Molecular Pain

Review

Purinergic mechanosensory transduction and visceral pain
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Abstract

In this review, evidence is presented to support the hypothesis that mechanosensory transduction occurs in tubes and sacs and can initiate visceral pain. Experimental evidence for this mechanism in urinary bladder, ureter, gut, lung, uterus, tooth-pulp and tongue is reviewed. Potential therapeutic strategies are considered for the treatment of visceral pain in such conditions as renal colic, interstitial cystitis and inflammatory bowel disease by agents that interfere with mechanosensory transduction in the organs considered, including $P2X_3$ and $P2X_{2/3}$ receptor antagonists that are orally bioavailable and stable *in vivo* and agents that inhibit or enhance ATP release and breakdown.

Introduction
Visceral pain is one of the most common forms of pain associated with pathological conditions like renal colic, dyspepsia, inflammatory bowel disease (IBD), angina, dysmenorrhoea and interstitial cystitis. While it is generally accepted that IBD is associated with pain (see [1,2]) there are reports that in some patients with IBD, there is hyposensitivity. $P2X_3$ (homomultimer) and $P2X_{2/3}$ (heteromultimer) receptors were cloned and shown to be largely located on small nociceptive sensory neurons in the dorsal root ganglia (DRG) in 1995 [3,4]. A schematic showing the initiation of nociception by ATP on primary afferent fibres in the periphery and purinergic relay pathways in the spinal cord are shown in Figure 1.

A hypothesis was proposed that purinergic mechanosensory transduction occurred in visceral tubes and sacs, including ureter, bladder and gut, where ATP released from epithelial cells during distension acted on $P2X_3$ homomeric and $P2X_{2/3}$ heteromeric receptors on subepithelial sensory nerves initiating impulses in sensory pathways to pain centres in the central nervous system (CNS) [5] (Figure 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. [6], who speculated about this being the basis of a sensory mechanism. Prolonged exposure to a desensitizing concentration of α,β-methylene ATP (α,β-meATP) significantly reduced the activity of mechanosensitive pelvic nerve afferents in an *in vitro* model of rat urinary bladder [7]. Later, it was shown that mice lacking the $P2X_3$ receptor exhibited reduced inflammatory pain and marked urinary bladder hyporeflexia with reduced voiding frequency and increased voiding volume, suggesting that $P2X_3$ receptors are involved in mechanosensory transduction underlying both inflammatory pain and physiological voiding reflexes [8]. Subsequently, using $P2X_2$ knockout mice and $P2X_2/P2X_3$ double knockout mice, a role for the $P2X_2$ subtype was shown to be involved in mediating the sensory effect of ATP [9]. 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 $P2X_3$ antagonists as well as in $P2X_3$ knockout mice; $P2X_3$ recep-
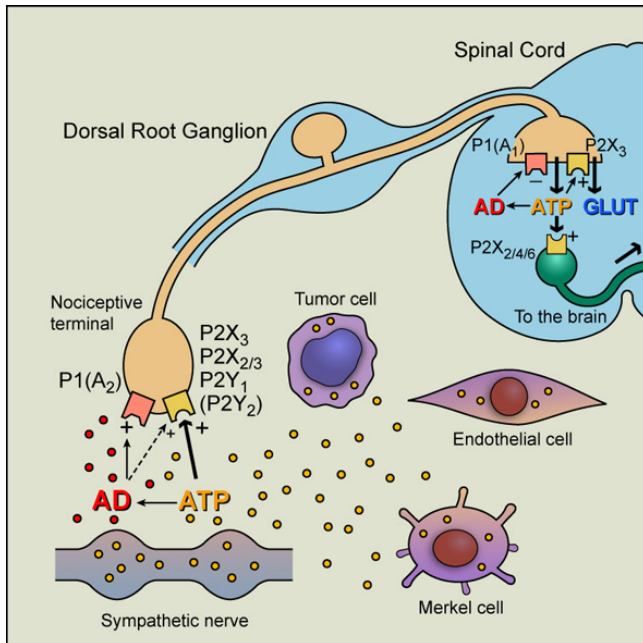
Figure 1
Hypothetical schematic of the roles of purine nucleotides and nucleosides in pain pathways. At sensory nerve terminals in the periphery, $P2X_3$ and $P2X_{2/3}$ receptors have been identified as the principal P2X purinoceptors present, although recent studies have also shown expression of $P2Y_1$ and possibly $P2Y_2$ receptors on a subpopulation of $P2X_3$ receptor-immunopositive fibers. Other known P2X purinoceptor subtypes (1--7) 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 $P1(A_2)$ purinoceptors; however, it also acts synergistically (broken black line) to potentiate $P2X_{2/3}$ receptor activation, which also may be true for 5-hydroxytryptamine, capsaicin, and protons. At synapses in sensory pathways in the CNS, ATP appears to act postsynaptically via $P2X_2$, $P2X_4$ and/or $P2X_6$ purinoceptor subtypes, perhaps as heteromultimers, and after breakdown to adenosine, it acts as a prejunctional inhibitor of transmission via $P1(A_2)$ purinoceptors. $P2X_3$ receptors on the central projections of primary afferent neurons in lamina II of the dorsal horn mediate facilitation of glutamate and probably also ATP release. Sources of ATP acting on $P2X_3$ and $P2X_{2/3}$ receptors on sensory terminals include sympathetic nerves as well as endothelial, Merkel, and tumor cells. Yellow dots, molecules of ATP; red dots, molecules of adenosine. (Reproduced from [114] and modified from [105], used with permission from the American Physiological Society.)

