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Adenosine 5′-triphosphate (ATP) is an excitatory cotransmitter with noradrenaline to the smooth muscle of the rat prostate gland

*,[1]Sabatino Ventura, [2]Ramila K. Dewalagama & [2]Linda C.L. Lau

[1]Department of Pharmaceutical Biology and Pharmacology, Victorian College of Pharmacy, Monash University, 381 Royal Parade, Parkville, Victoria 3052, Australia; [2]Department of Pharmacology, Monash University, Clayton, Vic. 3800, Australia

1 This study investigated whether adenosine 5′-triphosphate (ATP) is involved in neurotransmission to the rat prostate gland.

2 Fluorescence immunohistochemistry carried out on formaldehyde-fixed and frozen sections of rat prostate showed immunoreactivity for the $P2X_1$-receptor in the fibromuscular stroma surrounding the secretory acini but not in the glandular epithelium. $P2X_2$-, $P2X_3$-, $P2X_4$- and $P2X_7$-receptors were immunonegative in the rat prostate stroma. Double-staining procedures showed $P2X_1$-receptor immunoreactivity to be colocalized with α-actin immunoreactivity.

3 Isolated organ bath studies investigated whether drugs, which modify purinergic mechanisms, are able to affect contractility of the rat prostate gland. Suramin (100 μM) and αβmethylene ATP (10 μM) inhibited contractile responses to trains of electrical-field stimulation (70 V, 0.5 ms, 0.1 – 2 Hz) in the absence and presence of prazosin (300 nM). Responses to 5 – 20 Hz were unaffected by suramin or αβmethylene ATP.

4 Exogenous application of ATP analogues to unstimulated isolated preparations of rat prostate produced concentration-dependent suramin (100 μM) sensitive transient contractions with a relative order of potency: αβmethylene ATP > βγmethylene ATP > ATP.

5 Adenosine and adenosine 5′-monophosphate (AMP) did not produce contractile responses.

6 These results suggest that $P2X_1$-receptors for ATP, which mediate contractions are present in the fibromuscular stroma of the rat prostate. The relative order of potency of ATP analogues in producing contractions of the rat prostate is consistent with the activation of $P2X_1$-receptors. Inhibition by suramin and αβmethylene ATP of electrically evoked nerve-mediated contractions of the rat prostate implies that ATP contributes to this contractile response and is therefore a cotransmitter with noradrenaline during low-frequency stimulation.
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Keywords: Prostate; neuromuscular transmission; P2X-receptors; adenosine 5′-triphosphate; ATP; smooth muscle; stroma; cotransmission; sympathetic nervous system

Abbreviations: AMP, adenosine 5′-monophosphate; ATP, adenosine 5′-triphosphate; FITC, fluorescein isothiocyanate; PBS, phosphate-buffered saline

Introduction

The prostate is a sympathetically innervated gland of the qmale genitourinary tract. It is well accepted that stimulation of the nerves innervating the prostate produces α_{1L}-adrenoceptor-mediated contraction of the prostatic smooth muscle of a number of species (Pennefather *et al*., 2000). Using functional studies with prazosin, previous studies in our laboratory showed that noradrenaline acting at α_1-adrenoceptors, was the major neurotransmitter mediating electrical-field-stimulation-induced contractions in the rat (Lau *et al*., 1998). At present, antagonists of α_1-adrenoceptors are used to relieve lower urinary tract symptoms caused by benign prostatic hyperplasia (Kirby, 1989; Eri & Tveter, 1995; Hieble & Ruffolo, 1996). Benign prostatic hyperplasia is associated with increased sympathetic tone to the prostate that puts added pressure on the urethra and bladder and is a major contributor to the lower urinary tract symptoms associated with this disease. α_1-Adrenoceptor antagonists relieve symptoms by relaxing prostatic smooth muscle by blocking stimulation of postjunctional α_1-adrenoceptors.

Adenosine 5′-triphosphate (ATP) is known to be a cotransmitter with noradrenaline in postganglionic sympathetic nerve fibres innervating a variety of tissues including those of the genitourinary tract and most blood vessels (Abbracchio & Burnstock, 1998). In the rat, ATP is a known cotransmitter with noradrenaline in the vas deferens (Khakh *et al*., 1995) and cauda epididymis (Ventura & Pennefather, 1991). Despite this, very little attempt has been made to investigate ATP mechanisms in the prostate gland. This is surprising, considering the presence or expression of P2 receptors (Fang *et al*., 1992; Janssens *et al*., 1996; Longhurst *et al*., 1996; Wasilenko *et al*., 1997) and of ecto 5′-nucleotidase (Konrad *et al*., 1998) that has been reported in human prostate.

*Author for correspondence;
E-mail: Sab.Ventura@vcp.monash.edu.au

In addition, ATP has been shown to increase inositol phosphates in rat prostatic cultures (Guijarro *et al*., 1996). P2X-receptors for ATP have also been demonstrated in the rat prostate (Lee *et al*., 2000; Slater *et al*., 2000). However, these studies produced conflicting results as to the subtype of these P2 receptors. The first study suggested that the P2-receptor present in the fibromuscular stroma of the rat prostate was of the $P2X_1$-receptor subtype (Lee *et al*., 2000), while the second study suggested the presence of $P2X_2$-receptors in the fibromuscular stroma of the rat prostate (Slater *et al*., 2000).

