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Adenosine 5′ -triphosphate and noradrenaline are excitatory cotransmitters to the fibromuscular stroma of the guinea pig prostate gland

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Abstract

Immunohistochemical studies demonstrated abundant $P2X_1$-receptor immunoreactivity colocalized with α-actin within the fibromuscular stroma of the guinea pig prostate. $P2X_2$-, $P2X_3$- and $P2X_4$-receptor immunoreactivity was absent. αβmethylene Adenosine 5′ -triphosphate (ATP) attenuated contractile responses to electrical field stimulation (50 V, 0.5 ms, 5–20 Hz) in the absence and presence of prazosin (0.3 μM). Responses to 1–2 Hz were unaffected. ARL 67156 (6-*N*, *N*-Diethyl-β-γ-dibromomethylene-D-adenosine-5-triphosphate; 100 μM) enhanced contractile responses to electrical field stimulation (50 V, 0.5 ms, 10–20 Hz). Concentration–response curves to exogenously applied ATP analogues on unstimulated preparations elicited concentration-dependent suramin (100 μM)-sensitive contractions. The rank order of potency was: αβmethylene ATP>2methylthio ATP=βγmethylene ATP>adenosine 5′ -diphosphate (ADP)=ATP. Adenosine and adenosine 5′ -monophosphate (AMP) did not produce contractile responses. These results demonstrate the presence of functional $P2X_1$-receptors within the fibromuscular stroma of the guinea pig prostate and suggest a cotransmitter role for ATP with noradrenaline during high-frequency stimulation.

Keywords: Adenosine 5′ -triphosphate; Fibromuscular stroma; Neuromuscular cotransmission; Prostate; P2X-receptor; Smooth muscle

1. Introduction

The prostate is predominantly a sympathetically innervated male accessory gland which produces and expels complex secretions into the male genitourinary tract during ejaculation. Benign prostatic hyperplasia is a result of androgen-dependent growth in glandular and stromal elements of the gland as well as an increase in sympathetic tone to the prostatic smooth muscle (Oesterling, 1995). In humans, the prostate surrounds the urethra just beneath the bladder so that the development of benign prostatic hyperplasia results in lower urinary tract symptoms.

Although the pathogenesis of benign prostatic hyperplasia remains largely unknown, it is clear that two clinically distinct components exist. A static component is associated with hypertrophy of the stroma and epithelium and results in mechanical obstruction reflected by symptoms of straining, hesitancy, incomplete emptying, intermittency and terminal dribbling (Barry and Roehrborn, 1997). Conversely, a dynamic component involves an increase in smooth muscle tone due to alterations in neural control (Lepor et al., 1996) and results in irritative symptoms of the disease (frequency, urgency and nocturia; Barry and Roehrborn, 1997).

Current treatments for benign prostatic hyperplasia involve surgical intervention (invasive) or pharmacotherapy (noninvasive) to provide symptomatic relief for sufferers of benign prostatic hyperplasia. The most successful class of drugs used to combat benign prostatic hyperplasia in humans are the α_1-adrenoceptor antagonists such as terazosin and tamsulosin (Barry and Roehrborn, 1997). These drugs block postjunctional α_1-adrenoceptors thereby

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reducing smooth muscle tone and alleviating urinary obstruction. However, they have little effect in relieving the storage (irritative) symptoms of benign prostatic hyperplasia. Adverse side effects associated with this line of treatment persist due to the presence of these receptors in the vascular system (Andersson et al., 1997).

A substantial amount of research on the prostate has been conducted in various animal model species such as the rat. However, guinea pigs and humans have a similar ratio of prostatic smooth muscle to epithelium, making the guinea pig prostate a good model for human prostate function. Furthermore, their prostates exhibit similar age-related ultrastructural changes (Horsfall et al., 1994). Moreover, hormonal responses and alterations in the epithelium of the guinea pig prostate closely resemble that which occurs in the prostate of ageing humans (Lau et al., 1998). This suggests that the guinea pig prostate provides a more suitable animal model for human comparison than the rat prostate.

