Purine-mediated signalling in pain and visceral perception

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Receptor subtypes for purines have been identified in a variety of tissues, increasing interest in the roles of purine-mediated signalling in pathophysiological processes. Growing evidence supports the involvement of one of the purinoceptor subtypes, $P2X_3$, in nociception. In this article, recent studies of purine-mediated nociception and visceral pain will be discussed. Furthermore, a novel hypothesis is proposed for purine-mediated mechanosensory transduction where ATP released during distension from epithelial cells lining tubes (such as ureter and gut) and sacs (such as the bladder) acts on $P2X_3$ receptors on a subepithelial nerve plexus to initiate impulses that are relayed via the spinal cord to pain centres in the brain.

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The early history of purine-mediated signalling has been reviewed recently[1]. The first demonstration of extracellular roles for purines was reported in 1929 in the seminal paper of Drury and Szent-Györgyi, who described the potent actions of ATP and adenosine in the cardiovascular system. In 1959, a landmark paper by Pamela Holton reported that ATP is released during antidromic stimulation of sensory nerves to the ear artery in sufficient concentrations to change vascular tone, suggesting that ATP might be a neuronal messenger. In 1970, my own group proposed that ATP was a non-adrenergic, non-cholinergic (NANC) neurotransmitter in the gut, and later this concept was extended to NANC nerves in the bladder and vascular system[2]. In 1976, Burnstock proposed the cotransmitter hypothesis and subsequently ATP has been shown to be a cotransmitter with classical transmitters, neuropeptides and nitric oxide in both peripheral and central nerves[3]. Purinoceptor subtypes have also been identified on many non-neuronal cell types, including endothelial, epithelial, endocrine, bone, immune, pancreatic and hepatic cells[4].

A basis for distinguishing P1 (adenosine) from P2 (ATP/ADP) receptors was proposed in 1978. However, it was not until seven years later that Burnstock and Kennedy proposed a basis for distinguishing two types of P2 purinoceptor, P2X and P2Y, based largely on pharmacological criteria. Soon after, Gordon proposed two further P2 receptor subtypes, P2T on platelets and P2Z on macrophages, and receptors that responded to pyrimidines, as well as purines, were named P2U receptors. In the early 1990s, studies of transduction mechanisms and the cloning of both P2Y and P2X receptors led Abbracchio and Burnstock[5] to propose a new nomenclature system, which is now widely accepted: two families of P2 purinoceptors, P2X ionotropic ligand-gated ion channel receptors and P2Y metabotropic G-protein-coupled receptors (Box 1 and Table 1).

$P2X_3$ receptors in pain and nociception

The first description of pain produced by ATP was when ATP was introduced into human blister base preparations[6]. Later, ATP was implicated in the pathophysiology of migraine[7], and several laboratories carried out experiments suggesting that ATP had actions on the spinal cord nociceptive sensory inputs[8–10].

The $P2X_3$ receptor was cloned in 1995 (Refs 11,12) and was shown to be present in a subset of dorsal root ganglion (DRG) sensory neurones that express peripherin, a cytoskeletal protein associated with small-diameter sensory neurones[12]. These results, together with the fact that a high proportion of these small-diameter neurones is nociceptive, suggested that ATP might play an important role in nociceptor activation (Fig. 1).

Studies of rat sensory neurones in culture have demonstrated a molecular mechanism for the pain-inducing actions of ATP. Electrophysiological analysis has shown that 40–96% of DRG neurones in culture respond to ATP by increasing the intracellular free Ca^{2+} sensory concentrations, or by depolarization[14–16]. Messenger RNA transcripts of $P2X_{1–6}$ receptors have been shown to be expressed in sensory neurones of the DRG, nodose and trigeminal ganglia[17]. However, only one subtype, $P2X_3$, is expressed selectively in cell populations enriched in nociceptors, as judged by positive labelling using *in situ* hybridization of small-diameter neurones. Thus, other P2X purinoceptors probably account for the depolarizing actions of ATP on large-diameter neurones. The level of expression of mRNA detected by *in situ* hybridization and northern blots suggests that the $P2X_3$ receptor is present in relatively greater amounts in small-diameter sensory neurones than are other P2X receptor subunits, such as $P2X_2$; this has been supported by immunohistochemical localization of the receptor protein[18].