In a previous study from our laboratory, we have shown that adenosine and other breakdown products of ATP are able to inhibit nerve-mediated contractions of the rat prostate by stimulation of prejunctional adenosine receptors (Preston *et al*., 2000). This study failed to show any evidence of a neurotransmitter role for ATP in the rat prostate. Similarly, our earlier study showed that the P2-receptor antagonist suramin is unable to modify electrical-field-stimulation-induced contractions of the rat prostate (Lau *et al*., 1998).

This study aimed to clarify the P2-receptor subtype present in the fibromuscular stroma of the rat prostate using a combination of immunohistochemical and pharmacological methods. The study also aimed to further investigate a role of purine nucleotides in neurotransmission to the smooth muscle of the rat prostate gland.

Methods

Animals and tissues

Male Sprague – Dawley rats (250 – 300 g) were housed at 22°C and exposed to a photoperiod of 12 h light/12 h dark. Rats were allowed access to food and water *ad libitum*. Rats were killed by cervical dislocation. An abdominal incision was made, exposing the male urogenital tract and the left and right lobes of the prostate were removed providing two prostate preparations from each rat. Ethical approval was obtained from the Monash University Standing Committee of Animal Ethics in Animal Experimentation (Ethics number RSV1/2001).

Immunohistochemistry

The dissected prostate lobes from six rats were placed in a Petri dish containing phosphate-buffered saline (PBS; mM: NaCl 137.0, KCl 3.0, KH_2PO_4 2.0, Na_2HPO_4 8.0) and the prostatic capsule was removed along with any excess fat and connective tissue. Prostates were placed in a fixative solution containing 4% formaldehyde in PBS for 2 h at room temperature. For cryoprotection, the fixed prostates were then washed in PBS containing 7% sucrose and 0.01% sodium azide. Tissues were washed four times for 10 min each time and then stored in the same solution for 48 h at 4°C. Tissues were placed in a plastic mould and embedded in Tissue Tek. They were then snap frozen in liquid nitrogen and stored at −80°C. Frozen 12 μm sections were cut using a Leica CM1850 cryostat at −20°C and thawed onto gelatin-coated slides.

Slide-mounted sections were incubated for 18 – 20 h at room temperature with rabbit polyclonal antibodies for $P2X_1$-, $P2X_2$-, $P2X_4$- or $P2X_7$-receptors (Chemicon) or $P2X_3$-receptors (Oncogene), which were diluted 1 : 300 (determined as optimal by preliminary studies using a wide range of dilutions). Antibodies were diluted in a medium consisting of 0.1% w v^{-1} sodium azide, 0.01% w v^{-1} bovine serum albumin, 0.1% w v^{-1} lysine and 0.1% v v^{-1} Triton in PBS. For negative control studies, the primary antibody was excluded or replaced with preimmune serum. Tissues were then washed in PBS four times for 10 min each time, before incubation with fluorescein isothiocyanate (FITC)-anti-rabbit immunoglobulin (Vector) at a dilution of 1 : 250 in antibody diluting medium for 1 h at room temperature. Tissues were again washed four times in PBS for 10 min each time, and mounted in 'Vectashield' (Vector) and coverslipped. Prostate sections were then examined by use of an Olympus BX60 fluorescence microscope fitted with an Olympus U-MWB filter cube consisting of a DM500 dichroic filter, BP450-480 exciter filter and BA515 barrier filter. Micrographs were taken using an Olympus PM30 automatic photographic system and Kodak Ektachrome P1600 film.

Double labelling

In a further set of experiments, prostate sections from six rats were immunoprocessed for double labelling. In these studies, fixed-frozen slide-mounted sections of rat prostate were simultaneously incubated with the rabbit polyclonal antibody for $P2X_1$-receptors (1 : 300) and a mouse monoclonal antibody for actin (ICN) at a dilution of 1 : 250 for 18 – 20 h at room temperature. The primary antibody to actin was used to detect prostatic smooth muscle. Tissue sections were washed in PBS four times for 10 min each time before a 1 h incubation with FITC-conjugated anti-rabbit immunoglobulin (1 : 250) and Texas Red-conjugated anti-mouse immunoglobulin (Vector) (1 : 200) at room temperature. These secondary antibodies were raised in goat and horse, respectively. Tissues were washed in PBS four times every 10 min, then mounted in 'Vectashield', coverslipped and examined by use of an Olympus BX60 fluorescence microscope fitted with an Olympus U-MWB filter cube consisting of a DM500 dichroic filter, BP450 – 480 exciter filter and BA515 barrier filter to view FITC staining and an Olympus U-MWIY filter cube consisting of a DM600 dichroic filter, BP545 – 580 exciter filter and BA610IF barrier filter to view Texas Red staining. In order to prevent crossreaction with endogenous immunoglobulins in double-labelling experiments, primary and secondary antibody incubations were carried out in antibody-diluting medium containing 2% normal rat serum.

Isolated organ bath studies

The prostate lobes were placed in a Petri dish containing Krebs – Henseleit solution (mM: NaCl 118.1, KCl 4.69, KH_2PO_4 1.2, $NaHCO_3$ 25.0, glucose 11.7, $MgSO_4$ 0.5, $CaCl_2$ 2.5) and the prostatic capsule was removed along with excess fat and connective tissue. The isolated prostates were mounted in separate 10 ml organ baths. The organ baths contained Krebs – Henseleit solution, bubbled with 5% CO_2 in O_2 and maintained at 37°C. One end of the prostate was attached to an isometric Grass FT03 force – displacement transducer, which was connected to a PowerLab data acquisition system run on a Power Macintosh 5500/225 computer. The lower end of the preparation was attached to a perspex tissue holder

incorporating two parallel vertical platinum electrodes. Tissues were equilibrated for 60 min, under a resting force of 0.5 *g*. During the 60 min equilibration period, nerve terminals within the tissues were field stimulated using the electrodes connected to a Grass S88 stimulator to deliver trains of 0.5 ms pulse duration, 70 V, at 0.01 Hz. The bath medium was changed every 10 min because of the frothing that occurred in the organ bath as a result of spontaneous prostatic secretions (Lau *et al*., 1998).

