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Activation of ureter nociceptors by exogenous and endogenous ATP in guinea pig

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Abstract

This study was conducted to determine whether adenosine 5′-triphosphate (ATP) contributes to nociceptor activity induced by ureter distension. Multifibre recordings of ureter afferents were made using the guinea pig ureter preparation perfused in vitro. Distension of the ureter resulted in an initial rapid and later maintained increase in afferent nerve discharge. Intraluminal application of ATP (10–1000 μM, 0.1 ml/min for 3 min) or α,β-meATP (10–1000 μM) mimicked these increases in afferent activity. The afferent responses consisted of fast and slow components. Both agonists caused a sensitisation of the afferents to ureter distensions. TNP-ATP (30 μm), a $P2X_3$ receptor antagonist, and the non-specific P2 antagonist, PPADS (100 μm), blocked the rapid and reduced the slower response to ATP. The remaining responses were blocked by the selective A1 receptor antagonist, DPCPX. TNP-ATP and PPADS reduced distension-induced afferent activity. The selective ecto-ATPase inhibitor, ARL-67156 (100, 200 μM) and suramin (100, 200 μM), an ecto-nucleotidase inhibitor as well as a P2 receptor antagonist, produced an increase in baseline and distension-induced discharge. These results indicate that the ureter epithelium may tonically (at rest) as well as phasically (on distension) release ATP, which stimulates afferent terminals by interacting with multiple P2 and P1 receptors.

Keywords: ATP; P2X receptors; Ureter; Nociceptors

1. Introduction

Pain and discomfort are frequent complaints of patients suffering from disorders of visceral organs such as the stomach, the gall bladder, the gut and the urinary tract. Pain arises from activation of nociceptive primary afferents innervating these organs. Visceral nociceptors are small diameter A_δ and C fibres sensitive to mechanical distension, ischemia and inflammation (Janig and Morrison, 1986). Importantly, it has been established that sensitisation of visceral nociceptors by various inflammatory and non-inflammatory mediators contributes to visceral hyperalgesia (Al-Chaer and Traub, 2002).

Whilst the anatomical arrangement of the innervation of visceral organs is relatively homogenous, functional differences predominate in different visceral organs. For example, in the urinary bladder, activation of low threshold mechanosensitive afferent fibres is needed to generate micturition, whereas in the ureter, the transport of urine is via peristaltic contractions that does not require a functional innervation (Amann, 1993). Thus, low threshold mechanosensitive fibres constituted over 70% of primary afferents from the bladder and only 20% of afferents were high threshold fibres (Sengupta and Gebhart, 1994). In contrast, over 90% of ureter afferents in guinea pigs were high threshold fibres for detecting noxious stimuli (Cervero and Sann, 1989; Sann, 1998). This indicates that the ureter might be a useful model to study visceral nociception.

There is a growing body of evidence that ATP is involved in nociceptive processes. It has been proposed that the underlying mechanism is purinergic mechanosensory transduction (Burnstock, 1999), where ATP

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released from epithelial cells acts on homomeric $P2X_3$ and heteromeric $P2X_{2/3}$ receptors on subepithelial sensory nerve terminals (Burnstock, 2001). ATP has also been shown to activate visceral primary afferents such as those innervating the intestine (Kirkup et al., 1999; Wynn et al., 2003). In an in vitro mouse urinary bladder/pelvic nerve preparation, we demonstrated that distension of the urinary bladder results in a pressure-dependent release of ATP, and that ATP and α,β-methyleneATP (α,β-meATP) can activate and sensitise both low and high threshold bladder afferent fibres (Vlaskovska et al., 2001; Rong et al., 2002). In contrast, P2X antagonists such as suramin, PPADS and TNP-ATP were shown to inhibit distension-induced bladder afferent discharge (Vlaskovska et al., 2001; Rong et al., 2002; Namasivayam et al., 1999). Furthermore, Cockayne et al. (2000) demonstrated that $P2X_3$ knockout mice had a hyporeflexic urinary bladder. These observations indicate that ATP released by epithelial cells in response to distension contributes to both innocuous (physiological) and nociceptive mechanosensory transduction through interactions with $P2X_3$ (and/or $P2X_{2/3}$) receptors on nerve terminals in the subepithelial layer of the urinary bladder.

The ureteric colic induced by the passage of a kidney stone causes severe pain. Arguably, the impact of the stone on the ureter wall and an increase in intraluminal pressure proximal to the site of impact cause pain. However, little is known about the transduction mechanisms leading to the activation of ureter nociceptors. Recently, Knight et al. (2002) demonstrated that distension of the guinea pig ureter resulted in ATP release from the epithelium in a pressure-dependent manner. The aim of the current study is to determine further whether ATP contributes to the excitation of nociceptors induced by ureter distension.

