<tr>
<td>Tetramethylpyrazine</td>
<td>✓ ✓</td>
<td>✓</td>
<td>192</td>
</tr>
<tr>
<td>RO-3 (orally bioavailable, stable)</td>
<td>✓ ✓</td>
<td>✓</td>
<td>59</td>
</tr>
<tr>
<td>IpI</td>
<td>✓ ✓</td>
<td>✓</td>
<td>8</td>
</tr>
<tr>
<td>β-carboxymethylene ATP</td>
<td>✓ ✓</td>
<td>✓</td>
<td>8</td>
</tr>
<tr>
<td>β-chlorophosphonomethylene ATP</td>
<td>✓ ✓</td>
<td>✓</td>
<td>8</td>
</tr>
</tbody>
</table>

| P2X7                                            |      |        |
|                                                |      |        |
| KN62                                            | ✓ ✓ ✓| 59     |
| KN04                                            | ✓ ✓ ✓| 59     |
| MRS2427                                         | ✓ ✓  | 8      |
| α-ATP                                           | ✓ ✓  | 59     |
| Coomassie BBG                                   | ✓ ✓  | 59     |
| RN6189                                          | ? ✓  | 8      |
| AZ11645373 (selective)                          | ✓ ✓ ✓| 251    |
| A-740003 (selective)                            | ✓ ✓ ✓| 217    |
| A-438079 (selective)                            | ✓ ✓ ✓| 220    |

| P2Y12                                           |      |        |
|                                                |      |        |
| CT50547                                         | ✓ ✓  | 252    |
| AR-C69931MX (selective)                         | ✓ ✓ ✓| 253    |
| INS49266                                        | ✓ ✓ ✓| 8      |
| AZD6140                                         | ✓ ✓ ✓| 253    |
| PSB0413                                         | ? ✓  | 8      |
| AR-C66096 (selective)                           | ✓ ✓ ✓| 254    |
| 2-MeSAMP                                        | ✓ ✓  | 252    |
| Carba-nucleosides                               | ✓ ✓ ✓| 255    |

Ticks represent qualitative assessment of potencies.
involved in retinal cholinergic neuron density regulation. P2X<sub>2</sub>/3 receptors are expressed postsynaptically on horizontal cell processes as well as presynaptically on photoreceptor synaptic terminals in both rat and marmoset retinas [69]. P2X<sub>3</sub> receptors are present on Müller cells. Müller cells release ATP during Ca<sup>2+</sup> wave propagation.

**Sensory Nerve Fibres and Terminals**

Sensory nerve terminals express purinoceptors and respond to ATP in many situations. 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 (see [8]).

In terminals of sensory neurons P2X<sub>2/3</sub> and/or P2X<sub>3</sub> receptors are intimately involved in pain sensation and temperature sensitivity. During purinergic mechanosensory transduction (see below), the ATP that acts on P2X<sub>3</sub> and P2X<sub>2/3</sub> receptors on sensory nerve endings, is released by mechanical distortion from urothelial cells during distension of bladder and ureter and from mucosal epithelial cells during distension of the colorectum. It is probably also released from odontoblasts in tooth pulp, from epithelial cells in the tongue, epithelial cells in the lung, keratinocytes in the skin and glomus cells in the carotid body (see [37]). Released ATP is rapidly broken down by ectoenzymes to ADP (to act on P2Y<sub>1</sub>, P2Y<sub>12</sub> and P2Y<sub>13</sub> receptors) or adenosine (to act on P1 receptors).

In the gut, ATP and α,β-methylene ATP (α,β-meATP) activate P2X<sub>3</sub> and/or P2X<sub>2/3</sub> receptors on subepithelial terminals of intrinsic sensory neurons in the guinea pig intestine [70], supporting the hypothesis of Burnstock [19] that ATP released from mucosal epithelial cells has a dual action acting on the terminals of low-threshold intrinsic enteric sensory neurons to initiate or modulate intestinal reflexes and acting on the terminals of high-threshold extrinsic sensory fibres to initiate pain. Further support comes from the demonstration that peristalsis is impaired in the small intestine of mice lacking the P2X<sub>3</sub> subunit [71]. 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<sub>3</sub> receptors [72]. Intranglionic laminar nerve endings are specialized mecha-no sensory endings of vagal afferent nerves in the rat stomach, arising from the nodose ganglion; they express P2X<sub>3</sub> receptors and are probably involved in physiological reflex activity, especially in early postnatal development [73].

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 [74] and subsequently α,β-meATP [75]. Large amounts of adenine nucleotides are localized in glomus cells, stored within specific granules together with catecholamines and proteins. Evidence of ATP release from carotid chemoreceptor cells has been reported [76], and corelease of ATP and acetylcholine (ACh) is the likely mechanism for chemosensory signaling in the carotid body in vivo [77]. ATP coreleased with ACh from type I glomus chemoreceptor cells during hypoxic and mechanical stimulation was shown to act on P2X<sub>2/3</sub> receptors on nerve fibres arising from the petrosal ganglion mediating hypoxic signaling at rat and cat carotid body chemoreceptors [78, 79]. Immunoreactivity for P2X<sub>2</sub> and P2X<sub>3</sub> receptor subunits has been localized on rat carotid body afferents [78]. These findings were confirmed and extended in a study where P2X<sub>3</sub> 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 [80]. ATP mimicked the afferent discharge, and suramin and pyridoxalphosphate-6-azonphenyl-2',4'-disulphonic acid (PPADS) blocked the hypoxia-induced discharge. Immunoreactivity for P2X<sub>2</sub> and P2X<sub>3</sub> receptor subunits was detected on afferent terminals surrounding clusters of glomus cells in wild-type but not in P2X<sub>2</sub> and/or P2X<sub>3</sub> receptor-deficient mice. Sensory afferent fibres within the respiratory tract, which are sensitive to ATP, probably largely via P2X<sub>2/3</sub> receptors, have been implicated in vagal reflex activity [81] and in the cough reflex [82].