To examine whether ATP contributed to the contractile responses elicited by nerve stimulation, frequency – response curves (0.5 ms pulse duration, 70 V, 0.1 – 20 Hz) to electrical-field stimulation were constructed using a frequency progression ratio of approximately a third of a log unit. Trains of pulses were delivered for 10 pulses at low frequencies ($\leqslant$1 Hz) and for 10 s at higher frequencies ($\geqslant$1 Hz). This protocol was used to obtain a true indication of the contractile response to each frequency. Trains of electrical-field stimulation were delivered at intervals of 10 min.

Frequency – response curves were conducted on pairs of prostate lobes from the same animal, in parallel. Frequency – response curves were carried out on one prostate lobe in the presence of tetrodotoxin (1 μM), guanethidine (10 μM), prazosin (300 nM), suramin (100 μM), $\alpha\beta$methylene ATP (10 μM) or combinations of prazosin (300 nM) and suramin (100 μM) or prazosin (300 nM) and $\alpha\beta$methylene ATP (10 μM). The frequency – response curve conducted on the other prostate lobe served as a contralateral internal control and was carried out in the absence of any drug. Each drug or combination of drugs was left in contact with the tissue for the entire 60 min equilibration period and throughout the frequency – response curve. Drugs were replaced following bath washes. Only one frequency – response curve was obtained from each tissue.

$\alpha\beta$methylene ATP (10 μM) initially elicited a contractile response. However, subsequent applications did not produce contractile responses. This implied that this protocol was sufficient to desensitize P2X-receptors in this tissue.

Effects of agonists

In a separate set of experiments, tissues were not stimulated but instead after the 1 h equilibration period, discrete concentration – response curves to adenosine, adenosine 5′-monophosphate (AMP), ATP, $\alpha\beta$methylene ATP and $\beta\gamma$methylene ATP were constructed. When the response to each concentration of agonist had reached a plateau, the tissue was washed and allowed 15 min to recover before the next concentration was applied. Only one concentration – response curve was obtained from each tissue and only one agonist was applied to any one tissue. A dose progression ratio of half a log unit was used in the construction of these concentration – response curves. Concentration – response curves from pairs of unstimulated preparations of rat prostates from the same animal were conducted in the presence and absence of suramin (100 μM). Suramin (100 μM) was added at the beginning of the equilibration period and readded after each washout. This protocol was conducted to examine the influence of adenine nucleosides and nucleotides on the contractility of the rat prostate.

Measurement and analysis of data

The peak force of electrical-field-stimulation- or agonist-induced contractions was used as a measure of the contractile response. Mean log frequency – response and concentration – response curves were constructed by pooling data from individual curves constructed from tissues from six rats. Results are expressed as the mean $\pm$ standard error of the mean (s.e.m). The value of *n* represents the number of animals used.

Graphs showing mean log frequency – response and concentration – response curves were constructed, using Graph Pad Prism (version 3.02). Frequency – response curves were analysed by one-way analysis of variance (ANOVA) at each frequency followed by *post hoc* Tukey – Kramer test for multiple comparisons. This was carried out to compare differences between treatment groups at all frequencies on the frequency – response curve. This test was carried out using Instat (version 3.0). $P<0.05$ was considered significant.

Concentration – response curves were analysed by two-way repeated measures ANOVA. This was carried out to compare differences between control and treatment groups at all concentrations on the concentration – response curve. This test was carried out using Graph Pad Prism (version 3.02). The *P*-values used to evaluate statistical significance were the probabilities of a significant interaction between dose and treatment and in all cases, $P<0.05$ was considered significant. Estimates of the differences in agonist potency and shifts caused by antagonists were made by determining the mean concentration of agonist that produced a contractile response of 0.1 *g* ($EC_{0.1}$). This was determined by nonlinear regression using Graph Pad Prism (version 3.02). Mean and 95% confidence limits of this value for each agonist was then determined. The $EC_{0.1}$ value was arbitrarily chosen in place of the more traditional EC_{50} value as concentration – response curves to ATP and $\beta\gamma$methylene ATP failed to reach a clear maximum response making it impossible to calculate a true EC_{50} value. The affinity of suramin for the receptor being stimulated was quantified by calculating an apparent K_B using the dose ratio of agonist concentration required to elicit a contractile response of 0.1 *g* in the presence or absence of suramin.

Drugs

The following drugs were used: adenosine (Sigma, St. Louis, U.S.A.), AMP (Sigma, St. Louis, U.S.A.), ATP (Sigma, St. Louis, U.S.A.), guanethidine (Sigma, St. Louis, U.S.A.), $\alpha\beta$methylene ATP (Sigma, St. Louis, U.S.A.), $\beta\gamma$methylene ATP (Sigma, St. Louis, U.S.A.), prazosin (Sigma, St. Louis, U.S.A.), suramin (Sigma, St. Louis, U.S.A.) and tetrodotoxin (ICN, Costa Mesa, U.S.A.).