2. Methods

2.1. Ureter/afferent nerve preparation

A modified ureter/afferent nerve preparation as described by Cervero and Sann (1989) and Sann (1998) was used in this study. Briefly, 18 female guinea pigs weighing between 200 and 400 g were humanely killed by exposure to rising concentrations of CO_2, according to UK Home Office regulations covering Schedule 1 procedures. The ureters with associated hypogastric nerves and the inferior mesenteric ganglion were dissected and cut about 5 mm from the junctions with the renal pelvis and the bladder. One ureter was transferred to a recording chamber and was superfused with oxygenated Krebs (contents in mM: NaCl 120; KCl 5.9; NaH_2PO_4 1.2; $MgSO_4$ 1.2; $NaHCO_3$ 15.4; $CaCl_2$ 2.5; glucose 11.5). Chamber temperature was kept at 34 °C. Both proximal and distal ends of the ureter were catheterised for intraluminal perfusion (0.1 ml/min), application of drugs, distension and for monitoring intraluminal pressure. The other ureter was placed in oxygenated Krebs and was stored at 4 °C until use.

A small branch of the hypogastric nerve or a small nerve arising from the inferior mesenteric ganglion was carefully dissected and recordings were made from it with a suction electrode connected to a headstage (NL-100, Digitimer, UK). The signal was differentially amplified (NL104, Digitimer, UK) and filtered with a bandwidth of 200–3000 Hz. Nerve signals together with the pressure signal were recorded into a computer using the Spike 2 data capture and analysis program (Cambridge Electronic Design, UK).

2.2. Experimental protocols

After approximately a 60 min period of stabilisation, the ureter was distended with a reservoir to an intraluminal pressure of about 80 mmHg for 1 min. This was repeated at intervals of 10–15 min until a stable nerve response to distension was achieved (usually after 2–3 distensions). In pilot experiments involving five preparations, graded ramp distensions of the ureter (30 μl/min, up to 150 mmHg) were performed and we found that a pressure of greater than 30 mmHg was required to evoke ureteric afferent responses (e.g., Fig. 1A).

For chemical stimulation, ATP (0.01–1 mM) and α,β-meATP (0.01–1 mM) were applied intraluminally (0.1 ml/min) for 3–5 min and were then washed out with normal Krebs. Sufficient time was allowed for the recovery of nerve activity to baseline before the next

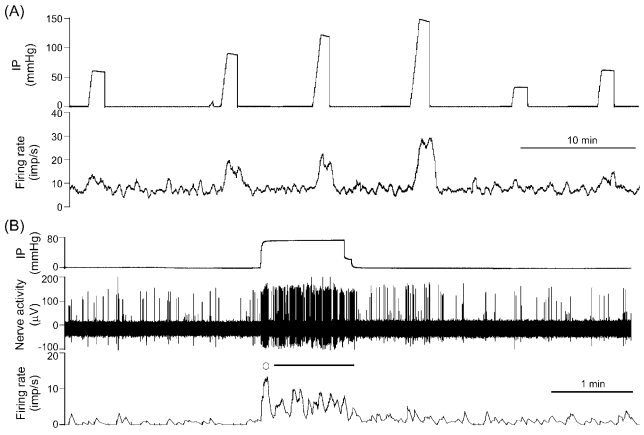

Fig. 1. Spontaneous and distension-induced activity in ureter afferent fibres. (A) Graded ramp distension (30 μl/min) of the ureter evokes pressure-dependent increases in multifibre afferent discharge. (B) Multifibre afferent responses to rapid distension. Note that background afferent activity occurs in bursts and that ureter distension results in an initial burst of discharge (circle) followed by a phase of maintained activity (bar). IP, intraluminal pressure.

challenge. In the studies with P2 and P1 antagonists, a control test with an effective concentration of an agonist (usually 0.1 mM for ATP, α,β-meATP and adenosine) was followed by treatment with the antagonist applied intraluminally for 10 min. Then the agonist was applied in the presence of the antagonist. Another test was performed after washing out the antagonist for 10 min.

To investigate the role of purinergic signalling in mechanosensory transduction, the responses of ureter afferents to distension were compared before and after application of P2 and P1 receptor antagonists. To achieve maximal blockade of P2 and P1 receptors, the antagonists were applied both intraluminally and into the bath. In some experiments, several increasing concentrations of antagonists were applied.

2.3. Data analysis

Nerve signals were fed into a Spike Processor (D130, Digitimer, UK) and were window discriminated. Spikes were counted and plotted as mean discharge frequency (imp/s) for every 10 s. Baseline level of nerve activity was obtained by averaging the mean discharge frequency over a 5 min period prior to a challenge. The effects of agonist or antagonist treatments on mechanosensory responses were quantified by comparing the total number of discharges per distension before and after the treatment. Values were expressed as mean ± SEM. Statistical analysis was performed using Student's *t*-test (where appropriate, paired *t*-test) with a *P* value of less than 0.05 indicating statistical significance.