Properties of $P2X_3$ receptors

An electrophysiological analysis of the properties of the expressed $P2X_3$ receptor channel showed many similarities with currents in rat sensory neurones in culture[16]. Thus, the channel rapidly desensitized and was activated by ATP congeners with the same rank order of potency as that described for sensory neurones in culture (2-methylthioATP >>ATP > α,β-methylene ATP > γ-thioATP > CTP > ADP >> UTP ≈ β,γ-methylene ATP > GTP)[12]. In addition, the

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Box 1. Purinoceptor subtypes – state of the art

The framework for subclassification of P2 receptor subtypes, proposed by Abbracchio and Burnstock in 1994, allowed for a logical expansion of the nomenclature as new receptors were identified, and there are currently seven subtypes of P2X receptors and six subtypes of P2Y receptors clearly recognized[a]. Earlier, four subtypes of adenosine (P1) receptors were cloned [A_1, A_{2A}, A_{2B} and A_3 (Ref. a)].

P2X receptor family

P2X receptor subtypes are characterized by two transmembrane domains, short intracellular N- and C-termini and an extensive extracellular loop with conservation of ten cysteines. It has become apparent that the pharmacology of the recombinant P2X receptor subtypes expressed in oocytes or other cell types is often different from the pharmacology of P2X-receptor-mediated responses in naturally occurring sites. Several contributing factors are emerging to explain these differences. First, it is now recognized that three (or possibly four) P2X receptor units form the ionic pore and that heteromultimers as well as homomultimers are involved. For example, heteromultimers are now clearly established for $P2X_{2/3}$, $P2X_{4/6}$, $P2X_{1/5}$ and $P2X_{2/6}$ receptors[b]. $P2X_7$ receptors do not appear to form heteromultimers and $P2X_6$ receptors seem unable to form a functional homomultimer. Second, splice variants of P2X receptors exist; although these variants are non-functional on their own, they can modify the actions of full-length P2X receptors[c]. Third, powerful ectoenzymes that rapidly break down purines and pyrimidines are present in tissues but are of less importance when examining recombinant receptors[d].

Early studies of the distribution of P2X receptor subtypes based on northern blot and *in situ* hybridization studies[e] have been substantially extended (after antibodies to these receptors became available) using immunohistochemical localization at both light[f–k] and electron microscope[l–n] levels. For example, although it was originally thought that smooth muscle contained only $P2X_1$ receptors, there is now extensive evidence for the presence of $P2X_2$ and $P2X_4$ receptors in this tissue. $P2X_1$ receptors, which were previously not considered to be present in the brain, have now been clearly demonstrated at postjunctional sites in synapses in the cerebellum[m].

P2Y receptor family

P2Y receptors, which, in common with other G-protein-coupled receptors, have seven transmembrane domains, have an extracellular N-terminus and intracellular C-terminus. The conservation between the different subtypes is greatest in the transmembrane domains, the C-terminus showing the greatest diversity[a,o].

$P2Y_1$ receptors, which were first cloned from chick brain where they are ATP selective, now appear to be ADP selective in mammals and humans, with 2-methylthioADP being a potent agonist, whereas MRS2179 has been proposed as a potent antagonist, and MRS2269 and MRS2286 as selective antagonists[p]. At $P2Y_2$ and $P2Y_4$ receptors in the rat, ATP and UTP are equipotent but the two receptors can be distinguished with antagonists (i.e. suramin blocks $P2Y_2$ receptors and Reactive blue 2 blocks $P2Y_4$ receptors[q,r]). The $p2y_3$ receptor is regarded as an orthologue of the $P2X_6$ receptor by many workers in the field, whereas the earlier designated $P2Y_5$, $P2Y_9$ and $P2Y_{10}$ receptors appear to be orphan receptors with no evidence for a functional role and have been dropped. The $P2Y_6$ receptor is UDP selective whereas the $P2Y_7$ receptor turned out to be a leukotriene receptor. The $p2y_8$ receptor has been cloned from frog embryos, where all the nucleotides are equipotent[s], but no mammalian homologue has been identified to date. $P2Y_{11}$ receptors are unusual in that there are two transduction pathways: cyclic AMP and inositol (1,4,5)-trisphosphate, which is the second messenger system used by the majority of the P2Y receptors. The $P2Y_{12}$ receptor found on platelets was not cloned until recently[t] and probably represents one of a sub-group of P2Y receptors where transduction is entirely through adenylyl cyclase; a receptor on C6 glioma cells and possibly a receptor in the midbrain that is selective for a diadenosine polyphosphate might also act via adenylyl cyclase.

