Research Signpost
37/661 (2), Fort P.O., Trivandrum-695 023, Kerala, India

Aspects of Pharmacology of the Pyeloureter with Clinical Perspectives, 2009: 77-83
ISBN: 978-81-308-0328-9 Editors: Jens Mortensen, Frederik Andreasen and Ulf Simonsen

6 Purinoceptors in the upper urinary tract

Geoffrey Burnstock
Autonomic Neuroscience Centre, Royal Free and University College Medical School, Rowland Hill Street, London NW3 2PF, United Kingdom

Abstract

There is now abundant evidence for ATP acting as an extracellular signalling molecule. Receptors for ATP have been cloned and characterised, including seven P2X ionotropic and eight P2Y metabotropic receptor subtypes, as well as four subtypes of P1 receptors sensitive to adenosine, a breakdown product of ATP. P2 purinoceptor subtypes are expressed on ureteral smooth muscle, urothelium and blood vessels involved in fast signalling and probably also slower, trophic signalling during development and regeneration. ATP released from the urothelial

Correspondence/Reprint request: Prof. Geoffrey Burnstock, Autonomic Neuroscience Centre, Royal Free and University College Medical School, Rowland Hill Street, London NW3 2PF, United Kingdom
E-mail: g.burnstock@ucl.ac.uk

cells during distension of the ureter has been shown to act on $P2X_3$ receptors expressed on suburothelial sensory nerves, sending nociceptive messages via dorsal root ganglia to pain centres in the central nervous system. $P2X_3$ receptor antagonists are being explored for alleviating renal colic. ATP contracts while adenosine relaxes ureteric smooth muscle. $P2X_7$ receptors have been implicated in macrophage infiltration, collagen deposition and apoptosis in response to ureteral obstruction in mice.

Introduction

Purinergic signalling, where ATP released from nerves and from many non-neuronal cells, acts as an extracellular signalling molecule, is a rapidly expanding field (see [1]). Receptors for purines and pyrimidines have been cloned and characterised, including seven P2X ionotropic and eight P2Y metabotropic receptor subtypes, as well as four subtypes of P1 receptors sensitive to adenosine, a breakdown product of ATP (see [2,3]). There is widespread expression of these receptor subtypes on both neuronal and non-neuronal cells (see [4]).

The first evidence presented for the involvement of purines as neurotransmitters or neuromodulators in vesicoureteral reflex activity was described for the cat ureter [5]. ATP was shown to constrict the pig ureter, while intravesical adenosine via A_{2B} receptors on smooth muscle [6]. ATP and adenosine produced transient decreases in ureteral peristaltic frequency and in the spontaneous firing of the renal nerve. Theophylline blocked the effect of adenosine, but not ATP, so both P1 and P2 receptors are likely to be involved. P2 purinoceptors were first identified immunohistochemically in the ureter by Lee et al. [7]. The authors showed expression of $P2X_1$ receptors on smooth muscle membranes, $P2X_5$ and probably $P2X_7$ receptors on uroepithelium and $P2X_6$ receptors in the layer beneath the urothelium of the rat ureter. $P2X_3$ receptors were shown to be localised on subepithelial sensory nerves. In addition $P2X_1$, $P2X_2$, $P2X_4$ and $P2X_7$ receptors were localised on the smooth muscle of blood vessels in the rat ureter and by analogy with other visceral blood vessels it is likely that P2Y and $P2X_4$ receptors are expressed by vascular endothelial cells [4]. It is likely that some of the purinoceptors present in the ureter participate in long-term (trophic) events during development and regeneration, such as cell proliferation, migration, differentiation and cell death (see [8]).

Purinergic mechanosensory transduction and pain

Burnstock [9] proposed that in tubes (e.g. ureter, salivary duct, bile duct, vagina and intestine) and in 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. Evidence to support the mechanosensory role of ATP was first shown in the bladder [10,11]. $P2X_3$ receptor immunoreactivity was found on nerves in the suburothelial plexus in the rat bladder [7]. Increased electrical activity was recorded in the afferent pelvic nerves of rats during slow distension of the bladder and this activity was inhibited by desensitising the $P2X_3$ receptor with α, β-methylene ATP and also by receptor antagonism with suramin [12]. There was also reduced sensory nerve activity in $P2X_3$ receptor-deficient mice [13,14]. ATP was shown to be released during distension of the mouse bladder, proportionate to the degree of distension and corresponding to the activity of multi-fibre pelvic nerve afferents.