ATP has been shown to be an auditory afferent neurotransmitter, alongside glutamate (see [83]). There are ~50,000 primary afferent neurons in the human cochlear and about one-half express P2X<sub>3</sub> (or P2X<sub>2</sub> variants) and, debatably, P2X<sub>3</sub> receptors. ATP is released from K<sup>+</sup>-depolarized organ of Corti in a Ca<sup>2+</sup>-dependent manner, and an increase in ATP levels in the endolymph has been demonstrated during noise exposure, perhaps released by exocytosis from the marginal cells of the stria vascularis [84]. The P2 receptor antagonist, PPADS, attenuated the effects of a moderately intense sound on cochlear mechanics [85]. Spiral ganglion neurons, expressing P2X<sub>2</sub> receptors, located in the cochlear, convey to the brain stem the acoustic information arising from the mechanoelectrical transduction of the inner hair cells [86] and are responsive to ATP [87].

Odorant recognition is mediated by P2X<sub>2</sub>, P2X<sub>3</sub> and P2X<sub>2/3</sub> receptors predominantly situated on the microvilli of olfactory receptor neurons in the olfactory epithelium and vomeronasal organ [88, 89]. Nucleotides also act on sustentacular supporting cells. Purinergic receptors appear to play an integral role in signaling 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, initiating a stress-signaling cascade involving heat shock proteins for neuroprotection [90].

Taste sensations appear to be mediated both by P2Y<sub>1</sub> receptor-activated impulses in sensory fibres in the chorda tympani [91] and by P2X<sub>2</sub> and P2X<sub>3</sub> and, perhaps, P2X<sub>2/3</sub> receptors [92]. These authors showed that genetic elimination of P2X<sub>2</sub> and P2X<sub>3</sub> 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. Ectonucleotidases are known to be abundantly present in taste buds [93]. Other papers present data that suggest that P2Y<sub>2</sub> and P2Y<sub>4</sub> receptors also play a dominant role in mediating taste cell responses to ATP and UTP [94].
E. PURINERGIC MECHANOSENSORY TRANSDUCTION AND PAIN

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 P2X₃ homomeric and P2X₂₃,₅ heteromeric receptors on subepithelial sensory nerves initiating impulses in sensory pathways to pain centres in the CNS [37] (Fig. 2a). Evidence supporting this hypothesis in various organs is reviewed below.

Urinary Bladder

Early evidence for ATP release from rabbit urinary bladder epithelial cells by hydrostatic pressure changes was presented by Ferguson et al. [95], who speculated about this being the basis of a sensory mechanism. Prolonged exposure to a desensitizing concentration of α,β-meATP significantly reduced the activity of mechanosensitive pelvic nerve afferents in an in vitro model of rat urinary bladder [96]. Later, it was shown that mice lacking the P2X₂ receptor exhibited reduced inflammatory pain and marked urinary bladder hyporeflexia with reduced voiding frequency and increased voiding volume, suggesting that P2X₃ receptors are involved in mechanosensory transduction underlying both inflammatory pain and physiological voiding reflexes [97]. Subsequently, using P2X₂ knockout mice and P2X₂/P2X₃ double knockout mice, a role for the P2X₂ subtype was shown to be involved in mediating the sensory effect of ATP [98]. 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 activity initiated in pelvic sensory nerves was mimicked by ATP and α,β-meATP and attenuated by P2X₃ antagonists as well as in P2X₃ knockout mice; P2X₃ receptors were localized on suburothelial sensory nerve fibres [99]. It appears that the bladder sensory DRG neurons, projecting via pelvic nerves, express predominantly P2X₂₅,₃ heteromultimer receptors [100]. 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 [101]. It was also shown that purinergic agonists increase the excitability of afferent fibres to distension. Botulinum toxin A, which has antinociceptive effects in treating interstitial cystitis, inhibits distension-mediated urothelial release of ATP in conditions of bladder inflammation [102]. The roles of ATP released from urothelial cells and suburothelial myo-fibroblasts on various bladder functions have been considered at length in several reviews [103, 104], and evidence presented that urothelial-released ATP alters afferent nerve excitability [105].

ATP given intravesically stimulates the micturition reflex in awake freely moving rats, probably by stimulating suburothelial C-fibres, although other mediators are likely to be involved [106]. Studies of resiniferatoxin desensitization of capsaicin-sensitive afferents on detrusor overactivity induced by intravesicle ATP in conscious rats supported the view that increased extracellular 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 [97, 107]. ATP has also been shown to induce a dose-dependent hyperreflexia in conscious and anesthetized mice, largely via capsaicin-sensitive C-fibres; these effects were dose-dependently inhibited by PPADS and 2',3'-O-(2,4,6-trinitrophenyl)-ATP (TNP-ATP) [108] (Fig. 3a). P2X₃ and P2X₂ receptors play a fundamental role in the micturition reflex in female urethane-anesthetized rats; P2X₃ receptor blockade by phenol red raised the pressure and volume thresholds for the reflex, while P2X₂ receptor blockade diminished motor activity associated with voiding [109].