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Table 1. Characteristics of purine-mediated receptors[a]

Receptor	Main distribution	Agonists	Antagonists	Transduction mechanisms
P1 (adenosine)				
A_1	Brain, spinal cord, testis, heart, autonomic nerve terminals	CCPA, CPA	DPCPX, CPX, XAC	G_i (1–3): ↓cAMP
A_{2A}	Brain, heart, lungs, spleen	CGS21680	KF17837, SCH58261	G_s: ↑cAMP
A_{2B}	Large intestine, bladder	NECA	Enprofylline	G_s: ↑cAMP
A_3	Lung, liver, brain, testis, heart	IB-MECA, DBX RM	MRS1220, L268605	G_i, $G_{q/11}$; ↓cAMP, ↑(Ins(1,4,5)P_3
P2X				
$P2X_1$	Smooth muscle, platelets, cerebellum, dorsal horn spinal neurones	α,βmeATP = ATP = 2meSATP (rapid desensitization)	TNP-ATP, IP_5I, NF023	Intrinsic cation channel (Ca^{2+} and Na^+)
$P2X_2$	Smooth muscle, CNS, retina, chromaffin cells, autonomic and sensory ganglia	ATP ≥ ATPγS ≥ 2meSATP >> α,βmeATP (pH + zinc sensitive)	Suramin, PPADS	Intrinsic ion channel (particularly Ca^{2+})
$P2X_3$	Sensory neurones, NTS, some sympathetic neurones	2meSATP ≥ ATP ≥ α,βmeATP (rapid desensitization)	TNP-ATP, suramin, PPADS	Intrinsic cation channel
$P2X_4$	CNS, testis, colon	ATP >> α,βmeATP	–	Intrinsic ion channel (particularly Ca^{2+})
$P2X_5$	Proliferating cells in skin, gut, bladder, thymus, spinal cord	ATP >> α,βmeATP	Suramin, PPADS	Intrinsic ion channel
$P2X_6$	CNS, motor neurones in spinal cord	(does not function as homomultimer)	–	Intrinsic ion channel
$P2X_7$	Apoptotic cells in immune cells, pancreas, skin, among others	BzATP > ATP ≥ 2meSATP >> α,βmeATP	KN62, KN04, Coomassie brilliant blue	Intrinsic cation channel and a large pore with prolonged activation
P2Y				
$P2Y_1$	Epithelial and endothelial cells, platelets, immune cells, osteoclasts	2meSADP > 2meSATP = ADP > ATP	MRS2279, MRS2179	G_q/G_{11}, PLC-β activation
$P2Y_2$	Immune cells, epithelial and endothelial cells, kidney tubules, osteoblasts	UTP = ATP	Suramin	G_q/G_{11} and possibly G_i, PLC-β activation
$P2Y_4$	Endothelial cells	UTP ≥ ATP	RB2, PPADS	G_q/G_{11} and possibly G_i, PLC-β activation
$P2Y_6$	Some epithelial cells, placenta, T cells, thymus	UDP > UTP >> ATP	RB2, PPADS, suramin	G_q/G_{11}, PLC-β activation
$P2Y_{11}$	Spleen, intestine, granulocytes	ARC67085MX > BzATP ≥ ATPγS > ATP	Suramin, RB2	G_q/G_{11} and G_s, PLC-β activation
$P2Y_{12}$	Platelets	ADP	ARC67085MX, ARC69931MX	G_i, inhibition of adenylyl cyclase

[a]Abbreviations: α,βme-ATP, α,β-methylene ATP; 2meSATP, 2-methylthioATP; BzATP, 2′,3′-*O*-(4-benzoyl-benzoyl) ATP; CCPA, 2-chloro-*N*^{6}-cyclopentyladenosine; CPA, *N*^{6}-cyclopentyladenosine; CPX, 8-cyclopentyl-1,3-dipropylxanthine; DBX RM, 1,3-dibutylxanthine-1-riboside-5′-*N*-methylcarboxamide; DPCPX, 8-cyclopentyl-1,3-dipropylxanthine; Ins(1,4,5)P_3, inositol (1,4,5)-trisphosphate; IB-MECA, *N*^{6}-(3-iodobenzyl)-5′-*N*-methylcarboxamidoadenosine; IP_5I, diinosine pentaphosphate; NECA, *N*-ethylcarboxamidoadenosine; NTS, nucleus of the solitary tract; PLC-β, phospholipase C-β; PPADS, pyridoxalphosphate-6-azophenyl-2′,4′-disulfonic acid; RB2, Reactive blue 2; TNP-ATP, 2′,3′-*O*-(2,4,6-trinitrophenyl) adenosine 5′-triphosphate; XAC, xanthine amine congener.

channel is blocked by suramin, a general antagonist of P2X receptors (with the exception of rat $P2X_4$ and $P2X_6$ receptors). However, it is unclear whether the $P2X_3$ receptor exists as a homomultimer or a heteromultimer with $P2X_2$ in sensory neurones *in vivo*. In a recent study of rat nodose ganglion neurones using 2′,3′-*O*-trinitrophenylATP (TNP-ATP) as a selective P2X receptor antagonist, it was concluded that some neurones used homomeric $P2X_2$ receptors whereas others used heteromeric $P2X_{2/3}$ receptors[19]. Capsaicin-sensitive, small-sized DRG neurones of the rat have been reported to express mainly the homomeric $P2X_3$ receptor subunit, whereas the capsaicin-insensitive, medium-sized neurones have been shown to express the heteromultimeric $P2X_{2/3}$ receptor[20]. The differences in properties of nodose and dorsal root ganglia might be related to the fact that the nodose is a vagal ganglion, not regarded as being associated with pain, in contrast to the dorsal root spinal ganglia.