In a later study, it was shown that distension of the guinea-pig ureter increased spike discharge in sensory neurons, which was mimicked by ATP and reduced by ATP antagonists [15] (Figure 1). The afferent responses consisted of both fast and slow components. The P2 receptor antagonist trinitrophenyl-ATP (TNP-ATP) and pyridoxalphosphate-6-azonphenyl-2',4'-disulfonic acid (PPADS) reduced distension-induced afferent activity 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 (DPCPX). The ecto-ATPase inhibitor (ARL-67156) produced an increase in base-line and distension-induced sensory discharge.

Knight et al. [16] 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 (ABC) 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 2) and that human ureteric suburothelial sensory nerves express $P2X_3$ receptors [17].

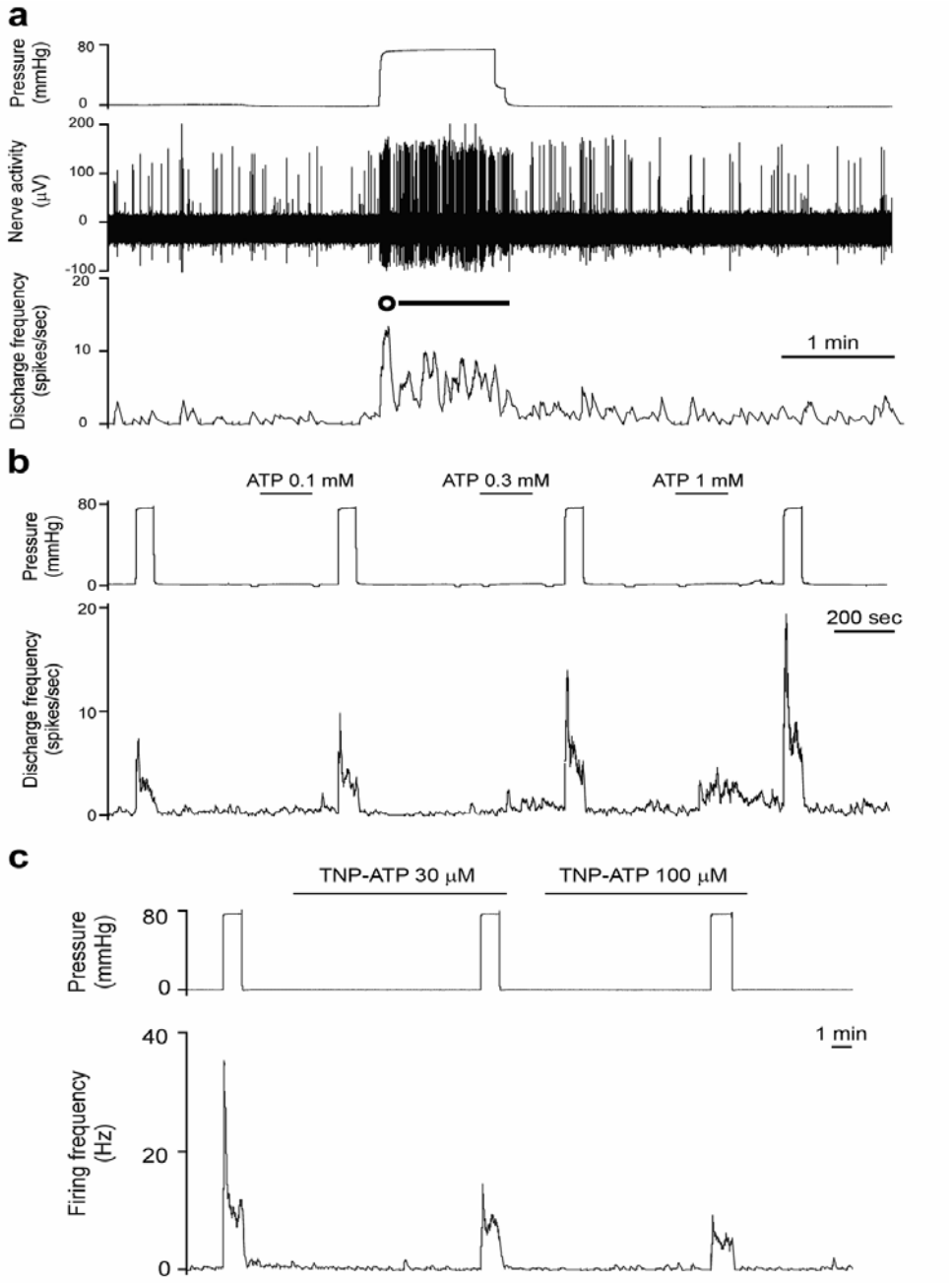

Figure 1. a. Spontaneous and distension-induced activity in ureter afferent fibres. Multifibre afferent responses to rapid distension. Note that background afferent activity occurs in bursts and that ureter distension results in an initial burst of discharge (circle) followed by a phase of maintained 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 [15] with permission of Elsevier.)