Ureter

The uroteric colic that is induced by the passage of a kidney stone causes severe pain. Immunostaining of P2X₃ receptors in sensory nerves in the subepithelial region was reported [110]. Distension of the ureter resulted in substantial ATP release from the urothelium in a pressure-dependent manner [38]. 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. Multifibre recordings of ureter afferent nerves were made using a guinea pig preparation perfused in vitro [111]. 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.

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 noiception via extrinsic sensory fibres [19, 112] (Fig. 2b). Evidence in support of this hypothesis was obtained from a rat pelvic sensory nerve-colorectal preparation [113]. 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 attenuated by the selective P2X₃ and P2X₂₅,₃ antagonist TNP-ATP and by PPADS. The sensory discharge was potentiated by ARL-67156, an ATPase inhibitor. Single fibres analysis showed that high-threshold fibres were particularly affected by α,β-meATP. The interactions of ATP with other mediators that activate pelvic afferent fibres in the rat colorectum, including 5-hydroxytryptamine (5-HT), bradykinin, prostaglandins and substance P (SP), have been described [114]. Lumbar splanchnic (LSN) and sacral pelvic (PN) nerves convey different mechanosensory information from the colon to the spinal cord. Forty percent of LSN afferents responded to α,β-meATP compared with only 7% of PN afferents [115].

ATP release and P2X₃ and P2X₂₅,₃ receptor-mediated nociceptive sensory nerve responses were enhanced in a model of colitis [116]. The excitability of visceral afferent nerves is enhanced following injury or ischemia and during inflammation, for example, in irritable bowel syndrome. 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 (see [117, 118]). α,β-MeATP was shown to stimulate mechanosensitive mucosal and tension receptors in mouse stomach and oesophagus leading to activity in vagal afferent nerves [119]. The sensitizing effects of P2X3 receptor agonists on mechanosensory function are induced in oesophagitis [120]. Purinergic mechanosensory transduction has also been implicated in reflex control of intestinal secretion, whereby ATP released from mucosal epithelial cells acts on P2Y1 receptors on enterochromaffin cells to release 5-HT, which leads to regulation of secretion either directly or via intrinsic reflex activity [121].

**Skin**

ATP and α,β-meATP activate nociceptive sensory nerve terminals in the skin, which increase in magnitude in inflammatory conditions due to increase in number and responsiveness of P2X3 and P2X2/3 receptors [122]. Calcium waves in human epidermal keratinocytes mediated by extracellular ATP produce \([\text{Ca}^{2+}]\) elevation in DRG neurons, suggesting a dynamic cross-talk between skin and sensory neurons mediated by extracellular ATP [123]. Skin cell damage caused action potential firing and inward currents in nociceptive fibres, which was eliminated by enzymatic degradation of ATP or blockade of P2X receptors, indicating release of cytosolic ATP [124]. Locally released ATP can sensitize large mechanosensitive afferent endings via P2 receptors, leading to increased nociceptive responses to pressure or touch; it was suggested that such a mechanism, together with central changes in the dorsal horn, may contrib-

![Purinergic mechanosensory transduction](Image)

**Fig. (2).** Purinergic mechanosensory transduction. (a) Schematic representation of hypothesis for purinergic mechanosensory transduction in tubes (e.g. ureter, vagina, salivary and bile ducts, 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 P2X3 and/or P2X2/3 receptors on subepithelial sensory nerves to convey sensory/nociceptive information to the CNS. (From [37], reproduced with permission from Blackwell Publishing). (b) Schematic of a novel hypothesis about purinergic mechanosensory transduction in the gut. It is proposed that ATP released from mucosal epithelial cells during moderate distension acts preferentially on P2X3 receptor subepithelial intrinsic sensory nerve fibres (labelled with calbindin) to modulate peristaltic reflexes. ATP released during extreme (colic) distension also acts on P2X3 receptors on high-threshold extrinsic sensory nerve fibres (labelled with isolectin B4 (IB4)) that sends messages via the dorsal root ganglia (DRG) to pain centres in the central nervous system. (From [256], reproduced with permission from Wiley-Liss, Inc.).
ute to touch evoked pain [125]. ATP appears to be involved in fast nociceptive signals, while persistent pain after tissue damage involves other algogenic compounds, notably bradykinin, prostaglandin and serotonin. However, persistent pain during inflammation may also involve sensitization and/or spread of P2X receptors. Nocifensive behaviours induced by administration of ATP and 2′,3′-O-(4-benzoylbenzoyl)-ATP to the hindpaw appear to recruit an additional set of fibres that are not activated by α,β-meATP and which trigger the spinal release of SP and are capsaicin selective [126].

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 versus normal skin [122]. This was reported to be due to upregulation of P2X2 and P2X3 receptors on DRG neurons [127]. Enhanced expression of glial cell-derived neurotrophic factor in the skin can change the mechanical sensitivity of IB4-positive nociceptive afferents (which express P2X3 and P2X2,3 receptors) [128]. Evidence has been presented that P2Y2 receptors in the terminals of capsaicin-sensitive mouse sensory neurons mediate nociceptive transmission and further that P2Y signaling may contribute to mechanotransduction in low-threshold Aβ fibres [129]. Treatment with oxidized ATP, a selective inhibitor of P2X7 receptors, reduced the hyperalgesia produced by complete Freund’s adjuvant (CFA) and carrageenan-induced inflammation in rats [130]. Data have been presented to support a pathogenic role for keratinocyte-derived ATP in irritant dermatitis [131]. A family of G protein-coupled receptors, named mas-related G protein-coupled receptors (Mrgrpdr, also sensory neuron-specific receptors) has been cloned, which show high expression in sensory neurons of the DRG and trigeminal ganglia, predominantly in small-diameter neurons [132]. It has been reported recently that cutaneous sensory neurons expressing the Mrgrpdr receptor sense extracellular ATP and are putative nociceptors [133]. They suggest that these nociceptors in the outer epidermis respond indirectly to external stimuli by detecting ATP release in the skin.