Indirect evidence for heteromultimeric channels in sensory neurones has been provided by coexpression of P2X receptor subunits in *Xenopus* oocytes. Some nodose ganglion neurones desensitize slowly in response to α,β-methylene ATP, but $P2X_3$ receptors exhibit a rapid desensitization in oocytes. However, coexpression of $P2X_2$ receptors and $P2X_3$ receptors produces a channel that has a slowly desensitizing response[11]. This confirms that the heteromultimeric channels are able to form, and might account for, some slowly desensitizing responses in subsets of sensory neurones.

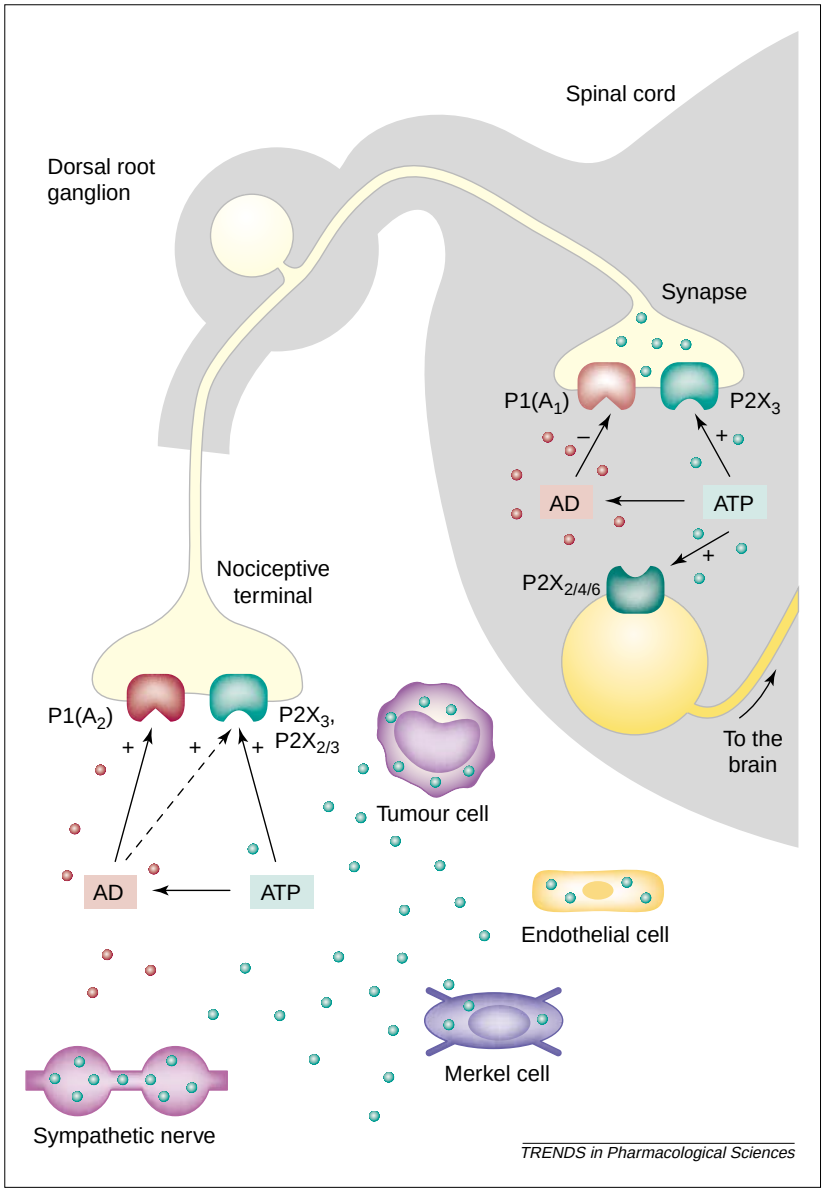

Fig. 1. The roles of purine nucleotides and nucleosides in pain pathways. At sensory nerve terminals in the periphery, the $P2X_3$ receptor has been identified as the principal P2X receptor present. The $P2X_3$ receptor is found predominantly in sensory ganglia, and it might work in heteromultimeric combination with other P2X receptors, such as $P2X_2$. Other known P2X receptor subtypes are also expressed at low levels in dorsal root ganglia. Although less potent than ATP, adenosine (AD) also appears to act on sensory terminals, probably directly via adenosine A_2 receptors; however, it might also act (broken arrow) to potentiate $P2X_3$ or $P2X_{2/3}$ activation. At synapses in sensory pathways in the CNS, ATP appears to act both presynaptically via $P2X_3$ receptors to enhance glutamate release and postsynaptically via $P2X_2$ and/or $P2X_4$ and $P2X_6$ receptor subtypes, and after breakdown to adenosine, it acts as a prejunctional inhibitor of transmission via adenosine A_1 receptors. Sources of ATP acting on $P2X_{2/3}$ sensory terminals include sympathetic nerves and endothelial, Merkel and tumour cells. Molecules of ATP are represented by green circles, and molecules of adenosine are represented by red circles. Modified from Ref. 13.

Although $P2X_3$ receptors are not sensitive to pH, recombinant $P2X_2$ receptors have been shown to be strongly pH sensitive[21] and $P2X_{2/3}$ receptors are also pH sensitive but to a lesser extent[22]. This suggests that the sensitivity of nociceptive $P2X_{2/3}$ receptors might be enhanced in inflammatory conditions with slow acidosis. Acid pH has also been shown to augment the excitatory actions of ATP on dissociated mammalian sensory neurones[23]. By contrast, physiological concentrations of extracellular Mg^{2+} inhibit ATP-activated current in rat nodose ganglion cells[24]. A more recent study of dissociated neurones from 1–4-day-old rat DRG showed that the relative actions of various ATP analogues and, in particular, the very low activity of β,γ-methylene-L-ATP confirmed the presence of $P2X_3$ receptors[15]. Furthermore, synergistic interactions between ATP and other known nociceptive agents on sensory terminals in the periphery have been proposed[21,25–27].