Lau et al. (1998) reported a significant decrease in electrically evoked contractile responses following the addition of prazosin to rat and guinea pig prostates. This suggests that neurotransmission to the prostatic stroma in both species is primarily noradrenergic in nature, and that released noradrenaline acts via α_1-adrenoceptors to mediate this effect. Furthermore, Haynes and Hill (1997) demonstrated that guinea pig prostatic preparations contracted in response to the α_1-adrenoceptor agonist phenylephrine. More recent investigations revealed that it is the α_{1L}-adrenoceptor subtype, a variant or low-affinity state of the α_{1A}-adrenoceptor, which mediates noradrenaline-induced contractile responses in the guinea pig prostate (Pennefather et al., 1999).

A number of studies have shown that residual contractile responses to trains of electrical field stimulation persist in the guinea pig prostate in the presence of prazosin, suggesting that another neurotransmitter may contribute to the contractile response (Haynes and Hill, 1997; Lau et al., 1998). Attenuation of electrical field stimulation-induced contractions in the guinea pig prostate in the presence of suramin has been demonstrated (Haynes and Hill, 1997; Lau et al., 1998), implicating adenosine 5′ -triphosphate (ATP) in neuromuscular transmission. The relevance of such findings involving ATP to the human prostate is reinforced by the presence of ecto 5′ -nucleotidase, an enzyme responsible for the catabolism of ATP (Konrad et al., 1998), and the expression of P2 receptors for ATP (Janssens et al., 1996; Longhurst et al., 1996; Wasilenko et al., 1997) in the human prostate. It is well established that noradrenaline and ATP are co-released from sympathetic nerve terminals to induce contraction in a number of genitourinary tissues and blood vessels (Abbrachio and Burnstock, 1998; Dunn, 2000) and we have recently shown ATP to be an excitatory cotransmitter with noradrenaline in the rat prostate (Ventura et al., 2003).

Despite this, it is important to investigate the actions of ATP and its receptors in the guinea pig prostate. A principal objective of this study was to identify the receptors for ATP within the fibromuscular stroma of the guinea pig prostate gland using immunohistochemical techniques. Isolated organ bath studies were used to examine whether ATP contributes to the neurogenic/contractile response in the guinea pig prostate and to identify the subtype of P2X-receptors which mediate contractions.

2. Methods

2.1. Animals and tissues

Adult Tricolor Monash strain male guinea pigs (0.5–1 kg) were housed at 22 °C with a photoperiod of 12-h light–dark. Guinea pigs had free access to water and a high-energy pellet diet ad libitum. Lucerne chaff (a vitamin C supplement) was also provided on a daily basis. Guinea pigs were killed by cervical dislocation and exsanguination. A lower abdominal incision revealed the male urogenital tract. The left and right lobes of the prostate were removed from beneath the seminal vesicles and urethra; hence, each guinea pig provided two preparations of entire lobes. The mass of prostatic strips used was in the range of 0.1–0.4 g. The Standing Committee of Ethics in Animal Experimentation of Monash University provided ethical approval for the completion of these experiments. Ethics number: RSV1/2001.

2.2. Immunohistochemistry

Immunohistochemical studies used the same protocols as those previously described by us (Ventura et al., 2003). Primary antibodies to $P2X_1$-, $P2X_2$-, $P2X_4$- and $P2X_7$-receptors were purchased from Chemicon and used at a dilution of 1:400. Antibodies to the $P2X_3$-receptor subtype were purchased from Oncogene and used at a dilution of 1:400.

2.2.1. Double labelling

Immunohistochemical studies used the same protocols as those previously described by us (Ventura et al., 2003). Rabbit polyclonal antibody for $P2X_1$-receptors was obtained from Chemicon and used at a dilution of 1:300. Mouse monoclonal antibody for actin was purchased from ICN and used at a dilution of 1:250.

2.3. Isolated organ bath studies

The left and right lobes of the prostate were removed along with the vas deferens, seminal vesicles, urethra and bladder. These structures were placed in a petri dish containing Krebs–Henseleit solution of the following

composition: 118.1 mM NaCl, 4.69 mM KCl, 1.2 mM KH_2PO_4, 25.0 mM $NaHCO_3$, 11.7 mM glucose, 0.5 mM $MgSO_4$, 2.5 mM $CaCl_2$. The prostate was dissected out with the careful removal of the surrounding structures and any excess fat and connective tissue. Each guinea pig provided two prostate preparations. Guinea pig prostates are spongy masses of tissue whose smooth muscle has no specific directional orientation. Thus, prostatic smooth muscle contractions can be measured in any direction. Tissues were mounted in 10 ml organ baths containing Krebs–Henseleit solution, maintained at 37 °C and bubbled with 5% CO_2 in O_2. One end of the tissue was fixed to a perspex tissue holder and the other to a Grass FTO3C-transducer for recording of isometric contractions. Force developed was recorded with a PowerLab data acquisition system (Chart 3.6) run on a Power Macintosh 5500/225 computer. Preparations were equilibrated for a period of 1 h under a resting tension of 0.5–1.0 g. During this time, the bathing solution was regularly replaced every 10 min to prevent the accumulation of prostatic secretions.