P2X2, P2X3 and P2Y1 receptors are abundantly present on sensory nerve terminals in the tongue [91, 134]. 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 and a purinergic mechanosensory transduction mechanism for the initiation of pain was proposed [135].

**Heart**

In the heart, an ATP-triggered vagal reflex has been described leading to suppression of sinus node automaticity and atioventricular nodal conduction [136]. This is probably mediated by P2X2/3 receptors located on vagal sensory nerve terminals in the left ventricle, supporting the hypothesis that ATP released from ischemic myocytes is a mediator of atrio-pine-sensitive bradyarrhythmias associated with left ventricular myocardial infarction [137]. A recent paper has implicated P2X2 receptors on sensory fibres in the heart originating in the nodose ganglion in nociception associated with myocardial ischaemic injury [138].

**Lung**

In the lung, pulmonary neuroepithelial bodies (NEBs) and more recently subepithelial receptor-like endings associated with smooth muscle (SMARs) [139] have been shown to serve as sensory organs in the lung, and P2X3 and P2X2/3 receptors are expressed on a subpopulation of vagal sensory fibres that supply NEBs and SMARs with their origin in the nodose ganglia. 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 [140]. NEBs are oxygen sensors especially in early development, before the carotid system has matured [141]. 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 <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 [142]. Both the TRPV1 receptor and P2X receptors mediate the sensory transduction of pulmonary reactive oxygen species, especially H₂O₂ and OH, by capsaicin-sensitive vagal lung afferent fibres [143].

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. Vagal afferent purinergic signaling may be involved in the hyperactivity associated with asthma and chronic obstructive pulmonary disease [144]. Th1 and Th2 cytokines reciprocally regulate P2X7 receptor function, suggesting a role for P2X7 receptors in pulmonary diseases, particularly lung hypersensitivity associated with chronic inflammatory responses [145].

**Musculoskeletal Systems and Joints**

Pain related to the musculoskeletal system (myofascial pain) is very common and ATP has been claimed to excite or sensitize myofascial nociceptors [146]. Prolonged muscle pain and tenderness was produced in human muscle by infusion of a combination of ATP, serotonin, histamine and prostaglandin E₂ [147]. Strenuous exercise of muscle, as well as inflammation and ischaemia, are associated with tissue acidosis. P2 receptors on the endings of thin fibre muscle afferents play a role in evoking both the metabolic and mechanoreceptor components of the exercise pressor reflex [148]. 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 [149]. ATP was shown to enhance the muscle pressor response evoked by mechanically sensitive muscle stretch, which was attenuated by PPADS [149]. Intramuscular injections of acidic phosphate buffer at pH 6 or ATP excited a subpopulation of unmyelinated (group IV) muscle afferent fibres [150], perhaps implicating P2X2 or
P2X3 receptors that are sensitive to acidic pH. ATP induces sustained facilitation of cranial nociception through P2X receptors on neck muscle nociceptors in mice [151]. Nociceptive information arising from the orofacial region via P2X3 receptors on afferent fibres are carried to trigeminal brainstem sensory nuclei [66].

ATP has been shown to be a stimulant of articular nociceptors in the knee joint via P2X3 receptors [152, 153] and also to some extent in lumbar intervertebral disc, but not as prominently as in the skin [154]. P2Y2 receptor mRNA is expressed in both cultured normal and osteoarthritic chondrocytes taken from human knee joints, and ATP is shown to be released by mechanical stimulation [155]. Masseter muscle pain is recognised as a prominent symptom in temporomandibular disorders. A recent report claims that ATP acting on P2X3 receptors on sensory nerve fibres in masseter muscle plays an important role in pressure pain and mechanical hyperalgesia caused by excessive muscular contraction [156]. 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 P2X3 receptors, respectively [157]. When monoarthritis was induced by injection of CFA into the unilateral temporomandibular joint of the rat, the pain produced was associated with an increase in P2X3 receptor-positive small neurons in the trigeminal ganglion [158]. Activation of P2X receptors in rat temporomandibular joint induces nociception, and that antagonism by PPADS decreases carrageenan-induced inflammatory hyperalgesia [159]. Oxidized ATP inhibits inflammatory pain in arthritic rats by inhibition of the P2X3 receptor for ATP localized in nerve terminals [160]. Accumulation is assuming to suggest that blockers of P2X3 receptors may have a future as anti-inflammatory drugs [161].

P2X3 and P2X2/3 receptors on sensory afferents in tooth pulp appear to mediate nociception [162-164], perhaps from ATP released by mechanical distension or inflammation of odontoblasts. Mustard oil application to the tooth pulp in anesthetized 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 [165].

Tumours

Purinergic mechanisms are beginning to be explored in relation to cancer pain [17, 166, 167]. It was suggested that the unusually high levels of ATP contained in tumour cells [168] may be released by mechanical rupture to activate P2X3 receptors on nearby nociceptive sensory nerve fibres [17]. There is increased expression of P2X3 receptors on calcitonin gene-related peptide (CGRP) immunoreactive epidermal sensory nerve fibres in a bone cancer pain model [167] and in other cancers that involve mechanically sensitive tumours [166]. 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 peristium might reduce movement-associated pain. The hyperalgesia associated with tumours appears to be linked to increase in expression of P2X3 receptors in nociceptive sensory neurons expressing CGRP by analogy with that described for increased P2X3 receptor expression in a model of inflammatory colitis [116]. Increased expression of P2X3 receptors was also reported associated with thermal and mechanical hyperalgesia in a rat model of squamous cell carcinoma of the lower gingival [169].