Distribution of $P2X_3$ receptors

Immunohistochemical studies of $P2X_3$ receptors on sensory ganglia have been carried out at both light microscope and electron microscope levels[28], and show that $P2X_3$ receptors are predominantly located in the non-peptidergic subpopulation of small nociceptive neurones that label with the lectin IB4.

In trigeminal ganglia, $P2X_3$ receptor immunoreactivity occurs in both small and large nerve cell bodies and their processes. $P2X_3$ receptors are expressed in approximately equal numbers of sensory neurones projecting to the skin and viscera but in very few of those innervating skeletal muscle[18]. The central projections of $P2X_3$-receptor-labelled nerves in DRG neurones are located in inner lamina II of the dorsal horn of the spinal cord[29]. For the labelled nerve profiles in lamina II, $P2X_3$ receptors were located largely in terminals that have the ultrastructural characteristics of sensory afferent terminals, which suggests that ATP is released onto primary afferent terminals, thereby modulating sensory input coming from the periphery[29]. In the solitary nucleus of the brain stem where there are many afferent fibres from the viscera and tongue, and in the nucleus tractus solitarius, $P2X_3$-receptor-positive boutons synapse on dendrites and cell bodies and have complex synaptic relationship with other axon terminals and dendrites. The peripheral projections of nociceptive neurones in the skin, tongue and tooth pulp are immunopositive for $P2X_3$ receptors[30,31]. After sciatic nerve axotomy, $P2X_3$ receptor expression dropped by more than 50% in L4/5 DRG neurones. Glial cell-derived neurotrophic factor (GDNF), delivered intrathecally, completely reversed axotomy-induced downregulation of the $P2X_3$ receptor[18]. By contrast, the $P2X_3$ receptor was transiently upregulated and anterogradely transported in trigeminal primary sensory nerves after nerve injury[32].

Function of $P2X_3$ receptor activation

In vivo studies of the functional consequences of P2X receptor activation of peripheral neurones in animal models are beginning to emerge. Behavioural indices of acute nociception were monitored in the conscious rat following subplantar injection of ATP and α,β-methylene ATP into the hindpaw[33]. Signs of overt nociception (i.e. hindpaw lifting and licking) were apparent following injection of α,β-methylene ATP;