Prostate tissue was stimulated via two parallel platinum electrodes incorporated in the tissue holder. These electrodes were attached to a Grass S88 stimulator. During the equilibration period, prostates were electrically field stimulated at 0.01 Hz, 0.5 ms duration, 50 V to allow continuous monitoring of tissue viability.

To examine whether ATP contributes to nerve-mediated contractile responses, frequency–response curves (0.5 ms duration, 50 V, 1–20 Hz) to electrical field stimulation were constructed. Trains of pulses were delivered for 10 s (1–20 Hz) allowing for an accurate representation of the contractile response to each frequency. Trains of electrical field stimulation were delivered at intervals of 10 min to allow for adequate tissue recovery between stimulations throughout the course of experimentation.

Frequency–response curves were constructed on paired tissues. One hour later, a second frequency–response curve was constructed with one tissue being treated with a single drug or combination of drugs while the tissue from the contralateral control side served as a time control and received no drug treatment. The following drug treatments were tested: tetrodotoxin (1 μM), atropine (10 μM), αβmethylene ATP (10 μM), prazosin (0.3 μM), ARL 67156 (6-*N*, *N*-Diethyl-β-γ-dibromomethylene-D-adenosine-5-triphosphate; 100 μM) and a combination of αβmethylene ATP (10 μM) and prazosin (0.3 μM). Concentrations were chosen according to previously published work with concentrations of approximately 100-fold more than previously determined pK_i or IC_{50} values being used to achieve a substantial but selective block. Tissue preparations were incubated with drug throughout the second 1 h equilibration period and the subsequent construction of the second frequency–response curve.

αβmethylene ATP (10 μM) typically generates a contractile response in the guinea pig prostate; however, following prolonged exposure this response is lost. This is indicative of P2X-receptor desensitization.

2.3.1. Effects of agonists

The effects of purinoceptor agonists on unstimulated prostatic tissue were observed in the presence and absence of suramin (100 μM). The effects of the purine agonist adenosine 5′-diphosphate (ADP) were also tested on unstimulated tissues in the presence and absence of the desensitizing agent αβmethylene ATP (10 μM). Suramin (100 μM) or αβmethylene ATP (10 μM) was added at the beginning of the 1 h equilibration period and was re-added after each washout. Following the 1 h equilibration period, discrete log concentration–response curves to various agonists on unstimulated tissues were constructed using 0.5 log unit increments. Each concentration was added 10 min apart and remained in contact with the tissue until the contraction had peaked prior to being washed out and replaced with fresh bathing medium. Preparations from each animal were administered the same agonist in order to provide relevant controls, with only one in every paired tissue being administered suramin (100 μM) or αβmethylene ATP (10 μM). Only one concentration–response curve was obtained from each tissue. This protocol was used to identify the P2X-receptor subtype responsible for mediating the contractile effects of ATP and its analogues in the guinea pig prostate.

2.3.2. Measurement and analysis of data

The peak force (g) of contractile responses was measured at each concentration of agonist and a mean concentration–response curve was constructed. Frequency–response curves constructed in the presence of various drugs are shown in terms of area under the curve (AUC; g.s.) in order to compare drug responses which varied in area but not height. The AUC was measured at each frequency and a mean frequency–response curve was constructed. Calculation of the AUC began when stimulation caused the contractile force to deviate from the baseline. The end of the measurement was when the contractile force returned to baseline. The AUC calculation allowed for a more accurate comparison between drug treatments. The results were expressed as mean ± standard error of the mean (S.E.M.). The *n* value represents the number of animals used. Mean frequency–response and −log concentration–response curves were constructed by pooling data from experiments using tissues from six guinea pigs.