F. NEUROPATHIC AND INFLAMMATORY PAIN

Activation of P2X3 receptors in the spinal cord was shown to elicit allodynia [170] and in a seminal study published in Nature in 2004, P2X3 receptors on spinal cord microglia were shown to be upgraded in neuropathic pain, which was significantly reduced after use of P2X4 antisense oligonucleotides [171]. This study has led to an explosion of work focussed on purinergic signalling in neuropathic pain [18, 172-174]. Importantly, it has been shown that P2X7 receptors on microglia are also involved in neuropathic pain, although the underlying mechanisms involving P2X4, P2X7 and P2Y12 receptors are still not clear. Adenosine has also been considered as a potential analgesic target for inflammatory and neuropathic pain [21, 175].

P2X3 and P2X2/3 Receptors

There is evidence that P2X3 receptors are involved in neuropathic pain, probably those located on primary afferent nerve terminals in inner lamina 2 of the spinal cord [176] and in the trigeminal brainstem sensory nuclei [66]. P2X2, P2X4 and P2X6 receptors have also been located on dorsal horn neurons relaying nociceptive information further along the pain pathway [177]. In addition, ATP coreleased with γ-aminobutyric acid in spinal interneurons is probably involved in modulation of nociceptive pathways [178].

P2X3 receptors on terminals of primary afferent nerves in the inner lamina 2 of the dorsal horn of the spinal cord mediate facilitation of glutamate release [179] and it has been suggested that thermal hyperalgesia may be mediated by spinal P2X3 receptors via activation of N-methyl-D-aspartate receptors [180]. Supraspinal P2X3 and P2X2/3 receptors play an inhibitory role in pain transmission [181]. Intrathecal administration of ATP produces long-lasting allodynia, most likely via P2X2/3 receptors [182]. The involvement of spinal P2X3 and P2X3 receptors in neuropathic pain in a mouse model of chronic constriction injury has been claimed [183]. A recent study suggests that P2X3/P2X2/3 receptor-dependent cytosolic phospholipase A2 (cPLA2) activity in primary sensory neurons is a key event in neuropathic pain and that cPLA2 might be a potential target for treating neuropathic pain [184]. It is claimed that sensitisation of P2X3 receptors rather than a change in ATP release is responsible for neuropathic pain and allodynia [185]. Data has been presented to suggest that the P2X3 and P2X2/3 receptor antagonism that reduces inflammatory hyperalgesia and chemogenic nociception is mediated by the spinal opioid system [186]. A recent review covering the role of P2X3 receptor involvement in acute pain, inflammatory pain, chronic neuropathic pain, migraine and cancer pain is available [187].

TNP-ATP (Fig. 3a) is the most potent P2X3 receptor antagonist [188]. TNP-ATP has low nanomolar affinity for blocking P2X3 receptors but also has high affinity for P2X1.
receptors and is rapidly degraded in situ [50]. Thus, in vivo studies of TNP-ATP as a pharmacological tool have been limited to direct intrathecal administration [18] or direct administration into a site of peripheral tissue damage [189]. Several novel non-nucleotide small molecule P2X3 antagonists have been reported. A-317491 (Fig. 3b) has nanomolar affinity for blocking both P2X3 and P2X2/3 receptors and is a competitive antagonist [190, 191]. Unlike TNP-ATP, A-317491 is not susceptible to metabolic degradation and shows high systemic bioavailability following subcutaneous administration but lacks oral bioavailability. Antagonism of P2X3 and P2X3 receptors by phenol red has been reported, and tetramethylpyrazine, a traditional Chinese medicine, used as an analgesic for dysmenorrhea, was claimed to block P2X3 receptor signalling [192]. Systemic administration of A-317491 effectively reduced nociception in inflammatory and neuropathic pain models [190]. A-317491 also effectively blocked persistent pain in the formalin and acetic acid-induced abdominal constriction tests but was generally inactive in models of acute noxious (thermal, mechanical and chemical) stimulation [193]. RO-3 (Fig. 3b) is another recently identified antagonist that potently blocks P2X3 receptors (pIC50 = 7.0) and exhibits at least 100-fold less activity across a wide range of kinases, receptors and ion channels [59]. RO-3 has lower protein binding (48%) compared with A-317491 (99%) and good CNS penetration. R0-3 also reduces nociceptive sensitivity in animal pain models [59].

Antisense oligonucleotides have been used to downregulate the P2X3 receptor, and in models of neuropathic (spatial sciatric nerve ligation) and inflammatory (CFA) pain, inhibition of the development of mechanical hyperalgesia as well as significant reversal of established hyperalgesia were observed [194-196]. P2X3 antisense oligonucleotides or antagonists appear to be less effective for treating discogenic (lumbar intervertebral disc) than cutaneous tissue pain [154]. Combined antisense and RNA interference-mediated treatment for specific inhibition of the recombinant rat P2X3 receptor appears to be promising for pain therapy [197]. P2X3 double-stranded short interfering RNA relieves chronic neuropathic pain and opens up new avenues for therapeutic pain strategies in humans [198].