Compounds were dissolved and diluted to the required concentrations in distilled water. All drugs were made up freshly on the morning of experimentation.

Results

Immunofluorescence localization of P2X-receptor subtypes

The distribution of P2X-receptor subtypes in the rat prostate was analysed by immunofluorescence and conventional

microscopy. In all studies, only background levels of immunofluorescence were observed when the primary antibody was replaced with preimmune serum or excluded from the diluting medium (not shown).

$P2X_1$-receptor immunoreactivity was absent from the prostatic epithelium, but was intensely distributed throughout the fibromuscular stroma of the rat prostate (Figure 1). Immunofluorescence was evenly distributed throughout the fibromuscular stroma, suggesting localization on the muscle rather than on innervating nerve fibres that are distinguished by a more varicose staining.

Immunoreactivity to $P2X_{2,3,4,7}$-receptors was not seen in prostatic epithelium or fibromuscular stroma (not shown).

Double labelling for $P2X_1$-receptors and actin

Simultaneous double immunostaining for $P2X_1$-receptors and smooth muscle actin (Figure 1) and superimposition of the resulting photographic images of the same sections taken with the two different filter cubes showed an impressive overlap and hence colocalization of the even $P2X_1$-receptor and actin staining. In double-labelling studies, the glandular epithelium was not fluorescent when observed with either filter cube. Consequently, the epithelium was considered immunonegative to both $P2X_1$-receptors and actin.

Isolated organ bath studies

Responses to electrical-field stimulation

Typical contractile responses to electrical-field stimulation (0.5 ms, 70 V, 0.1 – 20 Hz) of control isolated rat prostates and the effect on these contractile responses of suramin (100 μM), $\alpha\beta$methylene ATP (10 μM) and prazosin (0.3 μM) are shown in Figure 2. Contractile responses to electrical-field stimulation (0.5 ms, 70 V, 0.1 – 20 Hz) were frequency dependent and consistently returned to baseline after stimulation (Figure 2).

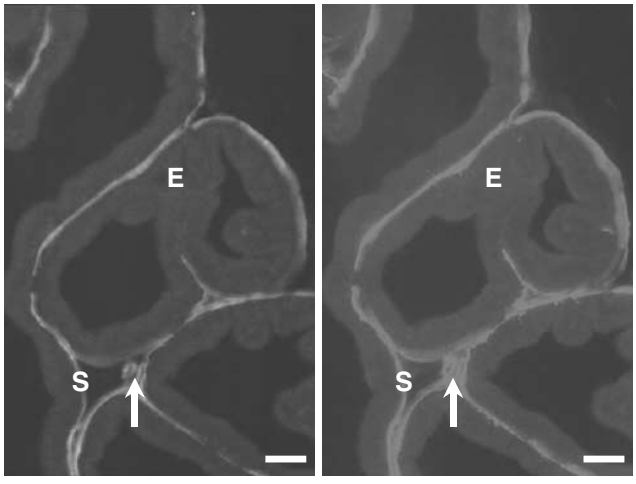

Figure 1 Representative photomicrographs showing microscopy of the same cross-section of rat prostate ($n=6$ rats) following double immunolabelling with rabbit polyclonal antibody to $P2X_1$-receptor (left panel) and mouse monoclonal antibody to actin (right panel). $P2X_1$-receptor and actin immunostaining (indicated by arrows) is colocalized in the fibromuscular stroma (S) between the glandular acini. The epithelium (E) that lines the lumen of the acini was immunonegative in both cases. Scale bar = 50 μM.

Responses to electrical-field stimulation (0.5 ms, 70 V, 0.1 – 20 Hz) were attenuated by tetrodotoxin (1 μM) and guanethidine (10 μM) (Figure 3) This attenuation was similar with both drugs and ranged from 100% inhibition at lower frequencies to only 53% at some of the higher frequencies (Figure 3).

Prazosin (0.3 μM) consistently attenuated responses to electrical-field stimulation by 30 – 50% at all frequencies tested (Figure 4). Increasing the concentration of prazosin had no further inhibitory effect on the contractile response to electrical-field stimulation.

Suramin (100 μM) attenuated responses to electrical-field stimulation by 45% at frequencies of 0.5 and 1 Hz (Figure 4; $P<0.05$, $n=6$). Contractile responses to electrical-field stimulation at all other frequencies were not different in the presence of suramin (100 μM) when compared to control (Figure 4, $P>0.05$, $n=6$). Furthermore, suramin (100 μM) further attenuated responses to electrical-field stimulation in the presence of prazosin at frequencies of 0.5, 1 and 2 Hz but not at the other frequencies tested (Figure 4). Increasing the concentration of suramin did not cause any further inhibition of electrical-field-stimulation-induced responses.

$\alpha\beta$Methylene ATP (10 μM) attenuated responses to electrical-field stimulation by 40 – 48% at frequencies of 0.1, 0.2, 0.5 and 1 Hz (Figure 4; $P<0.05$, $n=6$). Contractile responses to electrical-field stimulation at all other frequencies were not different in the presence of $\alpha\beta$methylene ATP (10 μM) when compared to control (Figure 4, $P>0.05$, $n=6$). Furthermore, $\alpha\beta$methylene ATP (10 μM) further attenuated responses to electrical-field stimulation in the presence of prazosin by 50 – 70% at all frequencies (Figure 4). Maximum attenuation (68.7%) of the contractile response to electrical-field stimulation in the presence of prazosin (0.3 μM) by $\alpha\beta$methylene ATP (10 μM) was seen at a frequency of 1 Hz (Figure 4).