For all frequency–response and concentration–response curves, statistical analysis was performed using a two-way repeated measure analysis of variance (ANOVA) which compared two treatment groups (drug and control; GraphPad Prism, version 2.01). In electrical field stimulation

studies, Bonferroni posttests for multiple comparisons were performed to identify at which frequencies a difference was seen. P values that represented a probability of a significant interaction between the frequency or agonist concentration and the drug were used. In all instances, P values <0.05 were considered significant.

Differences in agonist potency were estimated by determining the mean concentration of agonist that produced a contractile response of 0.05 g ($EC_{0.05\ g}$). Nonlinear regression was used to determine $EC_{0.05\ g}$ values (GraphPad Prism; version 2.01). Mean and 95% confidence limits (CL) of this value for each agonist were determined. Traditional EC_{50} values could not be accurately determined as concentration–response curves to 2methylthio ATP, βγmethylene ATP, ADP and ATP failed to reach a maximum response. Alternatively, the $EC_{0.05\ g}$ was chosen to gain a more accurate estimate of agonist potency. Antagonist potency was determined by calculating the apparent pK_B value for the antagonist using the equation:

$$\text{Apparent } pK_B = \log(CR - 1) - \log(X)$$

where CR represents the concentration ratio obtained from the agonist $EC_{0.05\ g}$ values in the presence and the absence of a concentration of the antagonist (X) (Tuladhar et al., 2000).

2.3.3. Drugs

The following drugs were used: adenosine 5′-triphosphate (ATP, Sigma), adenosine 5′-diphosphate (ADP, Sigma), adenosine 5′-monophosphate (AMP, Sigma),