P2X3 Receptors

There have been a number of papers about the role of P2X3 receptors on spinal microglia in neuropathic pain following the initial discovery by Tsuda et al. [171] (see [174]). Brain-derived neurotrophic factor (BDNF) is released from microglia by the stimulation of P2X3 receptors by effecting E-anion in spinal lamina 1 neurons [199]. The involvement of increase in spinal fibronectin/integrin following peripheral nerve injury in upregulation of microglial P2X3 receptors and neuropathic pain has been considered [200]. Innate immune system sensor - like toll-like receptors (TLR’s) and nucleotide-binding oligomerization domain 2 (NOD2) receptors, are key receptors to sense pathogen-associated molecular patterns. Ligands for TLR’s and NOD2 receptors stimulate microglial P2X3 receptor upregulation, suggesting that microglia sense the presence of inflammatory stimulation using multiple recognition systems [201]. In a recent study, Lyn tyrosine kinase was shown to play an important role in the pathogenesis of neuropathic pain and is required to mediate nerve injury-induced upregulation of P2X4 receptors [202].

Enhancement of pain behaviour after nerve injury not only requires the P2X4 receptor, but also phospho38 (p38) mitogen-activated protein kinase (MAPK) [203]. ATP causes the activation of p38 or ERK1/2 MAPKs resulting in the release of tumour necrosis factor-α and interleukin (IL)-6. In rats displaying allostodya, the level of p38 was increased in microglia. Intraspinal administration of the p38 inhibitor, SB203580, suppressed allostodya, suggesting that neuropathic pain hypersensitivity depends on the activation of the p38 signalling pathway in microglia in the dorsal horn following peripheral nerve injury. Platelet activating factor, which is released from activated microglia, is a potent inducer of tactile allodynia and thermal hyperalgesia after intrathecal injection into the spinal cord and it was suggested that this response is mediated by ATP [204]. The possible mechanisms that underlie the role of P2X3 receptors in neuropathic pain and the involvement of inflammatory cytokines have been reviewed [174, 205, 206]. Systemic and intracerebroventricular injection of pro-inflammatory bacterial lipopolysaccharide (LPS) results in widespread thermal hyperalgesia and tactile allodynia. LPS has been shown to enhance responses to low concentrations of ATP mediated by P2X3 receptors [207].

The lack of selective P2X4 antagonists has hindered the pharmacological validation of the role for P2X4 receptors in pain. 5-(3-Bromophenyl)-1,3-dihydrop-2H-benzo-furo-[3,2-e]-1,4-diazepin-2-one was shown to block P2X4 receptor-mediated currents expressed in Chinese hamster ovary cells with an IC50 value of 0.5 μM [20]. Antidepressants have been shown to be effective in relieving neuropathic pain [208] and a recent communication by Kazu Inoue [174] suggests that some antidepressants, in particular paroxetine, is an effective P2X4 receptor antagonist in transfected cells and preliminary clinical studies suggested that it was effective against chronic pain. A series of benzofuro-1,4-diazepin-2-ones have been reported to be effective P2X4 antagonists in a Bayer Health Care, AG patent (see [209]). It remains to be seen whether novel selective P2X4 antagonists will elicit analgesic effects in neuropathic and inflammatory pain states.

P2X7 Receptors

P2X7 receptors were shown to be expressed in a mouse microglial cell line, NTW8, in 1997 [210] and later on microglia in rat brain [211]. Relief of inflammation-induced hyperalgesia in rats with the P2X7 receptor antagonist, oxidized ATP [160]. Chronic inflammatory and neuropathic pain was abolished in P2X7 knockout mice, as was release of IL-1β [212]. The authors hypothesised that the P2X7 receptor, via regulation of mature IL-1β production, plays a common upstream transductional role in the development of neuropathic and inflammatory pain.

Several P2X7 receptor antagonists have been proposed including: oxidised ATP (Fig. 3a), Brilliant Blue G, the tyro- sine derivatives KN-62 and KN-04, cyclic imides, adamantane and benzamide derivatives [213], compound 4g [214], chelerythrine and other benzophenanthidine alkaloids [215], U73122 and U73343 [216] and recently developed
compounds such as cyanoguanidines and aminotetrazoles [209]. More direct support for a role of P2X7 receptors in pain modulation is provided by studies using selective antagonists. Systemic administration of the P2X7 receptor-selective antagonists, A-438079 and A-740003 (Fig. 3c) dose-dependently reduced nociceptive responses in models of neuropathic [217-219] and inflammatory pain [217]. Consistent with their in vitro efficacies, A-740003 was more potent than A-438079 at reducing mechanical allodynia 2 weeks after spinal L5/L6 nerve ligation. The antinociceptive effects of P2X7 antagonists in inflammatory pain models do not appear to be secondary to an anti-inflammatory effect, because A-740003 was more efficacious in reducing nociception than paw edema. The antinociceptive action of A-438079 is related to blocking mechanical and thermal inputs to several different classes of spinal neurons [219]. A-438079 reduced noxious and innocuous-evoked activity of low threshold, nociceptive-specific, and wide dynamic range spinal neurons in neuropathic rats. Spontaneous activity of all classes of spinal neurons was also significantly reduced by A-438079 in neuropathic, but not sham rats. Blockade of P2X7 receptors significantly reduced nociception in animal models of persistent neuropathic and inflammatory pain [20, 220, 221]. Collectively, these data combined with growing evidence supporting the role of P2X7 receptor modulation in proinflammatory IL-1 processing [161] indicate a specific role for P2X7 receptors in neural-glial cell interactions associated with ongoing pain [222].

P2X4 and P2X7 receptor knockout mice share a common pain phenotype, although this phenotype appears to be conferred via different mechanisms [223]. There has been a recent report suggesting that P2X4 and P2X7 receptors form heteromultimers [63].