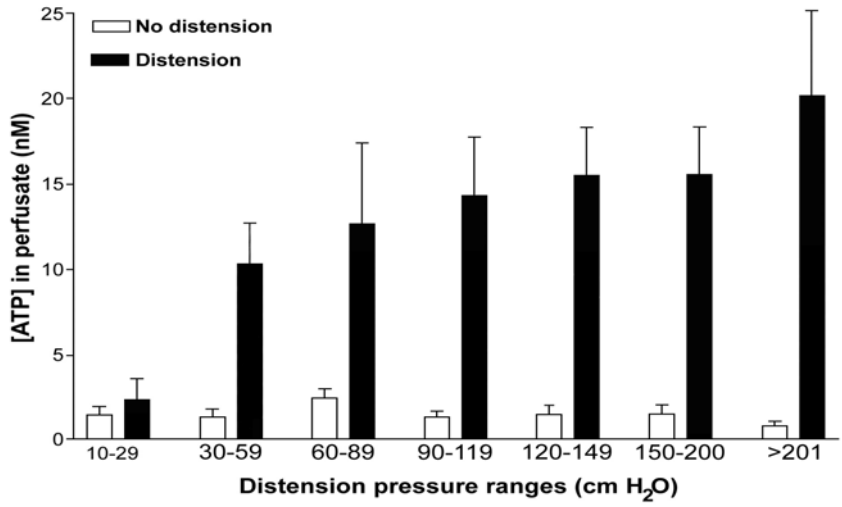

Figure 2. ATP concentration ([ATP]) in perfusate immediately before and after distension of the human ureter, grouped in pressure ranges. The mean [ATP] after distension is significantly greater than before distension in each pressure range $P<0.01$; n=7, error bars represent s.e.m. (reproduced from [17] with permission from Springer).

The release of ATP only occurred above a threshold of 25-30 cm H_2O. This is similar to the uroteric pressure threshold for pain measured by Risholm [18]. 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 [19].

$P2X_7$ receptors and ureteral inflammation and fibrosis

A study has been carried out to investigate the role of $P2X_7$ receptors in the inflammatory and fibrogenic responses of the kidneys to unilateral ureteral obstruction (UUO) by using $P2X_7$ knockout mice [20]. It was shown that 7 days after UUO of wild type (WT) mice there was increased expression of $P2X_7$ receptors associated with inflammation and fibrogenic responses in the cortex, although no positive cells were detected in the interstitium (Figure 3a). However, no $P2X_7$ receptor immunopositivity was seen after 14 days. $P2X_7$ receptor knockout mice did not exhibit the alterations seen in the WT mice. There were less macrophages in the interstitium, a lower population of myofibroblasts, diminished collagen deposition, as well as decreased transforming growth factor β (TGF-β) expression in the renal interstitium and less apoptotic cells. The authors suggest that there is a potential role for $P2X_7$ receptor antagonists to prevent renal interstitial fibrosis.