tors were localized on suburothelial sensory nerve fibres [10]. It appears that the bladder sensory DRG neurons, projecting via pelvic nerves, express predominantly $P2X_{2/3}$ heteromultimer receptors [11].

Sensory information from the urinary bladder is conveyed by both lumbar splanchnic (LSN) and sacral pelvic (PN) nerves to the spinal cord. A study comparing the mechanosensitive properties of single afferent fibres in these two pathways showed that both low and high threshold stretch-sensitive afferents were present in both pathways [12]. 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 [13]. It was also shown that purinergic agonists increase the excitability of afferent fibres to distension. The roles of ATP released from urothelial cells and suburothelial myofibroblasts on various bladder functions have been considered at length in several reviews [14,15], and evidence presented that urothelial-released ATP alters afferent nerve excitability [16]. Amiloride, a blocker of epithelial Na^+ channels, has been shown to suppress ATP release from cultured urothelial cells by a hypotonic (mechanical) stimulus [17] or by stretch of intact bladder [18]. Raising the intracellular Ca^{2+} concentration inhibits stimulation-evoked ATP release from urothelial cells [19].

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 [20]. 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 [8,21]. 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 pyridoxal-phosphate-6-azonphenyl-2',4'-disulfonic acid (PPADS) and 2',3'-O-(2,4,6-trinitrophenyl)-ATP (TNP-ATP) [22] (Figure 2a). $P2X_1$ and $P2X_3$ receptors play a fundamental role in the micturition reflex in female urethane-anesthetized rats; $P2X_3$ receptor blockade by phenol red raised the pressure and volume thresholds for the reflex, while $P2X_1$ receptor blockade diminished motor activity associated with voiding [23]. In TRPV1 receptor knock-out mice, release of ATP is significantly depressed [24] and afferent sensitivity to distension is attenuated, especially those effects mediated by low threshold fibres related to the micturition reflex, rather than the high threshold nociceptive fibres [25].

Four functionally distinct populations of bladder sensory neurons were identified with electrophysiological recordings when guinea-pig bladder was subjected to a range of

Figure 2 *(see legend on next page)*

Figure 2 *(see previous page)*
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, lung). It is proposed that distension leads to release of ATP from epithelium lining the tube or sac, which then acts on $P2X_3$ and/or $P2X_{2/3}$ receptors on subepithelial sensory nerves to convey sensory/nociceptive information to the CNS. (Reproduced from [5], with permission from Blackwell.) **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 $P2X_3$ and/or $P2X_{2/3}$ receptors on low-threshold subepithelial intrinsic sensory nerve fibers (labelled with calbindin) to modulate peristaltic reflexes. ATP released during extreme (colic) distension also acts on $P2X_3$ and/or $P2X_{2/3}$ receptors on high-threshold extrinsic sensory nerve fibers [labelled with isolectin B4 (IB4) or are peptidergic] that send messages via the dorsal root ganglia (DRG) to pain centres in the CNS. (Reproduced and modified from [115], published by John Wiley and Sons, Inc.)