P2-receptor classification

ATP, $\beta\gamma$methylene ATP and $\alpha\beta$methylene ATP applied exogenously, each produced concentration-dependent, transient contractions of isolated rat prostates (Figure 5). AMP and adenosine were inactive. $\alpha\beta$Methylene ATP produced a mean maximal response at a concentration of approximately 30 μM. The mean log concentration – response curves for each of the active agonists were parallel and are shown in Figure 6.

The order of potency of these purines in producing contractions of the rat prostate was: $\alpha\beta$methylene ATP > $\beta\gamma$ methylene ATP > ATP. The mean negative log $EC_{0.1}$ values determined from fitted regression lines and potencies relative to ATP are shown in Table 1. The contractions caused by these purine analogues could be attenuated by preincubation of the tissues in suramin (100 μM) (Figure 7; $n=6$, for each agonist). Calculated apparent K_B values for suramin at the receptor mediating these contractions are shown in Table 2.

Discussion

The results of this study indicate that $P2X_1$-receptors as well as α_1-adrenoceptors are present on the smooth muscle of the rat prostate. As with α_1-adrenoceptors, $P2X_1$-receptors appear to mediate excitatory effects, and both noradrenaline and ATP are released from sympathetic nerve terminals within the prostate gland in response to electrical-field stimulation.

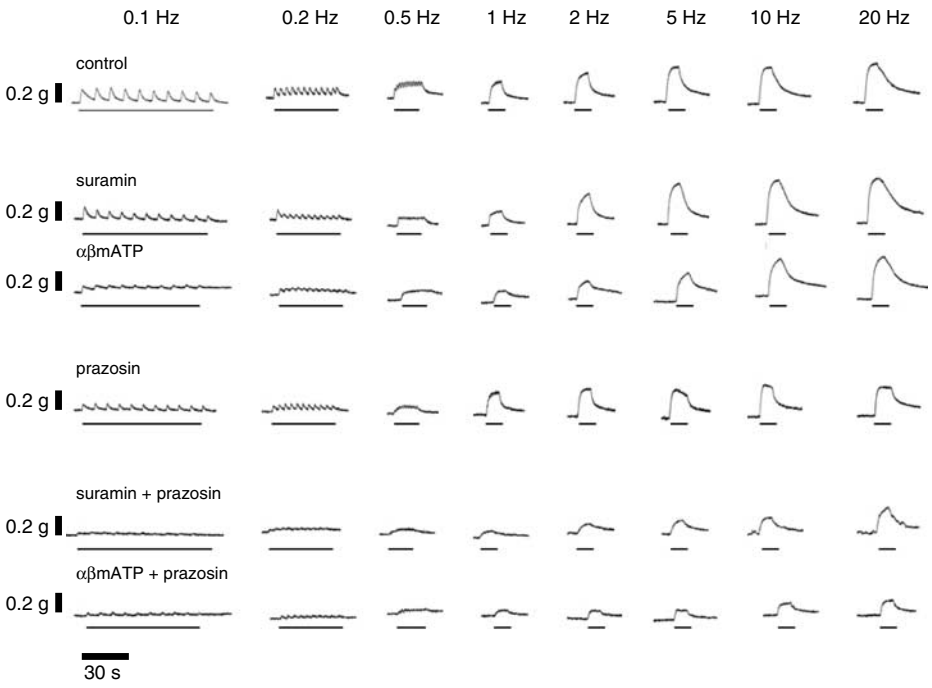

Figure 2 Representative traces showing the effects of suramin (100 μM), $\alpha\beta$methylene ATP (10 μM) and prazosin (300 nM) on responses to electrical-field-stimulation (—) (0.5 ms pulse duration, 70 V, 0.1 – 20 Hz for 10 pulses or 10 s)-induced contractions of isolated preparations of the rat prostate.

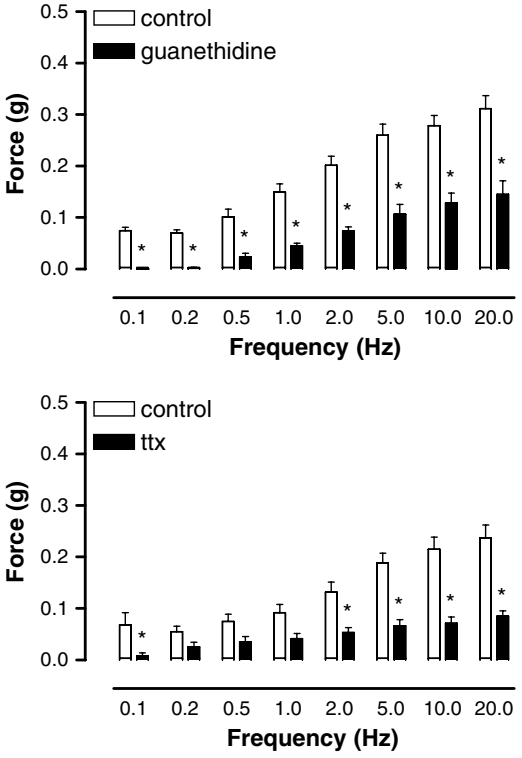

Figure 3 Mean contractile responses to electrical-field stimulation (0.1 – 20 Hz, 0.5 ms, 70 V for 10 pulses or 10 s) following administration of: (open bars) no drug or (closed bars) guanethidine (10 μM) (upper panel) or tetrodotoxin (1 μM) (lower panel). Each column represents the mean $\pm$ s.e.m. of six experiments. *Indicates a significant difference from corresponding control response (*$P<0.05$; ANOVA, followed by *post hoc* Tukey – Kramer correction).