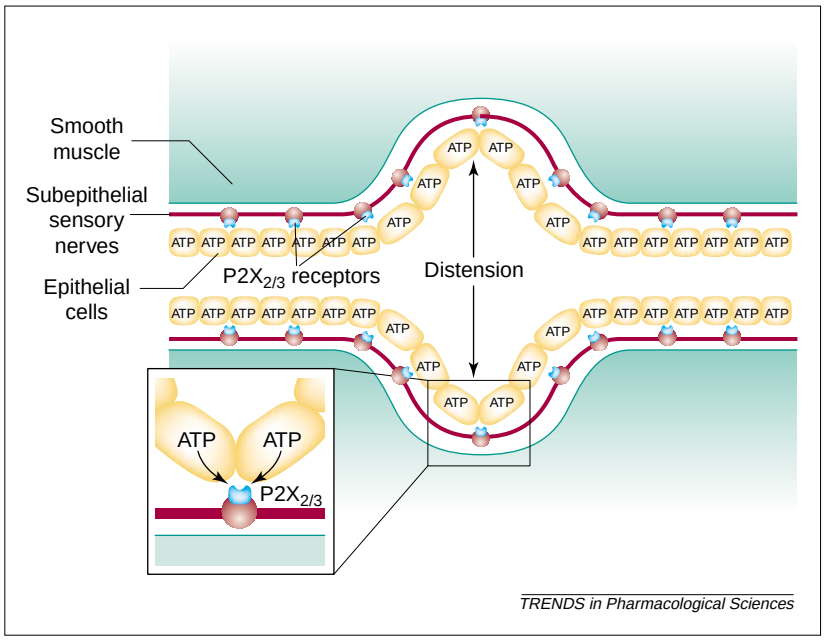

Fig. 2. Hypothesis for purinergic mechanosensory transduction in tubes (e.g. ureter, vagina, salivary and bile duct and gut) and sacs (e.g. urinary and gall bladders, and lung). It is proposed that distension leads to release of ATP from the epithelium lining the tube or sac, which then acts on $P2X_{2/3}$ receptors on subepithelial sensory nerves to convey sensory (nociceptive) information to the CNS. Modified from Ref. 41.

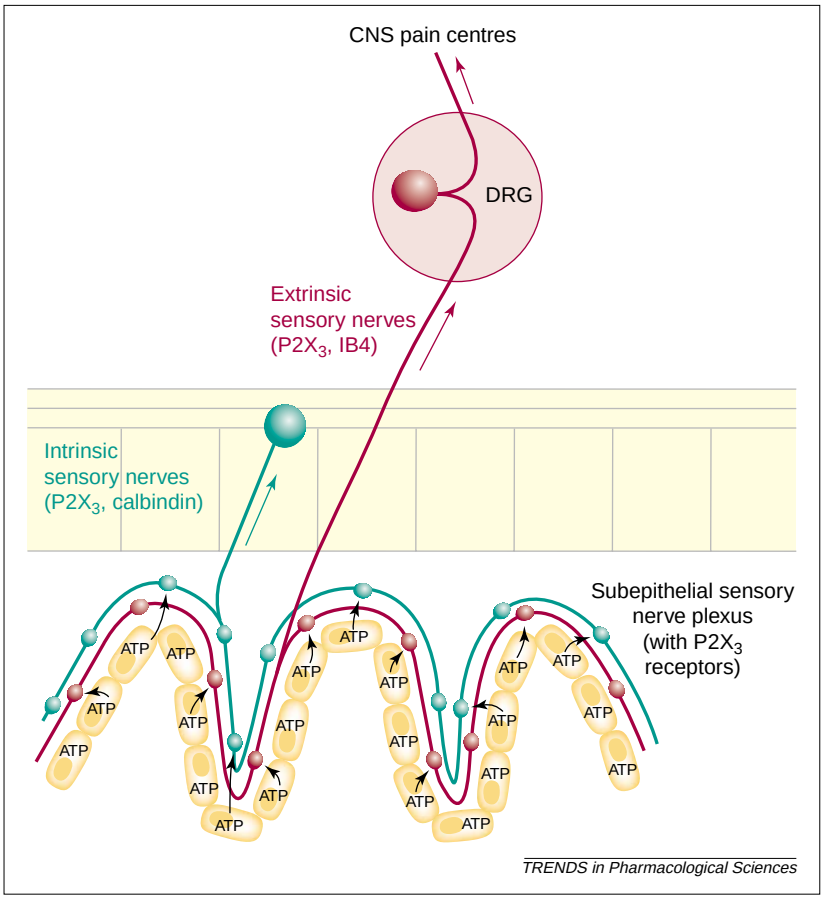

Fig. 3. A novel hypothesis of purine-mediated mechanosensory transduction in the gut. It is proposed that ATP released from mucosal epithelial cells during moderate distension acts preferentially on $P2X_3$ and/or $P2X_{2/3}$ receptors on low-threshold subepithelial intrinsic sensory nerve fibres (labelled with calbindin) contributing to peristaltic reflexes, whereas ATP released during extreme distension also acts on $P2X_3$ and/or $P2X_{2/3}$ receptors on high-threshold extrinsic sensory nerve fibres [labelled with isolectin B4 (IB4)][18] that send messages via the dorsal root ganglia (DRG) to pain centres in the CNS. Modified from Ref. 47.