mechanical stimuli (stretch, von Frey hair stroking and focal compression of receptive fields) and chemical stimuli (α,β-methylene ATP and capsaicin) [26]. Four different major classes of extrinsic sensory C fibres have been identified in the guinea-pig bladder: one mediates muscle mechanoresponses and was unaffected by removal of the urothelium; another was activated by stretch and α,β-meATP and was reduced by urothelial removal; the third were stretch insensitive, but could be activated by mucosal stroking with von Frey hairs or α,β-meATP and reduced by urothelium removal; while the fourth class were stretch insensitive, but could be weakly activated by mucosal stroking, but not by α,β-meATP [26].

Despite the compelling evidence in support of purinergic mechanosensory transduction from several independent laboratories (including stimulation by α,β-meATP of 2 of the 4 sensory afferents classes described by Zagorodnyuk et al. [26]), a recent paper from this group claims that urothelial release of ATP and stimulation of sensory fibres is not involved in mechanosensory transduction in the bladder, but that benzamil-sensitive stretch-activated ion channels are more likely to be involved [27]. Further experiments will hopefully resolve this issue.

In rats with detrusor overactivity induced by bladder outlet obstruction, there is an increase in expression of muscarinic receptors and an increase, but to a smaller extent, of $P2X_3$ receptor immunostaining [28]. Cyclophosphamide-induced bladder inflammation (a model for interstitial cystitis), sensitizes and enhances $P2X_3$ and $P2X_{2/3}$ receptor function in rat bladder sensory neurons [29]. Botulinum toxin A, which has antinociceptive effects in treating interstitial cystitis, inhibits distension-mediated urothelial release of ATP in conditions of bladder inflammation [30] as well as ATP release as a cotransmitter with acetylcholine from parasympathetic nerves [31].

In summary, there is now strong evidence from several different laboratories that ATP is released from urothelial cells during distension of the bladder wall. The ATP then activates sensory nerve endings beneath the urothelium,