2.4. Chemicals

ATP (sodium salt), α,β-meATP (lithium salt), adenosine, ARL-67156, pyridoxyl 5-phosphate 6-azophenyl-2′,4′-disulfonic acid (PPADS) and suramin (hexasodium salt) were all obtained from Sigma (Poole, UK) and were dissolved in water as 10 mM stock solution. 2′,3′-O-trinitrophenyl-ATP (TNP-ATP) was obtained from Molecular Probes (Leiden, Netherlands) as a 6.25 mM solution. DPCPX (Sigma, Poole, UK) was dissolved in DMSO as 10 mM stock solution. All chemicals were diluted in Krebs to required concentrations before use.

3. Results

3.1. Spontaneous and distension-induced afferent activity

A total of 46 multifibre preparations were studied. Consistent with previous reports (Cervero and Sann, 1989; Sann, 1998), ureter afferents exhibited spontaneous discharge that occurred in irregular bursts (Fig. 1). The level of spontaneous activity varied from preparation to preparation (ranging from 0.2 to 5.9 imp/s, mean 1.4 ± 0.3 imp/s), presumably due to variable numbers of active fibres in each recording. For each multifibre preparation, the level of spontaneous activity did not change significantly throughout the course of the experiment. Spontaneous contractions of the ureter were not observed, possibly due to the removal of the proximal ureter.

All nerve preparations reported here responded to a sustained pressure stimulus (80 mmHg, 1 min). This mechanosensory activity involved a burst of discharge that was followed by maintained activity (Fig. 1). The level of the maintained activity relative to the burst response showed considerable variation from preparation to preparation. After removal of the pressure stimuli, the level of nerve activity usually remained somewhat higher than the baseline level (after discharge). The after discharge lasted for 30–350 s. If unchallenged with other stimuli, the nerve responses to distension varied little for repeated trials over several hours. This suggests that the ureter preparation is stable and can be used as a model for investigating the molecular mechanisms underlying mechanosensory transduction and for examining the action of putative antagonists.

3.2. Responses of ureter afferents to ATP, αβ-meATP and adenosine

Intraluminal application of ATP (0.01–1 mM, 0.1 ml/min) produced rapid increases in afferent nerve discharge (latency 9–27 s) in a concentration-dependent manner. Three patterns of afferent responses were observed. In 5/16 preparations, the afferent responses consisted of a transient phase of intense discharge lasting 10–35 s, followed by a second phase of incrementing activity (Fig. 2A). In four preparations, ATP induced an increase in afferent activity, which reached a peak rapidly and then slowly decayed. In the other seven preparations, ATP caused an incrementing increase in afferent discharge. The nerve activity gradually returned to pre-treatment level following washing-out of the agonist. In some recordings, it was possible to distinguish the activity of several single units by using the spike-sorting function of the Spike2 software. Such analysis revealed that mechanosensitive units could be activated by ATP (Fig. 2B). Fig. 3 is a plot of the averaged change in afferent activity following intraluminal application of different concentrations of ATP in a total of 16 preparations.

ATP can interact with P2X and P2Y receptors. There is also evidence that ecto-nucleotidase activity is present in many tissues, which can rapidly break down ATP to ADP, AMP and adenosine. Thus, multiple

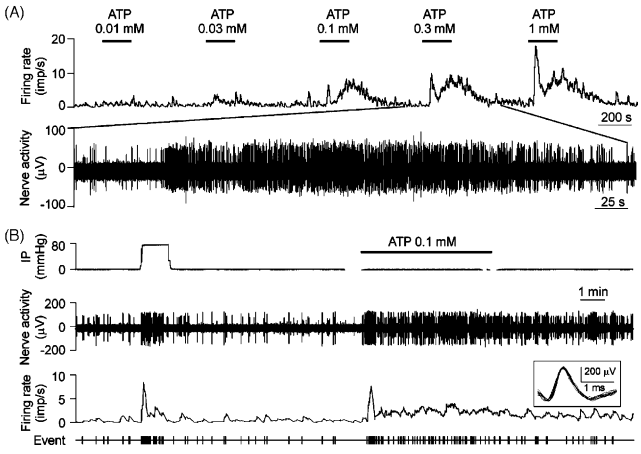

Fig. 2. Responses of ureter afferents to intraluminal application of ATP. (A) Typical biphasic (fast and slow) increases in afferent activity evoked by increasing concentrations of ATP applied intraluminally in a multifibre recording. The lower trace is an expanded view of the nerve activity in response to intraluminal ATP (0.3 mM); (B) Single unit analysis confirms ATP stimulates mechanosensitive afferent fibres. Intraluminal pressure (IP), multiunit activity and rate histogram of multiunits are shown. In inset are superimposed action potentials of a single unit which responded to ureter distension as well as ATP. The bottom trace (Event) shows when the single unit fired such that each line represents a spike.