$P2X_1$-receptors are the principle P2-receptors in smooth muscle from genitourinary tissues such as the rat vas deferens (Khakh *et al*., 1995), rat and guinea-pig bladder (Inoue & Brading, 1990; Bo & Burnstock, 1992) as well as from rat vascular smooth muscle (Bo & Burnstock, 1993; Evans & Kennedy, 1994). The function of $P2X_1$-receptors in these tissues is to mediate smooth muscle contraction. ATP is released from the nerve fibres innervating these tissues to act on these receptors. In the present study, we have also demonstrated the release of ATP in response to electrical-field stimulation, which is able to elicit contraction of the rat prostate gland. The possible significance of the role of ATP in prostatic function has been emphasized with reports of expression of P2-receptors (Fang *et al*., 1992; Janssens *et al*., 1996; Longhurst *et al*., 1996; Wasilenko *et al*., 1997) and of ecto 5′-nucleotidase (Konrad *et al*., 1998) in human prostate.

Previous studies, which have demonstrated P2X-receptors for ATP in the rat prostate (Lee *et al*., 2000; Slater *et al*., 2000), have produced conflicting results regarding the subtype of these P2-receptors. The immunohistochemical studies conducted in our study are in agreement with Lee *et al*. (2000) who also showed a predominance of the $P2X_1$-receptor subtype in the fibromuscular stroma of the rat prostate. Slater *et al*. (2000) reported a predominance of the $P2X_2$-receptor subtype in the stroma of the rat prostate, but we found no evidence of the presence of this P2-receptor subtype in the rat prostatic fibromuscular stroma in our study. Our immunohistochemical demonstration of the presence of a $P2X_1$-receptor subtype in the fibromuscular stroma of the rat prostate was supported further by its apparent colocalization with actin and our functional contractility studies.

Studies of the excitatory effects of the naturally occurring purines showed that ATP was able to elicit contractions of the

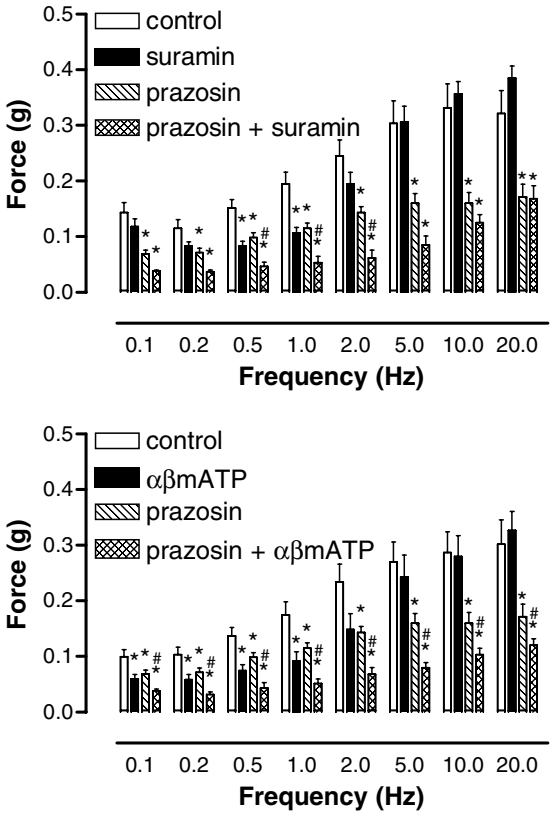

Figure 4 Mean contractile responses to electrical-field stimulation (0.1 – 20 Hz, 0.5 ms, 70 V for 10 pulses or 10 s) following administration of: (open bars) no drug, (closed bars) suramin (100 μM) (upper panel) or $\alpha\beta$methylene ATP (10 μM) (lower panel), (diagonally striped) prazosin (300 nM), (cross-hatched) suramin (100 μM) and prazosin (300 nM) (upper panel) or $\alpha\beta$methylene ATP (10 μM) and prazosin (300 nM) (lower panel). Each column represents the mean ± s.e.m. of six experiments. *Indicates a significant difference from control response (*$P<0.05$; ANOVA, followed by *post hoc* Tukey – Kramer correction). #Indicates a significant difference from response in the presence of prazosin (#$P<0.05$; ANOVA, followed by *post hoc* Tukey – Kramer correction).

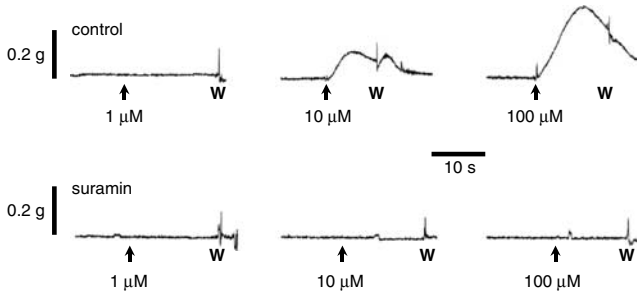

Figure 5 Representative traces showing the effects of $\beta\gamma$methylene ATP (1 – 100 μM) on unstimulated isolated preparations of rat prostate gland in the absence (upper panel) and presence (lower panel) of the P2-receptor antagonist suramin (100 μM). Arrows indicate administration of each concentration of $\beta\gamma$methylene ATP. W = washout.