these effects were dose related and inhibited by selective desensitization of the $P2X_3$ receptor after prolonged exposure to α,β-methylene ATP. In another study using this model the results suggested that endogenous concentrations of ATP are more likely to reach concentrations capable of exciting nociceptors in inflamed as opposed to normal skin[34]. In yet another study, ATP and α,β-methylene ATP were applied to the peripheral terminals of primary afferent articular nociceptors in rat knee joints and neural activity was recorded from the medial articular nerve in rats anaesthetized with pentobarbitone[35]. Rapid, short-lasting excitation of a subpopulation of C and Aδ nociceptive afferents was evoked, which as antagonized by pyridoxalphosphate-6-azophenyl-2′,4′-disulfonic acid (PPADS).

Experiments have also been carried out using the formalin rat paw model, which has two distinct components: an initial phase that reflects a direct sensory nerve activation and a later phase that might reflect an inflammatory component[36]. The P2 receptor antagonists, suramin, Evans blue, Trypan blue and Reactive blue 2, produced antinociception in this model when applied intrathecally[37]. Sawynok and Reid[38] concluded that their results provided evidence in support of a P2X-receptor-mediated augmentation of the pain signal and that the delayed time-course of the effects suggests that it might occur in concert with other mediators that are recruited by the inflammatory process. In another study, using the formalin and writhing tests in adult male albino mice, systemically administered ATP and ADP, which are rapidly degraded to adenosine, caused a reduction in the number of writhes and the time of licking the formalin-injected paw[39]. However, adenosine (P1) receptor antagonists, but not P2 receptor antagonists, reversed these reactions; thus, it was concluded that the antinociceptive effects of adenosine nucleotides were mediated by adenosine.

The sources of ATP involved in the initiation of pain have been discussed by Burnstock[40]; it was suggested that ATP is released as a cotransmitter from: (1) sympathetic nerves in causalgia and reflex sympathetic dystrophy; (2) endothelial cells of microvessels in vascular pain, including migraine and angina; and (3) tumour cells in cancer.

Visceral pain – purinergic mechanosensory transduction

It has been proposed[41] that in tubes (e.g. ureter, salivary duct, bile duct, vagina and intestine) and sacs (e.g. urinary bladder, gall bladder and lung) nociceptive mechanosensory transduction occurs where distension releases ATP from the epithelial cells lining these organs, which then activates $P2X_3$ and/or $P2X_{2/3}$ receptors on subepithelial sensory nerve plexuses to relay messages to the CNS pain centres (Fig. 2). Supporting evidence for this concept comes from studies of the rabbit bladder where ATP

has been shown to be released from the urothelial cells by hydrostatic pressure changes[42], and in rat where nerve discharges were produced in pelvic nerve afferents during slow distension of the bladder and infusion of α,β-methylene ATP, which were antagonized by suramin[43].

In a recent study of $P2X_3$ receptor knockout mice, it was shown that there was a loss of rapidly desensitizing inward currents induced by ATP in DRG neurones and a reduction of ATP-induced sustained currents in nodose ganglion neurones[44]. $P2X_3$-receptor-deficient mice show significantly reduced pain-related behaviour in response to intraplantar injections of ATP and formalin. However, behavioural responses to noxious mechanical and thermal stimuli appear normal[45]. $P2X_3$-receptor-deficient mice also exhibit a marked urinary bladder hyporeflexia following cystometric evaluation, characterized by decreased voiding frequency, increased bladder capacity and voiding volume, but normal bladder pressures. Immunohistochemical studies localize $P2X_3$ receptors to nerve fibres innervating the urinary bladder of wild-type mice and demonstrate that loss of $P2X_3$ receptors does not alter sensory neurone innervation density. Thus, the $P2X_3$ receptor is crucial not only to peripheral sensory functions such as nociception, but also to the normal physiological regulation of afferent pathways that control volume reflexes in the urinary bladder.

The presence of P2X receptors has also been reported on intestinal afferent nerve endings[46]; on the basis of recent unpublished data from our laboratory that $P2X_3$ receptors are present on both extrinsic and intrinsic sensory nerve fibres in the gut, a new hypothesis has been proposed for differential purine-mediated mechanosensory transduction in the gut[47] (Fig. 3).