receptors including P2X, P2Y and P1 (adenosine) receptors can potentially mediate the afferent responses to ATP. To characterise the receptors underlying the effects of ATP, α,β-meATP, a stable analogue of ATP selective for P2X receptors, was applied. In 7/16 preparations, α,β-meATP (0.01–1 mM) evoked a brief burst of activity lasting 10–45 s, followed by a second phase of maintained activity (Fig. 4A). In 5/16 preparations, α,β-meATP induced a slowly incrementing increase in nerve discharge. In the other four preparations, α,β-meATP up to 1 mM was without effect on the afferent activity, although these preparations all

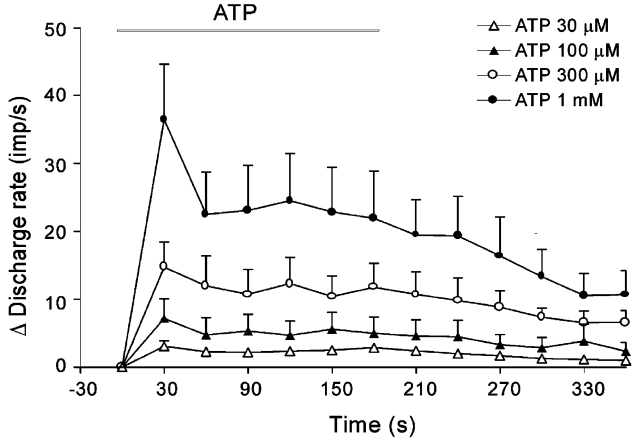

Fig. 3. Concentration-dependent changes in afferent activity following intraluminal application of ATP.

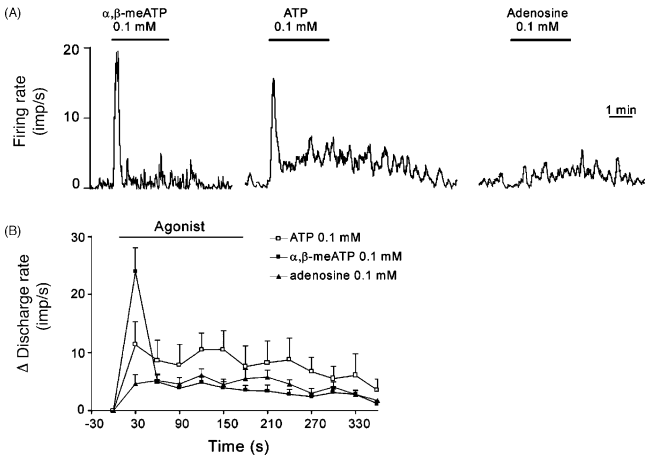

Fig. 4. Responses of ureter afferents to ATP analogues. (A), α,β-meATP (0.1 mM) evoked a prominent fast response followed by a slow increase in afferent discharge in a multifibre recording. Note that in the same preparation, ATP (0.1 mM) evoked a significant slow response as well as a fast response and adenosine (0.1 mM) induced a slow response only. (B) A comparison of the afferent responses following intraluminal administration of ATP (0.1 mM), α,β-meATP (0.1 mM) and adenosine (0.1 mM) in five preparations.

responded to ATP with slow maintained nerve discharges.

We then investigated whether the breakdown product of ATP, adenosine, contributed to the afferent activity in response to the agonist. Intraluminal application of adenosine (0.1 mM) resulted in a slowly incrementing increase in afferent discharge (e.g., Fig. 4A). We compared the effects of the same concentration of ATP, α,β-meATP and adenosine (0.1 mM) in five preparations (Fig. 4B). It was clear that adenosine could not solely account for the slow nerve response induced by ATP since the responses to adenosine were much smaller than those evoked by the same concentrations of ATP and because a small maintained response was present following the application of α,β-meATP, which does not breakdown to adenosine.

In addition to eliciting afferent discharges, ATP (and α,β-meATP) also resulted in sensitisation of ureter afferents. Thus, a greater response to ureter distension was elicited following application of ATP or α,β-meATP than the response prior to treatment with the agonists. The potentiation of distension-induced afferent nerve responses by ATP occurred in a concentration-dependent manner (Fig. 5A and B).

3.3. *Effects of P2 and P1 antagonists on afferent response to ATP analogues*

To obtain further evidence that P2X and P1 receptors mediate the afferent responses to ATP, we studied the effects of P2 and P1 receptor antagonists on the responses to ATP analogues. As illustrated in Fig. 6A,