via $P2X_3$ and $P2X_{2/3}$ receptors, that leads, via low threshold fibres, to modulation of the voiding reflex and via high threshold fibres to reach pain centres in the CNS.
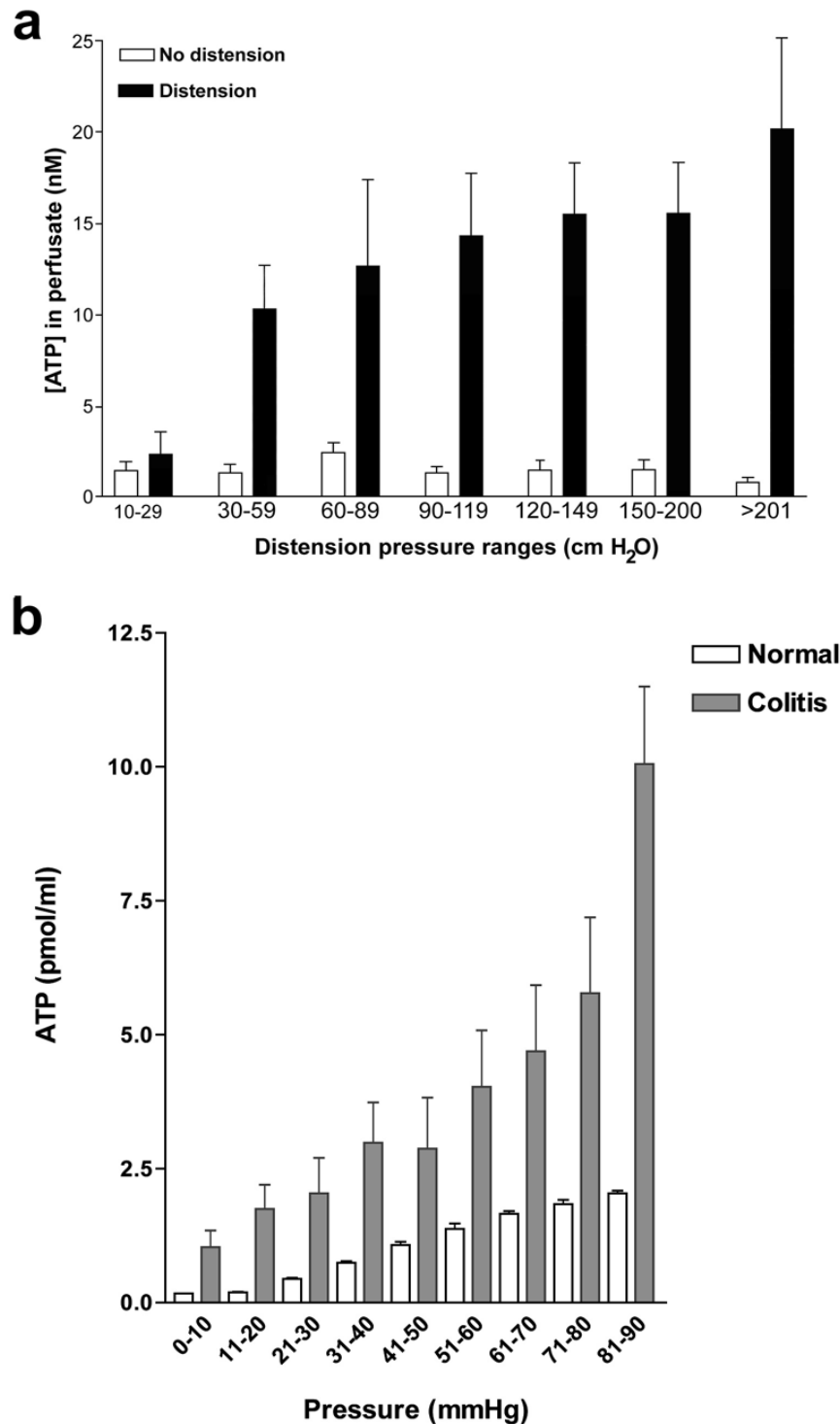
Ureter
The ureteric colic that is induced by the passage of a kidney stone causes severe pain. Immunostaining of $P2X_3$ receptors in sensory nerves in the subepithelial region was reported [32]. Multifibre recordings of ureter afferent nerves were made using a guinea pig preparation perfused *in vitro* [33]. Distension of the guinea-pig ureter increased spike discharge in sensory neurons, which was mimicked by ATP and reduced by ATP antagonists [33] (Figure 3a). The afferent responses consisted of both fast and slow components. The P2 receptor antagonists TNP-ATP and PPADS reduced distension-induced afferent activity (Figure 3b) and blocked the rapid and reduced the slower response to ATP, while the remaining responses were blocked by the selective A_1 receptor antagonist 8-cyclopentyl-1,3-dipropylxanthine. The ecto-ATPase inhibitor (ARL-67156) produced an increase in base-line and distension-induced sensory discharge.