P2Y Receptors

The contributions of the metabotropic P2Y receptors to normal and pathological pain have been less well-examined, compared to P2X receptors [see 224]. However, the expression of P2Y1, P2Y6, P2Y4 and P2Y6 mRNA in DRG neurons suggests that these receptors may be involved in peripheral somatosensory transmission [8, 225]. Activation of UTP-sensitive P2Y2 and/or P2Y4 receptors and the UDP-sensitive P2Y6 receptor, in contrast to P2X receptors, produces inhibition of spinal pain transmission [226]. P2Y1 and P2Y4 receptors were identified on predominantly small diameter sensory neurons, in a subpopulation of which P2X3 receptors [26] and TRPV1 receptors [28] were also expressed. Activation of P2Y1 receptors on DRG neurons modulates currents generated through N-type (Ca2.2) calcium channels and P2X3 receptors [227]. Indeed, activation of N-type calcium channels in cultured DRG neurons was inhibited by ATP and even more potently by the P2Y1,12,13 receptor agonist ADP [28]. The effects of ATP were blocked by the selective P2Y1 receptor antagonist MRS 2179 (Fig. 3d). The outcome of P2Y1-related inhibitions on N-type calcium channels or P2X3 receptors is likely to result in a decreased release of nociceptive transmitters into the spinal cord [8].

P2Y1 receptor mRNA is up-regulated in the lumbar DRG after peripheral axotomy, indicating that P2Y1 receptors may contribute to the heightened somatosensory sensitivity in this pathological state [228]. Early work with TRPV1-transfected human embryonic kidney 293 cells suggested that the P2Y1, and/or P2Y2 receptors were responsible for the ATP modulation of TRPV1 responses to heat, capsaicin and protons [225]. The contributions of P2Y2 receptors for pain transmission probably extend beyond interactions with TRPV1 receptors in primary afferent neurons. In the isolated skin-nerve preparation, 54% cutaneous C-fibres and 12% A- mechanoceptors responded to UTP (approximately 70-80% were capsaicin-sensitive) [129]. However, an additional 22 to 26% of large diameter Aβ fibres responded to UTP, suggesting that P2Y2 receptors also may be directly involved in the transmission of low-threshold mechanical inputs to the spinal cord. It is also possible that activation of the hetero-oligomeric P2Y2/4,1 receptor complex [229] may also negatively modulate the antinociceptive effects of A1 receptor agonists [172].

The rostral ventromedial medulla serves as a critical link in bulbo-spinal nociceptive modulation and it has been suggested that while on-cells preferentially express P2X receptors, off-cells express P2Y receptors in this region [230]. Activation of P2Y receptors inhibits P2X3 receptor channels via G protein-dependent facilitation of their desensitisation [231].

It has been reported recently that P2Y12 receptors expressed on microglia [232] are required for neuropathic pain after peripheral nerve injury, and intrathecal administration of the P2Y12 receptor antagonist, AR-C69931MX, prevented the development of tactile allodynia [233]. Activation of the P2Y12 receptor by released ATP is via the p38 MAPK pathway [234]. Nucleotide agonists selective for P2Y1, P2Y2, P2Y4 and P2Y12 receptors and nucleotide antagonists selective for P2Y1 (MRS2179, MRS2500), P2Y12/C (Congrelor, previously known as AR-C69931MX) and P2Y12 (ARL660-96, INS4266, AZD6140) have been identified (see [52]).

Migraine

The involvement of ATP in migraine was first suspected in conjunction with the vascular theory of this disorder with ATP released from endothelial cells during reactive hyperaemia associated with pain following cerebral vascular vasospasm (not associated with pain) [235]. More recently, P2X3 receptor involvement in neuronal dysfunction in brain areas that mediate nociception such as the trigeminal nucleus and thalamus have been considered [236, 237]. P2X3 receptors are the only ligand-gated channel known to be expressed exclusively by a subset of trigeminal and spinal sensory neurons [15] and may make a promising candidate for antimigraine drug development [238]. Slow up-regulation of nociceptive P2X3 receptors on trigeminal neurons by the migraine mediators CGRP and nerve growth factor (NGF) has been demonstrated [237, 239]. In an in vivo model of mouse trigeminal pain, anti-NGF treatment suppressed responses evoked by P2X3 receptor activation [240]. The interaction of P2Y1 receptors on trigeminal neurons with P2X3 receptors after sensitisation of these neurons with algogenic stimuli (e.g., NGF, BDNF or bradykinin) has been proposed and may also represent a new potential target for antimigraine drugs [241]. Peripheral sensitisation is considered as a contributor to mechanisms underlying migraine headache and a...
recent review concludes that ATP has a role in sensitisation of primary afferents at both peripheral and central terminals [242]. Evidence for the possible role of adenosine in migraine has been reviewed [243]. Plasma adenosine has been observed to rise during migraine attacks and adenosine has been reported to trigger migraine attack while dipyridamole, an adenosine uptake inhibitor, can increase migraine attack frequency. A1 receptor stimulation has also been considered for migraine treatment [244] and it has been claimed that A2A receptor gene variation may contribute to the pathogenesis of migraine [245].

G. CONCLUSIONS AND FUTURE DIRECTIONS

Multiple P2 receptor subtypes are involved in pain pathways both as an initiator and modulator. Activation of homomeric P2X3 receptors probably contributes to acute nociception and some aspects of acute inflammatory pain [8, 172]. In contrast, activation of heteromeric P2X2/3 receptors appears to modulate longer-lasting nociceptive sensitivity associated with nerve injury or chronic inflammation [194, 246]. Furthermore, under conditions of persistent nociceptive input activation of P2X4, P2X7 and P2Y12 receptors on microglia may serve to maintain nociceptive sensitivity through complex neural-glia interactions or via sensitisation (via P2Y2) of other nociceptive receptors such as TRPV1 channels. There is still an urgent need to understand the mechanisms underlying the successful application of P2X3, P2X4, P2X7 and P2Y12 receptor antagonists for the treatment of neuropathic pain.