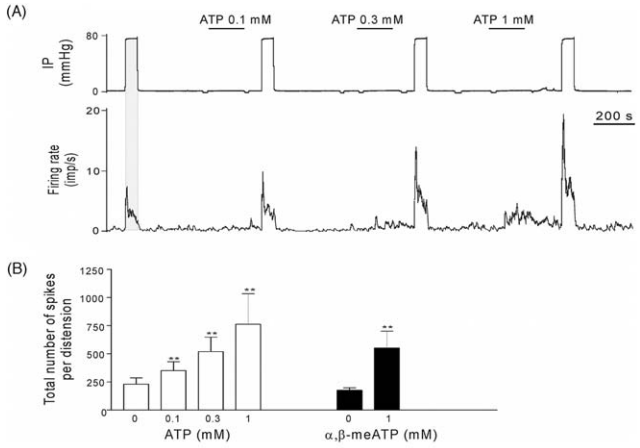

Fig. 5. ATP can sensitise ureter afferent fibres. (A) An example representative of distension-induced afferent activity before and following intraluminal application of increasing concentrations of ATP; (B) averaged number of spikes per distension (i.e., the total number of impulses in the shaded area) before and following intraluminal application of ATP ($n = 6$ for each data set) and α,β-meATP ($n = 5$). $^{**}P < 0.01$.

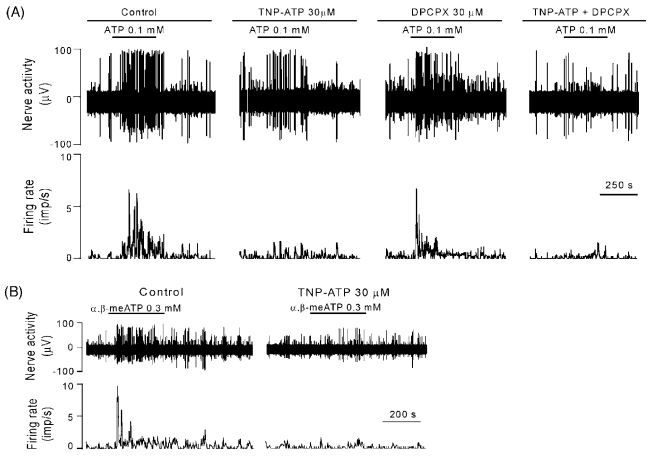

Fig. 6. Effects of P2 and P1 receptor antagonists on the afferent responses to ATP. (A) Multifibre recordings to show afferent nerve responses to ATP in control and in the presence of TNP-ATP or DPCPX or both antagonists; (B) representative traces to show afferent nerve responses to α,β-meATP in control and in the presence of TNP-ATP.

TNP-ATP (30 μM), a $P2X_1$, $P2X_3$ and $P2X_{2/3}$ selective antagonist (Lewis et al., 1998) completely blocked the initial phase of afferent activity and substantially reduced the slow phase of nerve discharge induced by ATP (0.1 mM). In contrast, co-application of TNP-ATP and DPCPX (30 μM), an A1 receptor antagonist, was able to completely block the ATP-evoked effects. DPCPX alone did not affect the initial phase of nerve discharge and partially blocked the slower responses to ATP. As shown in Fig. 6B, the effects of α,β-meATP were completely blocked by TNP-ATP.

Pretreatment with PPADS (100 μM) also prevented the fast response and reduced the slow response induced by ATP. Suramin, however, produced variable results. In two of five preparations, suramin (100 μM) resulted in a reduction in the slow afferent responses evoked by ATP. In the other three preparations, ATP evoked a larger increase in nerve discharge following application of suramin.

3.4. Effects of P2 antagonists on distension-induced afferent responses

The above observations demonstrate that exogenous ATP can stimulate and sensitise ureter nociceptors and suggest that the effects of ATP were mediated by $P2X_3$, $P2X_{2/3}$ and P1 receptors. We then explored the possibility that endogenous ATP contributes to distension-induced afferent activity by observing the effects of P2X antagonists on the nerve response to ureter distension. To maximally block the P2X receptors, TNP-ATP was applied both intraluminally and into the bath. At concentrations below 1 μM, TNP-ATP had little effect on the afferent responses to distension. With increasing concentrations, however, TNP-ATP caused an inhibition of the afferent activity. At 30 μM, TNP-ATP reduced spontaneous activity (from 1.7 ± 0.5 to 0.8 ± 0.2 imp/s, $n = 6$, $P < 0.05$), indicating there was a tonic action of ATP on afferent terminals. In addition, TNP-ATP led to a reduction in the afferent responses to distension. These effects of TNP-ATP were concentration-dependent (Fig. 7A and B). At 30 μM, TNP-ATP resulted in a 36.7% reduction in the mechanosensory responses ($n = 5$, $P < 0.01$). In one