Knight et al. [34] found that distending the perfused guinea-pig ureter at pressures from 20-700 cm H_2O caused a pressure-dependent release of ATP from urothelial cells, approximately 10 times the basal release levels. The ATP release was abolished by removal of the urothelium and scanning electronmicroscopy confirmed an intact urothelium after distension. ATP was not released due to activation of stretch-activated channels since gadolinium failed to affect ATP release, nor did glibenclamide, known to inhibit ATP-binding cassette proteins. However, both monensin and brefeldin A, which interfere with vesicular formation and trafficking, inhibited distension-evoked ATP release, which was Ca^{2+}-dependent, indicating that ATP release from ureter urothelium might be largely mediated by vesicular exocytosis. In a recent study in our laboratory, experiments have been carried out to show that ATP is released from the human ureter upon distension (Figure 4a) and that human ureteric suburothelial sensory nerves express $P2X_3$ receptors [35].
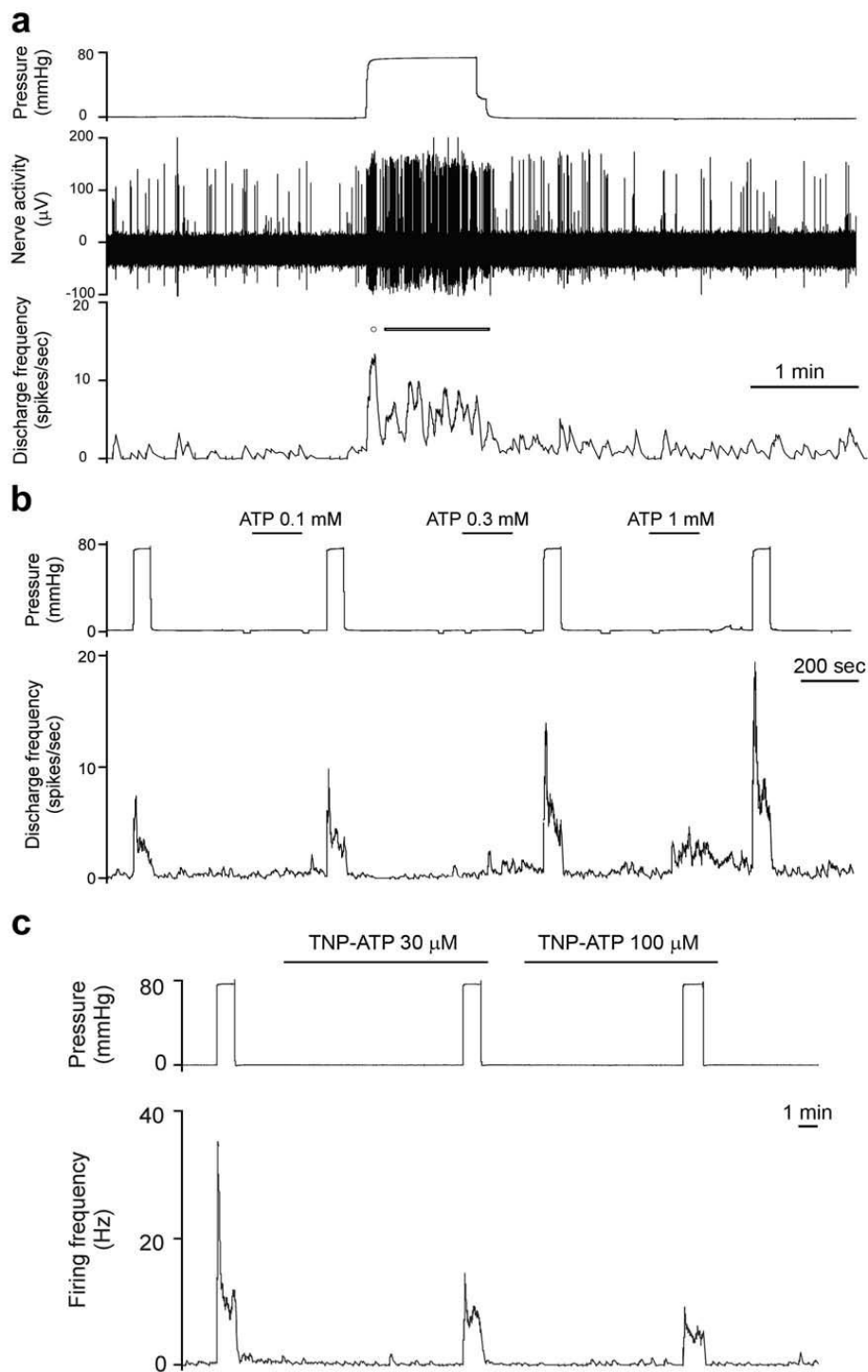
Figure 3
A. Spontaneous and distension-induced activity in ureter afferent fibres. Multifibre afferent responses to rapid distension. Note that background afferent activity occurs in bursts and that ureter distension results in an initial burst of discharge (circle) followed by a phase of maintained activity (bar). **B**. ATP can sensitise ureter afferent fibres. An example representative of distension-induced afferent activity before and following intraluminal application of increasing concentrations of ATP. **c**. TNP-ATP inhibits distension-induced afferent activity. A multifibre recording to show distension-induced afferent activity in control and in the presence of TNP-ATP. (Reproduced from [33], with permission of Elsevier.)

Figure 4
A. ATP concentration ([ATP]) in perfusate immediately before and after distension of the human ureter, grouped in pressure ranges. The mean [ATP] after distension is significantly greater than before distension in each pressure range P < 0.01; n = 7, error bars represent s.e.m. (Reproduced from [35], with permission from Springer.) **B**. ATP concentration in luminal fluid samples from normal and inflamed rat colorectum during distension. Values are means ± SE. (Reproduced from [67] and used with permission from the American Physiological Society.)