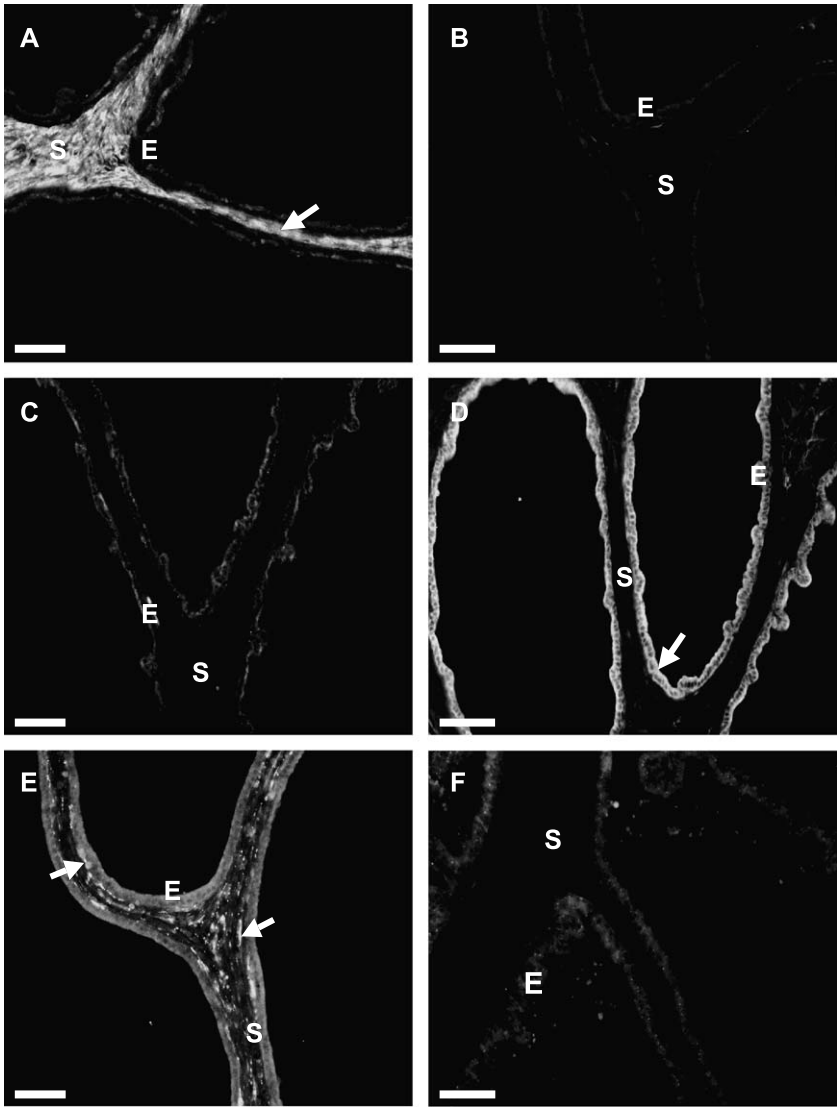

Fig. 1. Photo micrographs illustrating cross-sections of the guinea pig prostate (n=7) following immunolabelling with rabbit polyclonal antibodies to $P2X_{1,2,3,4,7}$ receptors. Positive immunostaining is indicated by arrows. Abundant $P2X_1$-receptor immunostaining (A) is restricted to the fibromuscular stroma (S). $P2X_2$- and $P2X_3$-receptor immunoreactivity (B and C, respectively) was absent in both stromal and epithelial (E) layers of the tissue. $P2X_4$-receptor immunoreactivity was apparent within the epithelium (D). Moderate $P2X_7$-receptor immunoreactivity (E) is demonstrated throughout the stromal and epithelial segments of the guinea pig prostate. Negative control sections (F) displayed no immunoreactivity. Scale bar=50 μm.

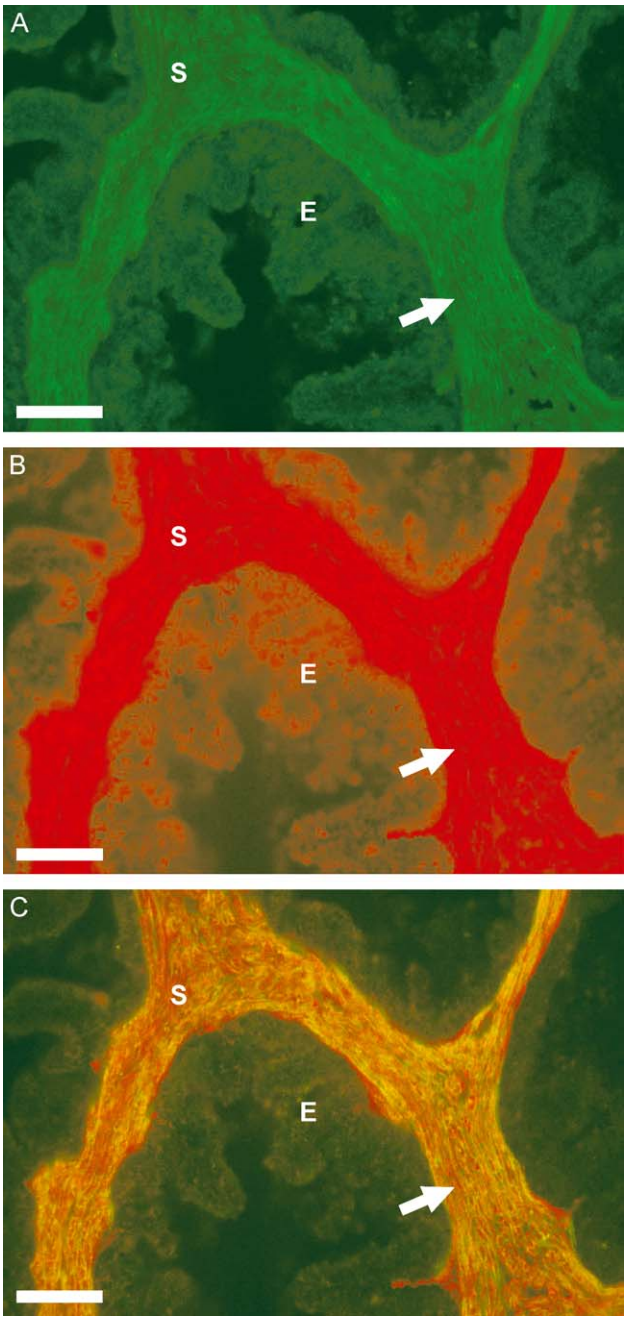

Fig. 2. Representative photo micrographs illustrating the same cross section of guinea pig prostate (n=7) following double labelling with rabbit polyclonal antibodies to the $P2X_1$-receptor (A) and mouse monoclonal antibody to α-actin (B). Positive immunoreactivity is indicated by arrows. Superimposition of the two micrographs (C) reveals colocalisation of $P2X_1$-receptors and α-actin, confirming that $P2X_1$-receptors are confined to the fibromuscular stroma surrounding the alveolar epithelium in the guinea pig prostate. Scale bar=50 μm.