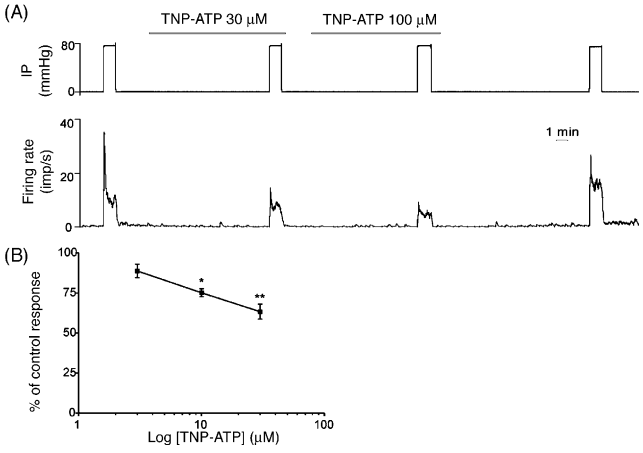

Fig. 7. TNP-ATP inhibits distension-induced afferent activity. (A) A multifibre recording to show distension-induced afferent activity in control and in the presence of TNP-ATP; (B) summary data from five multifibre preparations show that TNP-ATP inhibits distension-induced afferent activity in a concentration-dependent manner (the total numbers of discharge per distension are compared, see Fig. 5). One hundred micromolar TNP-ATP was used in one experiment only and is not included in the statistical analysis. $^{*}P < 0.05$; $^{**}P < 0.01$.

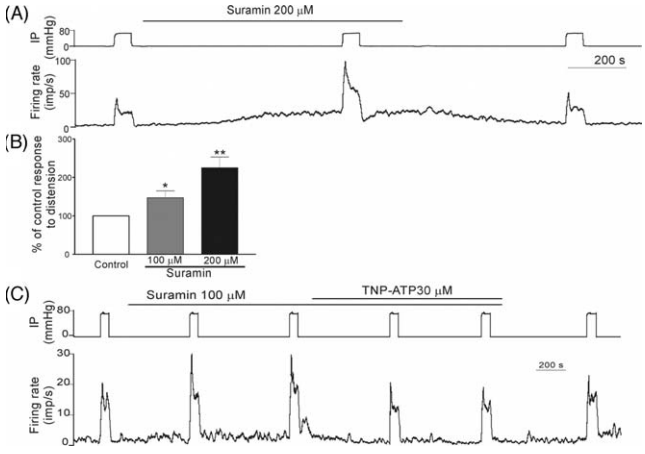

Fig. 8. The effects of suramin on distension-induced afferent activity. (A) A representative example of the changes in baseline and distension-induced afferent nerve activity following application of suramin (0.2 mM); (B) accumulative data from five multifibre preparations show that suramin potentiates distension-induced afferent responses; (C) typical example of TNP-ATP inhibiting the potentiating effects of suramin on distension-induced afferent nerve responses. $^{*}P < 0.05$; $^{**}P < 0.01$.

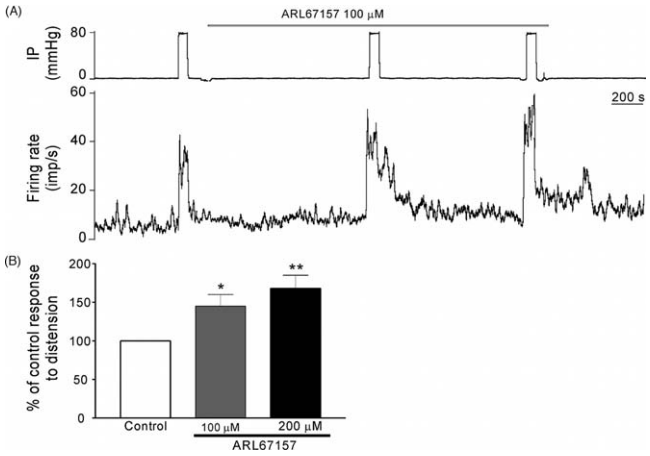

Fig. 9. Effects of ARL on distension-induced afferent nerve responses. (A) A representative example of the changes in distension-induced afferent responses following treatment with ARL67156 (100 μM); (B) accumulated data from four preparations show that ARL67156 augments afferent nerve responses to distension. $^{*}P < 0.05$; $^{**}P < 0.01$.

preparation (see Fig. 7A), 100 μM TNP-ATP was applied and it more than halved the distension-induced afferent activity.

In three nerve preparations, we investigated the effects of PPADS (100 μM) on the mechanosensory responses of ureter afferents. There was a slight decrease in the baseline nerve activity following application of PPADS. PPADS caused a 10.3%, 17.8% and 32.6% (mean $20.3 \pm 6.6\%$) reduction in the afferent responses to ureter distension.

We also tested the effects of suramin, another P2 receptor antagonist, on the mechanosensory responses. Following application of suramin, there was a gradual increase in nerve discharge as well as a potentiation of the responses to ureter distension (Fig. 8A). These effects of suramin appeared to be concentration-dependent (Fig. 8B) and could be reversed by TNP-ATP (30 μM, Fig. 8C) and PPADS (100 μM, data not shown), suggesting that the effects were mediated by P2X receptors.

Although the P1 antagonist, DPCPX, partially blocked the slow afferent responses to ATP, it did not have significant effects on the distension-induced nerve discharges.