Concluding remarks

There now seems to be sufficient evidence to connect purine-mediated signalling to visceral pain, particularly in the ureter, bladder and gut. The relation of purine-mediated signalling to 'pelvic' pain is less clear, although the ureter and trigone have been implicated (see http://www.pelvicpain.org/acute_chronic_visc.asp). It is hoped that new therapeutic strategies might arise from these findings.

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Chemical names

ARC67085MX: 2-propylthio-D-β,γ-dichloromethylene ATP
ARC69931MX: N^6-[2-(methylthio)-ethyl]-2-(3,3,3-trifluoropropyl)thio-5′-adenylic acid
CGS21680: 2-[4-(2-carboxyethyl)phenethylamino]-5′-*N*-ethylcarboxamidoadenosine
KF17837: (*E*)-1,3-dipropyl-8-(3,4-dimethoxystyryl)-7-methyl-3,7-dihydro-1*H*-purine-2,6-dione)
KN04: *N*-[1-[*N*-methyl-*p*-(5-isoquinolinesulfonyl)benzyl]-2-(4-phenylpiperazine)ethyl]-5-isoquinoline-sulfonamide
L268605: 3-(4-methoxyphenyl)-5-amino-7-oxo-thiazolo[3,2]pyrimidine
MRS1220: 9-chloro-2-(2-furyl)-5-phenylacetylamino-[1,2,4]-triazolo[1,5-*c*]quinazoline
MRS2179: N^6-methyl-2′-deoxyadenosine 3′,5′-bisphosphate
MRS2269: anhydrohexitol derivative of N^6-methyl-2′-deoxyadenosine 3′,5′-bisphosphate
MRS2279: *N*-methanocarbs-N^6-methyl-2-chloro-2′-deoxyadenosine-3′,5′-bisphosphate
MRS2286: acyclic derivative of N^6-methyl-2′ deoxyadenosine 3′,5′ bisphosphate
NF023: pyridoxal-5′-phosphate-6-azophenyl-4′-carboxylate
SCH58261: 5-amino-7-(2-phenylethyl)-2-(2-furyl)-pyrazolo-(4,3-*e*)1,2,4-triazolo(1,5-*c*)-pyrimidine

$GABA_A$ receptor subtypes: dissecting their pharmacological functions

Uwe Rudolph, Florence Crestani and Hanns Möhler

The enhancement of GABA-mediated synaptic transmission underlies the pharmacotherapy of various neurological and psychiatric disorders. $GABA_A$ receptors are pluripotent drug targets that display an extraordinary structural heterogeneity: they are assembled from a repertoire of at least 18 subunits (α1–6, β1–3, γ1–3, δ, ε, θ, ρ1–3). However, differentiating defined $GABA_A$ receptor subtypes on the basis of function has had to await recent progress in the genetic dissection of receptor subtypes *in vivo*. Evidence that the various actions of allosteric modulators of $GABA_A$ receptors, in particular the benzodiazepines, can be attributed to specific $GABA_A$ receptor subtypes will be discussed. Such discoveries could open up new avenues for drug development.

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The enhancement of neuronal inhibition by GABA is one of the most powerful therapeutic strategies for the treatment of CNS diseases such as generalized anxiety disorders, sleep disturbances, muscle spasms and seizure disorders. $GABA_A$ receptors are targets for many drugs in wide clinical use; these include ligands of the benzodiazepine site of the $GABA_A$ receptor, barbiturates, anesthetics and – currently at an experimental stage – neurosteroids. $GABA_A$ receptors are ubiquitous in the CNS (Ref. 1). Therefore, a major goal in neuropharmacology has been to target drugs selectively to defined $GABA_A$ receptor subtypes and thereby refine the therapeutic spectrum of the presently available drugs, reduce their side-effects and discover new therapeutic indications.

$GABA_A$ receptors are pentameric membrane proteins that operate as GABA-gated Cl^- channels. These receptors are most clearly distinguished by their subunit architecture, which in mammalian brain comprises seven different classes of subunits with mostly multiple variants (α1–α6, β1–β3, γ1–γ3, ρ1–ρ3, δ, ε, θ). Most $GABA_A$ receptors are composed of α-, β- and γ-subunits[2]. Mutational analyses of multiple recombinant $GABA_A$ receptors have generated valuable information on their drug sensitivity *in vitro*[3]. However, pharmacological analysis of $GABA_A$ receptor subtypes has had to wait for the generation of animal models in which particular $GABA_A$ receptor subunits are either inactivated (knockout strategy) or selectively point-mutated (knock-in strategy). The lessons for drug design learned from these approaches will be discussed in the present article.

$GABA_A$ receptors analyzed by gene-knockout strategies

In gene-knockout strategies, ablation of a particular receptor subunit would be expected to perturb the structure of a defined group of $GABA_A$ receptors and

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