The release of ATP only occurred above a threshold of 25-30 com H_2O. This is similar to the uroteric pressure threshold for pain measured by Risholm [36]. In a recent review of the physiology and pharmacology of the human ureter, it was suggested that purinergic receptors might be target analgesics for the treatment of ureteral colicky pain and that an additional advantage might be facilitating spontaneous ureteral stone passage [37].

Gut

A hypothesis was proposed suggesting that purinergic mechanosensory transduction in the gut initiated both physiological reflex modulation of peristalsis via intrinsic sensory fibres and nociception via extrinsic sensory fibres [38,39] (Figure 2b). Evidence in support of this hypothesis was obtained from a rat pelvic sensory nerve-colorectal preparation [40]. Distension of the colorectum led to pressure-dependent increase in release of ATP from mucosal epithelial cells (Figure 4b) and also evoked pelvic nerve excitation. This excitation was mimicked by application of ATP and α,β-meATP and attenuated by the selective $P2X_3$ and $P2X_{2/3}$ antagonist TNP-ATP and by PPADS. The sensory discharge was potentiated by ARL-67156, an ATPase inhibitor. Single fibres analysis showed that high-threshold fibres were particularly affected by α,β-meATP. In addition to release of ATP from mucosal epithelial cells in the rat gut in response to distension (see [40]), ATP has also been shown to be released from human intestinal epithelial cells in response to osmotic swelling [41,42]. The interactions of ATP with other mediators that activate pelvic afferent fibres in the rat colorectum, including capsaicin, 5-hydroxytryptamine (5-HT), bradykinin, prostaglandins and substance P (SP), have been described [43,44]. In addition, TRPV1 channels are activated and sensitised by ATP that is released during distension [45,46], especially in pathological states such as colitis [47-49]. Carvacral, an agonist for TRPV3 channels, caused increased ATP release from colonic epithelial cells [50] and TRPV4 channels have also been shown to mediate stretch-release of ATP from urothelial cells [51]. LSN and 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 [52].

Purinergic mechanosensory transduction has been described in other regions of the gastrointestinal tract. For instance, α,β-meATP was shown to stimulate mechano-sensitive mucosal and tension receptors in mouse stomach and oesophagus leading to activity in vagal afferent nerves [53]. The sensitizing effects of $P2X_3$ receptor agonists on mechanosensory function are induced in oesophagitis [54]. Vagal nodose (placode-derived) nociceptive fibres in guinea-pig oesophagus are exclusively C-fibres sensitive to $P2X_3$ receptor agonists and rarely express SP, while jugular (neural crest-derived) nociceptive fibres include both A- and C-fibres and are insensitive to $P2X_3$ agonists and mostly express SP [55]. Adenosine has been claimed to activate a subset of nociceptive vagal sensory nerves in guinea-pig oesophagus [56]. Visceral hypersensitivity may play a role in the pathogenesis of functional chest pain claimed to be of oesophageal origin. Theophylline ameliorated chest pain in 7 out of 8 patients in a clinical trial, perhaps by reducing adenosine-mediated nociception [57]. Purinergic mechanosensory transduction has also been implicated in reflex control of intestinal secretion, whereby ATP released from mucosal epithelial cells acts on $P2Y_1$ receptors on enterochromaffin cells to release 5-HT (and ATP, which is stored and co-released with 5-HT from enterochromaffin cells [58]), which leads to regulation of secretion either directly or via intrinsic reflex activity [59].

Subepithelial fibroblasts in intestinal villi are highly sensitive to mechanical stimulation and release ATP during touch or stretch and probably act as mechanosensors [60]. The ATP released activates $P2Y_1$ receptors on surrounding cells, which leads to intercellular propagation of Ca^{2+} waves and contractions in networks of subepithelial fibroblasts and a signal to sensory nerve terminals in the villi [61]. Intrinsic enteric sensory nerves express $P2X_3$ and $P2X_{2/3}$ receptors [62-66]. In $P2X_2$ or $P2X_3$ knock-out mice, intraluminal pressure-induced peristalsis is inhibited [65,66].