3.5. *Effects of ecto-nucleotidase inhibitor on spontaneous and mechanosensory afferent activity*

Two mechanisms could potentially explain the facilitating effects of suramin on ureter nociceptors, by blocking ecto-nucleotidase activity or by potentiating the effects of ATP on P2X receptors. We therefore explored the effects of blocking the breakdown of ATP on ureter afferents by using a selective ecto-nucleotidase inhibitor, ARL-67156 (Crack et al., 1995). Following application of ARL-67156, there was a slow increase in spontaneous nerve discharge and an increase in the responses to ureter distension (Fig. 9A and B).

4. Discussion

An in vitro ureter/afferent nerve preparation was previously used to study the mechano- and chemosensitivity of afferent fibres (Cervero and Sann, 1989; Sann, 1998). They demonstrated two populations of mechanosensitive afferents: low threshold mechanoreceptors (U-1 units), which were activated by contractions of the ureter and constituted a small minority of the sample(5/79); and high threshold mechanoreceptors (U-2 units, 74/79) that did not respond to contractions and had threshold to distension (39 mmHg) in the noxious range. Sann (1998) also analysed 22 multiunits and found that all multiunits did not respond to ureter contraction, suggesting that they mainly consisted of high threshold fibres.

In the present study, we investigated the possible role of purinergic signalling in mechanosensory transduction in ureter afferents employing a similar preparation. We made some minor modifications as compared to the preparation described previously (Cervero and Sann, 1989; Sann, 1998). We cut the proximal ureter about 5 mm from its junction with the renal pelvis and we never observed spontaneous ureter contraction (i.e., peristalsis). The ureter was constantly perfused intraluminally as well as superfused, since Cervero and

Sann (1989) reported evidence of sensitisation in preparations not perfused intraluminally. Afferent activity was recorded using suction electrodes. In our hands, suction recording gave greater signal/noise ratio and stability. To stimulate mechanosensitive afferent fibres, the ureter was distended intermittently (10–15 min) with a pressure pulse of 80 mmHg (i.e., about twice the threshold of U-2 units) lasting 1 min. We found that the ureter afferents responded to repeated distensions robustly and consistently for several hours, allowing prolonged pharmacological observations.

In keeping with Sann's report (1998), we found that the ureter afferents exhibited some irregular spontaneous discharge and responded to ureter distension in the noxious range. We do not consider the baseline activity observed in this study as an indication of sensitisation of the ureter afferents in in vitro situations. Rather, having some level of spontaneous activity might be the intrinsic property of certain visceral afferents. Sann (1998) found that U-1 (low threshold) fibres had no spontaneous activity whereas U-2 (high threshold) fibres were spontaneously active. High levels of baseline discharge were also observed in jejunal afferents in vivo (Kirkup et al., 1999) as well as in vitro (unpublished observations), but not in urinary bladder afferents in vitro (Vlaskovska et al., 2001; Rong et al., 2002).

4.1. Multiple P2 and P1 receptors mediate the responses of ureter nociceptors to ATP

One of the major findings from the present study is that ATP elicited excitation and sensitisation of mechanosensory afferents supplying the ureter. Our observations indicate that the effects of ATP were mediated by multiple P2 and P1 receptors. Firstly, the responses to ATP could be differentiated into fast (a transient phase of intense discharge) and slow components. Secondly, α,β-meATP, a stable analogue of ATP and a selective $P2X_1$, $P2X_3$ and $P2X_{2/3}$ agonist, induced a marked fast and a less significant slow response as compared to ATP. Adenosine, on the other hand, only evoked a slow response. These indicate that both rapidly acting $P2X_1$ or $P2X_3$ and slowly desensitising $P2X_{2/3}$ receptors as well as P1 adenosine receptors were involved. This conclusion is supported by the observation that P2X receptor antagonists (TNP-ATP and PPADS) blocked the fast and part of the slow components and that combined use of P2X and A1 receptor antagonists (DPCPX) resulted in complete blockade of ATP (and α,β-meATP) effects.

In situ hybridisation studies have shown that $P2X_{1-6}$ receptor mRNAs are expressed in DRG neurons, suggesting that multiple P2X receptors may be expressed in primary sensory neurons (Dunn et al., 2001). However, extensive patch-clamping studies on cultured sensory neurons indicate that the effects of ATP were largely mediated by homomeric $P2X_2$, $P2X_3$ and heteromeric $P2X_{2/3}$ receptors (Dunn et al., 2001). Therefore, we presume that homomeric $P2X_3$ receptor and heteromeric $P2X_{2/3}$ receptors might be responsible for the fast and part of the slow nerve responses to ATP observed in this study, respectively. In support of this, immunoreactivity for $P2X_3$ and $P2X_2$ subunits has been demonstrated on terminal fibres in the subepithelial layer of the rat ureter (Lee et al., 2000).