The search is on for selective P2X3, P2X2/3, P2X4, P2X7, and P2Y12 receptor antagonists that are orally bioavailable, can cross the blood-brain barrier and do not degrade in vivo.
for the treatment of pain (see [18, 59]). The P2X7 receptor in particular is now a major target for inflammatory neuropathic pain and a number of selective P2X7 receptor antagonists have been developed (see [20, 247]). A review describing recent progress in the development of adenosine receptor ligands as anti-inflammatory drugs is also available [248].

Other therapeutic approaches to pain are being considered, including the development of agents that control the expression of receptors and those that inhibit ATP breakdown by selective inhibition of the known ectonucleotidases. Further, while it is now clear that many different cell types release ATP physiologically in response to mechanical distortion, hypoxia, and various agents, we still await clear understanding of the mechanisms that underlie ATP transport. Hopefully, when this becomes clearer, agents will be developed that will be able to enhance or inhibit ATP release, another useful way forward as a therapeutic strategy. A-134974, a novel adenosine kinase (AK) inhibitor, alleviated tactile allodynia via spinal sites of action in peripheral nerve-injured rats, adding to the growing evidence that AK inhibitors may be useful analgesic agents [249].

There are no publications to date describing clinical evaluations of P2 receptor antagonists and related purinergic compounds for the relief of pain, although clinical trials for some compounds are in progress (see [59]) and reduced pain sensation was noted in a suramin phase 1 cancer clinical trial [250]. However, the emerging information about P2 receptor subtype selective antagonists is promising.

**ABBREVIATIONS AND CHEMICAL NAMES (WHERE AVAILABLE)**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Chemical Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>A-317491</td>
<td>5-[(3-Phenoxybenzyl)(1S)-1,2,3,4-tetrahydro-1-naphthalenyl]aminocarbonyl]-1,2,4-benzenzetircarboxylic acid</td>
</tr>
<tr>
<td>A-317491</td>
<td>5-[(3-Phenoxybenzyl)(1S)-1,2,3,4-tetrahydro-1-naphthalenyl]aminocarbonyl]-1,2,4-benzenzetircarboxylic acid; A438079, 3-(5-(2,3-dichlorophenyl)-1H-tetrazol-1-yl)methyl pyridine</td>
</tr>
<tr>
<td>A-740003</td>
<td>N-[(1-[(Cyanoimino][5,quino[1,4]nolylamino)methyl]amino]-2,2-dimethylpropyl]-2-(3,4-dimethoxyphenyl)acetamide</td>
</tr>
<tr>
<td>AR-C66096</td>
<td>2-Propylthio-D-β,γ-difluoromethylene-ATP</td>
</tr>
<tr>
<td>AR-C69931MX</td>
<td>N6-(2-Methylthioethyl)-2-(3,3,3-trifluoropropyl)-β-dichloromethylene-ATP (cangrelor)</td>
</tr>
<tr>
<td>AZD6140</td>
<td>3-{7-[2-(3,4-Difluoro-phenyl)-cyclopropylamino]-5-propylsulfanyl[1,2,3]triazolo[4,5-d]pyrimidin-3-yl]-5-(2-hydroxymethylene)-cyclopentane-1,2-diol</td>
</tr>
<tr>
<td>INS49266</td>
<td>6-Phenylnuclea-2',3'-phylacetaldehyde acetal</td>
</tr>
<tr>
<td>Ip3I</td>
<td>Diinosine pentaphosphate</td>
</tr>
<tr>
<td>KN62</td>
<td>1-N0,Obis[5-isouquinolinesulphonyl]-N-methyl-L-tyrosyl]-4-phenylpiperazine</td>
</tr>
<tr>
<td>2-MeSMAP</td>
<td>2-Methylthio AMP</td>
</tr>
<tr>
<td>MRS2159</td>
<td>Pyridoxal-a5-phosphate-6-phenylazo-4'-carboxylic acid</td>
</tr>
<tr>
<td>NF110</td>
<td>4,4',4''-(Carboxylbis<a href="bis%5Bcarboxylmino%5D">mino-5,1,3-benzenetetyl</a>)tetakis-benzenesulfinic acid</td>
</tr>
<tr>
<td>NF449</td>
<td>4,4',4''-(Carboxylbis{mino-5,1,3-benzenetetyl}-bis[carboxylmino]}tetakisbenzene-1,3-disulfonic acid</td>
</tr>
<tr>
<td>o-ATP</td>
<td>Oxidised ATP</td>
</tr>
<tr>
<td>PPADS</td>
<td>Pyridoxalphosphate-6-azophenyl-2',4'-disulphonate</td>
</tr>
<tr>
<td>RO3</td>
<td>5-(Methyl[2-methylthyl-4,5-dimethoxyphenyl]-2,4-pyrinediamine</td>
</tr>
<tr>
<td>TNP-ATP</td>
<td>2',3'-O-(2,4,6-Trinitrophenyl)-ATP</td>
</tr>
</tbody>
</table>

**REFERENCES**


[199] Morita K, Morioka N, Abdin J, Kitayama S, Nakata Y, Dohi T. Development of tactile allodynia and thermal hyperalgesia by in-


quinolinylamino)methyl]amino)-2,2-dimethylpropyl]-2-(3,4-