ATP release and $P2X_3$ and $P2X_{2/3}$ receptor-mediated nociceptive sensory nerve responses were enhanced in a model of colitis consisting of administration to adult rats of an intrarectal enema of 30% trinitro benzene sulfonic acid in ethanol at a dose of 80 mg/kg body weight [67]. An increase in the number of DRG neurons supplying the colorectum expressing $P2X_3$ receptors was also claimed and there was also a substantial increase in release of ATP with distension (Figure 4b). The excitability of visceral afferent nerves is enhanced following injury or ischemia and during inflammation, for example, in irritable bowel syndrome (IBS) [68]. 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 [69,70]). Chronic functional visceral hyperalgesia induced in a rat model for IBS, induced by colonic injection of 0.5% acetic acid, is associated with potentiation of ATP-evoked responses and an enhanced expression of $P2X_3$ receptors in colon-specific sensory neurons [71]. In addition, activation of spinal A_1 receptors with adenosine, following

breakdown of ATP, has been shown to modulate visceral hyperalgesia [72].

Non-erosive reflux disease shows the classic symptoms of gastro-oesophageal reflux, but in the absence of oesophageal mucosal injury. Visceral hypersensitivity plays an important role in the pathology of this disease [73]. ATP has been found to sensitise vagal afferents to mechanical stimuli in the ferret oesophagus [54] and the protein expression of P2X$_3$ receptors is increased in nodose and DRG with chronic oesophageal acid exposure in a rat model [74].

Lung

In the lung, pulmonary neuroepithelial bodies (NEBs) and more recently subepithelial receptor-like endings associated with smooth muscle (SMARs) [75] have been shown to serve as sensory organs in the lung, and P2X$_3$and P2X$_{2/3}$ receptors are expressed on a subpopulation of vagal sensory fibres that supply NEBs and SMARs with their origin in the nodose ganglia. 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 [76]. NEBs are oxygen sensors especially in early development, before the carotid system has matured [77]. 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 [78]. Both the TRPV1 receptor and P2X receptors mediate the sensory transduction of pulmonary reactive oxygen species, especially H$_2$O$_2$ and OH, by capsaicin-sensitive vagal lung afferent fibres [79].

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 [80]. Th1 and Th2 cytokines reciprocally regulate P2X$_7$ receptor function, suggesting a role for P2X$_7$ receptors in pulmonary diseases, particularly lung hypersensitivity associated with chronic inflammatory responses [81].

Uterus

It has been hypothesised that tissue stress or damage in the uterine cervix during late pregnancy and parturition leads to ATP release and sensory signalling via P2X receptors [82]. In support of this proposal, these authors have shown P2X$_3$ receptor immunoreactivity in axons in the cervix, in small and medium sized neurons in L6/S1 DRG and in lamina II of the L6/S1 spinal cord segments and increases in P2X$_3$ receptor expression between pregnancy day 10 and parturition (day 22/23) in the rat cervix, although not in DRG or spinal cord.

Tooth pulp

P2X$_3$ and P2X$_{2/3}$ receptors on sensory afferents in tooth pulp appear to mediate nociception [83-86], perhaps from ATP released by mechanical distension or inflammation of odontoblasts. Mustard oil application to the tooth pulp in anaesthetised rats produced long-lasting central sensitisation, reflected by increases in neuronal mechanoreceptive field size; TNP-ATP reversibly attenuated the mustard oil sensitisation for more than 15 minutes [87].

Tongue

P2X$_3$ receptors are abundantly present on sensory nerve terminals in the tongue [88] and ATP and α,β-meATP have been shown to excite trigeminal lingual nerve terminals in an *in vitro* preparation of intra-arterially perfused rat mimicking nociceptive responses to noxious mechanical stimulation and high temperature [89]. A purinergic mechanosensory transduction mechanism for the initiation of pain was considered. Taste sensations appear to be mediated both by P2Y$_1$ receptor-activated impulses in sensory fibres in the chorda tympani [90] and by P2X$_2$ and P2X$_3$ and, perhaps, P2X$_{2/3}$ receptors [91].

Potential Therapeutic Strategies

The search is on for selective P2X$_3$ and P2X$_{2/3}$ receptor antagonists that are orally bioavailable and do not degrade *in vivo* for the treatment of pain (see [92-96]).