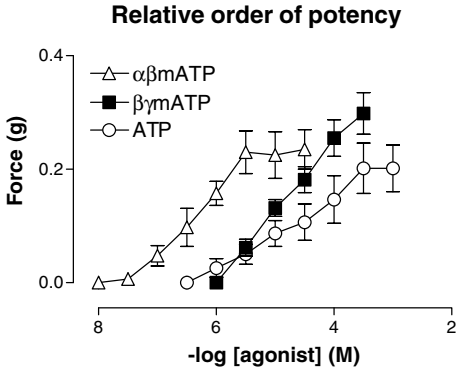

Figure 6 Mean log concentration – response curves for the excitatory effects of: ATP, $\beta\gamma$methylene ATP and $\alpha\beta$methylene ATP on unstimulated isolated rat prostatic preparations. Results are expressed as the mean peak force developed to each concentration of agonist. Each point represents the mean ± s.e.m. of six experiments.

rat prostate, while AMP and adenosine were inactive. This is consistent with actions at P2-receptors for ATP rather than adenosine receptors (Burnstock, 1978). The sensitivity of these contractile responses to suramin further implicates P2-receptors. The relative order of potency of ATP and its methyl phosphate isosteres in causing contraction of the tissue, namely, $\alpha\beta$methylene ATP > $\beta\gamma$methylene ATP > ATP is consistent with the original subclassification of the P2-receptors mediating these effects as the P2X-receptor subtype (Burnstock & Kennedy, 1985). The sensitivity and rapid desensitization to $\alpha\beta$methylene ATP in combination with suramin sensitivity is indicative of either a $P2X_1$- or $P2X_3$-receptor subtype (Humphrey *et al*., 1998). The greater potency of $\alpha\beta$methylene ATP when compared to ATP suggests that contractions are caused by the stimulation of $P2X_1$-receptors (Humphrey *et al*., 1998). In addition, our calculated mean apparent K_B values (2.5 – 5.4 μM) for suramin compare favourably with previously reported IC_{50} values (1 – 5 μM) at $P2X_1$-receptors (Humphrey *et al*., 1998).

Indeed, the relative potencies of these compounds on the prostate gland are similar to those observed previously by us in the rat cauda epididymis (Ventura & Pennefather, 1991). In that study, we found $\alpha\beta$methylene ATP to be 1150 times more potent than ATP. $\beta\gamma$Methylene ATP was approximately 17 times more potent than ATP. The differences in the magnitude of the potency ratios in the present study compared to those seen in our earlier study on the cauda epididymis may reflect between-tissue differences in the rate of catabolism of ATP.

Table 1 Mean negative log $EC_{0.1}$ values, potency ratios and mean maximum force developed at $P2X_1$-receptors on rat prostatic smooth muscle

Agonist	*−log $EC_{0.1}$ (M) (95% CI)*	*Potency ratio*[a]	*Mean maximum force developed (g; mean ± s.e.m.)*
$\alpha\beta$Methylene ATP	6.36 (6.12–6.60)	69	0.24 ± 0.04
$\beta\gamma$Methylene ATP	5.19 (2.70–7.68)	4.7	0.30 ± 0.04[b]
ATP	4.52 (3.58–5.46)	1	0.20 ± 0.04[b]

[a]Potency ratio = antilog ((neg log $EC_{0.1}$ value for analogue) − (neg log $EC_{0.1}$ value for ATP)). [b]Maximum contractile response for $\beta\gamma$methylene ATP and ATP was not reached; so maximum force developed is given as the force produced at the highest concentration used. $n=6$ animals for each agonist.