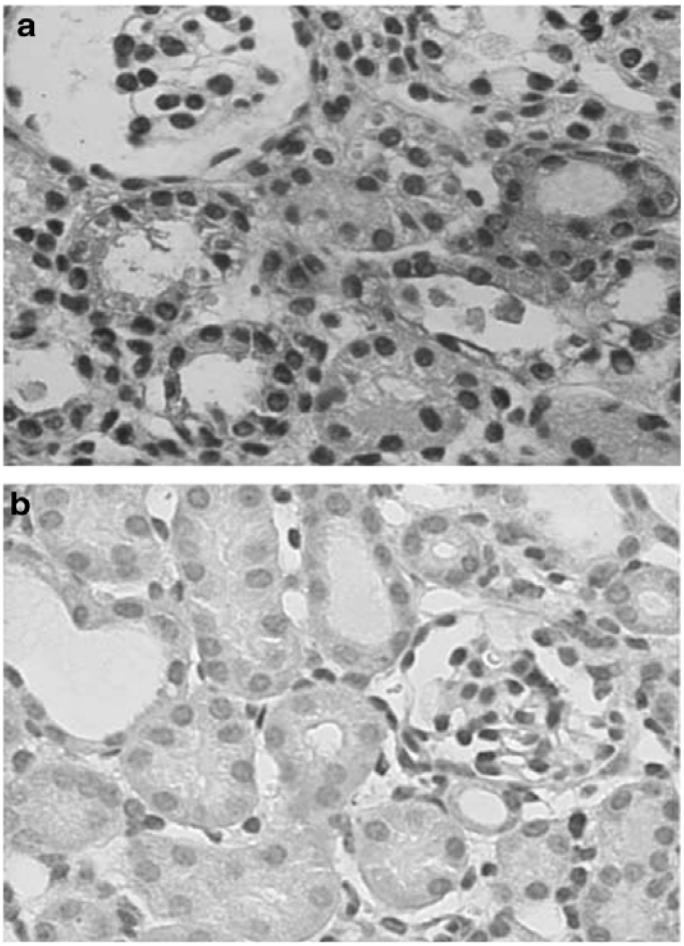

Figure 3. Representative pictures of immunohistochemical study for anti-$P2X_7$ receptor antibody. (a) $P2X_7$ receptor-positive tubular epithelial cells of the cortex of unilateral ureteral obstruction (UUO) WT mice at day 7 of obstruction. No positive cells were detected in the interstitium. (b) Kidney cortex of UUO WT mice at day 14, with no $P2X_7$ receptor-positive cells. (Reproduced from [20] with permission of Nature Publishing Group.)

References

1. Burnstock, G. 2007, Physiol. Rev., 87, 659.
2. Ralevic, V., and Burnstock, G. 1998, Pharmacol. Rev., 50, 413.
3. Burnstock, G. 2007, Cellular and Molecular Life Sciences, 64, 1471.
4. Burnstock, G., and Knight, G.E. 2004, Int. Rev. Cytol., 240, 31.
5. Theobald, R.J. 1986, Neurourol. Urodyn., 5, 493.
6. Hernández, M., Barahona, M.V., Bustamante, S., García-Sacrístan, A., and Orensanz, L.M. 1999, Br. J. Parmacol., 126, 969.
7. Lee, H.-Y., Bardini, M., and Burnstock, G. 2000, J. Urol., 163, 2002.
8. Abbraccho, M.P., and Burnstock, G. 1998, Jpn. J. Pharmacol., 78, 113.
9. Burnstock, G. 1999, J. Anat., 194, 335.

10. Ferguson, D.R., Kennedy, I., and Burton, T.J. 1997, J. Physiol., 505, 503.
11. Burnstock, G. 2001, Trends Pharmacol. Sci., 22, 182.
12. Namasivayam, S., Eardley, I., and Morrison, J.F. 1999, BJU Int., 84, 854.
13. Vlaskovska, M., Kasakov, L., Rong, W., Bodin, P., Bardini, M., Cockayne, D.A., Ford, A.P.D.W., and Burnstock, G. 2001, J. Neurosci, 21, 5670.
14. Cockayne, D.A., Hamilton, S.G., Zhu, Q.-M., Dunn, P.M., Zhong, Y., Novakovic, S., Malmberg, A.B., Cain, G., Berson, A., Kassotakis, L., Hedley, L., Lachnit, W.G., Burnstock, G., McMahon, S.B., and Ford, A.P.D.W. 2000, Nature, 407, 1011.
15. Rong, W., and Burnstock, G. 2004, Neuropharmacology, 47, 1093.
16. Knight, G.E., Bodin, P., de Groat, W.C., and Burnstock, G. 2002, Am. J. Physiol. Renal Physiol., 282, F281.
17. Calvert, R.C., Thompson, C.S., and Burnstock, G. 2008, Purinergic Signal., Epub ahead of print 26/9/08.
18. Risholm, L. 1954, Acta. Chir. Scand. Suppl., 184, 5.
19. Canda, A.E., Turna, B., Cinar, G.M., and Nazli, O. 2007, Urol. Int., 78, 289.
20. Gonçalves, R.G., Gabrich, L., Rosário, A., Takiya, C.M., Ferreira, M.L., Chiarini, L.B., Persechini, P.M., Coutinho-Silva, R., and Leite, M. 2006, Kidney Int., 70, 1599.