Table 1: P2X$_3$ and P2X$_{2/3}$ receptor antagonists

Antagonist	P2X$_3$	P2X2/3
Suramin and analogues NF449, NF110	√	√
PPADS and derivatives MRS2159 & MRS2257	√√	√
Reactive blue 2 and derivatives TNP-ATP	√	√
A-317491 (selective)	√√√	√√√
Phenol red	√√√	√√√
Tetramethylpyrazine	√√	-
RO4 (orally bioavailable, stable *in vivo*)	√√	?
Ip$_5$I	√√	√√
βγcarboxymethylene ATP	√√	-
βγchlorophosphnomethylene ATP	?	√√
	?	√√

From [93,95,112,113]

Table 1 summarises the drugs widely available. Suramin, PPADS and Reactive blue 2 have been used as non-selective antagonists at $P2X_3$ and $P2X_{2/3}$ receptors on nociceptive sensory nerve endings. PPADS has the advantage that it associates and dissociates approximately 100 to 10,000 times more slowly than other known antagonists [97]. The trinitrophenyl-substituted nucleotide, TNP-ATP, is a very potent antagonist at both $P2X_3$ and $P2X_{2/3}$ receptors. A-317491 (synthesised by Abbott Laboratories) and compound RO3 (synthesised by Roche Palo Alto) are both effective $P2X_3$ and $P2X_{2/3}$ antagonists, the latter being orally bioavailable and stable *in vivo*. Antagonism of $P2X_1$ and $P2X_3$ receptors by phenol red has been reported and tetramethylpyrazine, a traditional Chinese medicine, used as an analgesic for dysmenorrhoea, was claimed to block $P2X_3$ receptor signalling [98].

Antisense oligonucleotides have been used to down-regulate the $P2X_3$ receptor, and in models of neuropathic (partial sciatic nerve ligation) and inflammatory (complete Freund's adjuvant pain, inhibition of the development of mechanical hyperalgesia as well as significant reversal of established hyperalgesia, were observed within 2 days of treatment [99-101]. $P2X_3$ antisense oligonucleotides or antagonists appear to be less effective for treating discogenic (lumbar intervertebral disc) than cutaneous tissue pain [102]. Combined antisense and RNA interference-mediated treatment for specific inhibition of the recombinant rat $P2X_3$ receptor appears to be promising for pain therapy [103]. $P2X_3$ double-stranded short interfering RNA relieves chronic neuropathic pain and opens up new avenues for therapeutic pain strategies in man [104].

While $P2X_3$ and $P2X_{2/3}$ receptors, expressed in sensory neurons, were the predominant P2 receptor subtypes first recognised to be involved in the initiation of nociception (see [105,106]), it has become apparent more recently that P2Y receptors are also present [107] and that these are involved in modulation of pain transmission [108]. P2Y receptors appear to potentiate pain induced by chemical or physical stimuli via capsaicin sensitive TRPV1 channels and it has been proposed that the functional interaction between $P2Y_2$ receptors and TRPV1 channels in nociceptors could underlie ATP-induced inflammatory pain [45]. $P2Y_1$ receptor-mediated responses also enhance the sensitivity of TRPV1-mediated responses to capsaicin, protons and temperature in a protein kinase C-dependent manner [109]. ATP-induced hyperalgesia was abolished in mice lacking TRPV1 receptors.

It has been claimed that opioids inhibit purinergic nociception in rat sensory neurons and fibres via a G protein-dependent mechanism [110]. Cannabinoids act as inhibitory modulators of nociceptive responses produced by $P2X_{2/3}$ receptors [111].

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 [93,94]). Other therapeutic approaches to pain are being considered, including the development of agents that control the expression of receptors and those that enhance ATP breakdown. 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 inhibit ATP release, another useful way forward as a therapeutic strategy.

Conclusion
Compelling evidence has been presented for the role of purinergic mechanosensory transduction where ATP, released from epithelial cells lining the bladder, ureter and gut during distension, acts on $P2X_3$ and/or $P2X_{2/3}$ receptors on subepithelial sensory nerve terminals to relay nociceptive messages via sensory ganglia and spinal cord to pain centres in the CNS.

Antagonists to $P2X_3$ and $P2X_{2/3}$ receptors are being explored to treat visceral pain and the possibilities for development of agents that inhibit ATP transport from epithelial cells or enhance ATP breakdown after its release are discussed.

Competing interests
The author declares that he has no competing interests.

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