The responses of ureter afferents to ATP were blocked by TNP-ATP and PPADS. In contrast, suramin had variable effects on the responses to ATP. In 3/5 experiments, suramin caused an increase of the afferent responses to ATP. Suramin is known to have many other pharmacological effects apart from its well-characterised non-selective actions on P2X receptors. Therefore, caution must be taken in interpreting the data. However, we favour two mechanisms that might explain the potentiating effects of suramin on ATP (and distension) responses. Firstly, by blocking ectonucleotidase activity, suramin resulted in more ATP accessing afferent terminals. Secondly, suramin might potentiate the interaction of ATP with the receptors involved. In this regard, guinea pig P2X receptors have not yet been fully characterised. However, there have been several reports suggesting differences in pharmacology of guinea pig P2X receptors as compared to P2X receptors in rat or mouse sensory neurons. In particular, suramin has been shown repeatedly to potentiate ATP responses in guinea pig myenteric and DRG neurons (Dunn et al., 2001).

4.2. Endogenous ATP contributes to spontaneous and mechanosensory afferent activity

Vesical and non-vesical release of ATP from various types of cells has been demonstrated (Bodin and Burnstock, 2001). Ferguson and co-workers (1997) first demonstrated that in urinary bladder, epithelial cells release ATP in response to small changes in hypostatic pressure. In this study we did not investigate the source of ATP. However, it has been shown convincingly by Knight et al. (2002) that ATP is released from the urothelium but not smooth muscle upon distension of the ureter. They showed no release of ATP with distension after mechanical removal of the urothelium. Similarly, Bodin et al. (1991) demonstrated that increased flow induced ATP release from vascular endothelial but not smooth muscle cells.

The idea that ATP contributes to mechanosensory transduction was formulated by Burnstock (1999), who proposed that in tube and sac organs (such as the urinary bladder, the gut and the ureter), epithelial cells release ATP in response to distension (stretch) and ATP in turn stimulates afferent terminals in the

subepithelial layer via interaction with P2X receptors. Evidence supporting this hypothesis has been accumulating (Wynn et al., 2003; Vlaskovska et al., 2001; Namasivayam et al., 1999; Cockayne et al., 2000; Knight et al., 2002; Lee et al., 2000). In the present study, we found that TNP-ATP and PPADS both reduced the distension-induced afferent activity, thus providing strong evidence that ATP released during distension is involved in mechanosensory transduction in guinea pig ureter via interactions with P2X receptors. Furthermore, TNP-ATP and PPADS also reduced the spontaneous activity in ureter afferents, suggesting that the urothelium might tonically release ATP, which interacts with afferent terminals to elicit spontaneous discharges. We also found that inhibiting ecto-nucleotidase activity (by ARL67156 or suramin) resulted in increased spontaneous as well as distension-induced afferent activity. This raised the possibility that increased level of extracellular ATP (such as due to inflammation and reduced ecto-nucleotidase activity) might contribute to visceral nociception and hyperalgesia.

One criticism to the present observations might be that high concentrations of TNP-ATP were used so that its effects could be non-specific. Previous whole-cell recording studies have shown that TNP-ATP potently inhibits recombinant and native $P2X_1$, $P2X_3$ and $P2X_{2/3}$ receptor-mediated effects with an IC_{50} in the nanomolar range. However, TNP-ATP up to 1 μM was not effective on ATP (or α,β-meATP or distension)-induced ureteric afferent responses, while at higher concentrations, it clearly inhibited the afferent responses to P2 agonists and to ureter distension. This is in keeping with other whole tissue or in vivo studies where a high dose of TNP-ATP was needed to inhibit P2X receptor-mediated effects (Lewis et al., 1998). Reduced potency of TNP-ATP in whole tissues might be due to its breakdown by ecto-nucleotidases and/or poor accessibility to its sites of actions. Nevertheless, we cannot exclude the possibility of non-specific actions of the high concentrations of TNP-ATP used in the present study.

Visceral pain is a major clinical challenge and current therapies for the relief of pain are not satisfactory. Often, the presentation of visceral or pelvic pain remains without explanation. A better understanding of the transduction mechanism underlying nociceptor activation should result in better strategies for tackling visceral or pelvic pain. The current study revealed that ATP could activate and sensitise ureter afferents. More importantly, P2X receptor antagonists such as TNP-ATP and PPADS, inhibited mechanosensory responses of ureter afferent fibres. Since the majority (>90%) of ureter afferents are high threshold fibres for detecting noxious stimulation (Cervero and Sann, 1989; Sann, 1998), the current findings suggest that ATP may act as a transduction molecule in signalling visceral pain. For example, the ureteric colic induced by a renal stone may result in the release of ATP through the impact of the stone on the ureter wall and the increased pressure proximal to the site of impact. The present study also raised the possibility that changes in purinergic signalling might explain some of the visceral pains with unidentified causes, such that increased level of extracellular ATP (due to changes in the tonic release, the breakdown and reuptake) might cause the pain.

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