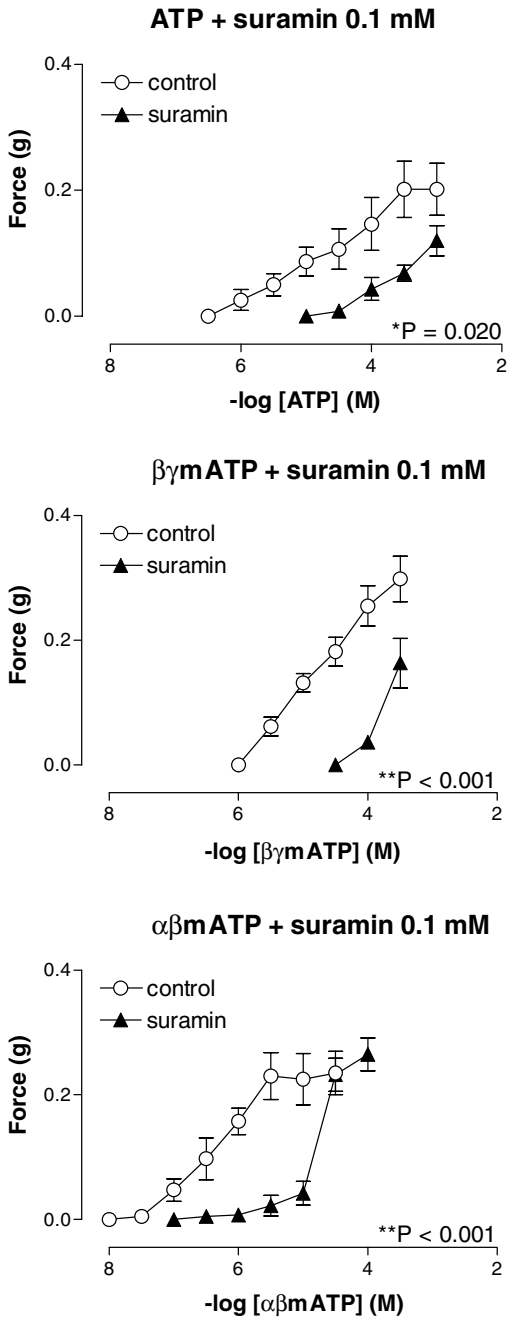

Figure 7 Mean log concentration – response curves for the excitatory effects of ATP (upper panel), $\beta\gamma$methylene ATP (centre panel) and $\alpha\beta$methylene ATP (lower panel) on unstimulated isolated rat prostatic preparations: in the presence and absence of suramin (100 μM). Results are expressed as the mean peak force developed to each concentration of agonist. Each point represents the mean $\pm$ s.e.m. of six experiments. *P*-values are for the concentration $\times$ treatment interaction of a repeated-measures ANOVA and represent the difference in the concentration – response curves in the absence and presence of suramin. Asterisks indicate a significant difference ($*P<0.05$; $**P<0.005$).

Investigations of the hypothesis that both noradrenaline and ATP are released from sympathetic nerve terminals within the rat vas deferens were prompted, in part, by observations that the responses of the tissue to electrical-field stimulation are biphasic. The fast component of the response was considered purinergic, whereas the slow component was considered noradrenergic (for review, see Ventura, 1998). In the present study, we observed that the contractions evoked by electrical-field stimulation of the prostate gland are monophasic and relatively slow or tonic. Nevertheless, studies using selective antagonists for α_1-adrenoceptors and P2 receptors were conducted to deduce whether as in the vas deferens, the electrical-field stimulation of preparations of rat prostate gland leads to the release of ATP as well as noradrenaline from sympathetic nerve terminals. Evidence that the electrical-field stimulation applied stimulated sympathetic nerve terminals was obtained in experiments using guanethidine that greatly attenuated stimulation-induced contractions. $\alpha\beta$Methylene ATP or suramin both partially inhibited the contractile response to electrical-field stimulation at low frequencies (<2 Hz). Moreover, the effects of prazosin and $\alpha\beta$methylene ATP or suramin were additive. These findings taken together suggest that electrical-field stimulation of preparations of the rat prostate leads to the release of ATP as well as noradrenaline from sympathetic nerve terminals within the tissue.

$\alpha\beta$Methylene ATP attenuated the electrical-field-stimulation-induced contractions of the rat prostate at more frequencies than was observed with suramin. This was seen both in the absence and presence of prazosin and demonstrates that $\alpha\beta$methylene ATP has a greater efficacy than suramin at inhibiting the effects mediated by P2X-receptors. The blocking effects of both $\alpha\beta$methylene ATP and suramin were more readily seen and seen at a greater number of different frequencies in the presence of prazosin, suggesting that removal of the noradrenergic component of contraction helps unmask the contribution of ATP to the nerve-mediated contraction of the rat prostate. In addition, the frequency-dependent nature of the sensitivity of the electrical-field-stimulation-induced contractions to $\alpha\beta$methylene ATP and suramin suggest that ATP release is proportionately greater at lower frequency stimulation. These phenomena may be the reason why we did not see a contribution of ATP to electrical-field-stimulation-induced contractions of the rat prostate in our earlier study, which used only one stimulation frequency of 10 Hz (Lau *et al*., 1998). This frequency was much greater than the frequencies in which we saw an effect of $\alpha\beta$methylene ATP and suramin in the absence of prazosin in the present study.

The residual response to electrical stimulation in the presence of $\alpha\beta$methylene ATP and prazosin or suramin and prazosin is probably not because of another neurotransmitter. The most likely explanation for the residual response is direct smooth muscle stimulation since tetrodotoxin was unable to abolish responses to electrical stimulation. Similarly, a

Table 2 Mean apparent $K_B \pm$ s.e.m values for suramin at $P2X_1$-receptors mediating contraction of rat prostatic smooth muscle

Agonist	*Mean apparent $K_B \pm$ s.e.m (μM)*[a]
$\alpha\beta$Methylene ATP	2.58 ± 0.18
$\beta\gamma$Methylene ATP	5.42 ± 0.22
ATP	2.58 ± 0.12

[a]Apparent K_B = [molar concentration of suramin (100 μM)]/((ratio of concentrations of agonist producing contractile response of 0.1 *g* in the presence and absence of suramin (100 μM))–1). $n=6$ animals for each agonist.

contribution of nonsympathetic nerves is unlikely since the guanethidine-resistant residual response to electrical stimulation was similar in magnitude to the residual responses to electrical-field stimulation in the presence of tetrodotoxin. Inspection of Figures 3 and 4 show that the residual responses to electrical field stimulation in the presence of prazosin and suramin or prazosin and $\alpha\beta$methylene ATP are similar in magnitude to the residual responses seen in the presence of guanethidine and tetrodotoxin. This suggests that a combination of prazosin and suramin or prazosin and $\alpha\beta$methylene ATP is capable of inhibiting the neurogenic component of the contractile response to electrical-field stimulation.

In conclusion, the major findings of this study are that electrical stimulation of the nerve terminals present in the preparations of the rat prostate gland leads to contractions that can be inhibited by either $\alpha\beta$methylene ATP, suramin or prazosin, indicating that both ATP and noradrenaline act as cotransmitters. Secondly, we have confirmed that the smooth muscle of this tissue contains postjunctional P2X-receptors of the $P2X_1$-receptor subtype.

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