adenosine (Sigma), atropine (Sigma), suramin (Sigma), prazosin (Sigma), αβmethylene ATP (Sigma), βγmethylene methylene ATP (Sigma), 2methylthio ATP (Sigma), 6-*N*, *N*-diethyl-β-γ-dibromomethylene-D-adenosine 5′-triphosphate (ARL 67156, Sigma) and tetrodotoxin (ICN). Drug compounds were dissolved and diluted to the appropriate concentrations in distilled water. All drugs were prepared on the day of experimentation.

3. Results

3.1. Immunofluorescence localization of P2X-receptor subtypes

The presence and distribution of P2X-receptor subtypes for ATP were analysed using immunolabelling and fluorescence microscopy. In all studies, control sections displayed no immunoreactivity (Fig. 1).

$P2X_1$-receptor immunoreactivity was abundant throughout the fibromuscular stroma of the guinea pig prostate but was absent in the alveolar epithelium (Fig. 1A). $P2X_2$- and $P2X_3$-receptor immunoreactivity was absent in both stromal and epithelial layers of the tissue (Fig. 1B and C). $P2X_4$-receptor immunoreactivity was apparent within the epithelium only (Fig. 1D). Moderate $P2X_7$-receptor immunoreactivity was demonstrated throughout the stromal and epithelial segments of the guinea pig prostate (Fig. 1E). The pattern of stromal distribution of immunoreactivity to the $P2X_7$-receptor was unlike that illustrated by $P2X_1$-receptor immunoreactivity (Fig. 1). Immunoreactivity was sparse throughout the stroma and appeared varicose. This pattern of staining is characteristic of nerve fibre staining rather than smooth muscle staining.

3.1.1. Double labelling for $P2X_1$-receptors and actin

Double labelling studies revealed positive immunostaining for both $P2X_1$-receptors and α-actin (Fig. 2, upper and centre panels, respectively). Superimposition of the images taken with two different filter cubes showed consistent colocalization, suggesting the presence of $P2X_1$-receptors in the stroma (Fig. 2, lower panel). Conversely, no immunoreactivity to α-actin or the $P2X_1$-receptor was observed within the epithelium with either filter cube (Fig. 2).

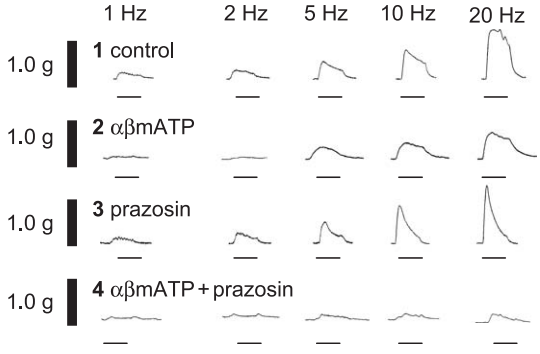

Fig. 3. Representative traces illustrating the effects of various antagonists (αβmethylene ATP, 10 μM; prazosin, 0.3 μM) on electrical field stimulation-induced (—) contractile responses in the guinea pig prostate (1–20 Hz, 0.5 ms, 50 V for 10 s).

3.2. Isolated organ bath studies

3.2.1. Responses to electrical field stimulation

Representative contractile responses to electrical field stimulation (0.5 ms, 50 V, 1–20 Hz) of isolated guinea pig prostates and the effects of αβmethylene ATP (10 μM) and prazosin (0.3 μM) on these responses are illustrated in Fig. 3. Contractile responses to electrical field stimulation (0.5 ms, 50 V, 1–20 Hz) seen in this tissue were frequency-dependent and returned to baseline values following stimulation. Preincubation of isolated guinea pig prostates with tetrodotoxin (1 μM) consistently attenuated electrical field stimulation-induced contractions (P<0.001, Fig. 4). The degree of inhibition was minimal at 1 Hz with a 40% reduction in response, while maximal inhibition was achieved at a frequency of 20 Hz with a 70% reduction in contractile response. Atropine (10 μM) had no effect on field stimulation-induced responses across the entire range of frequencies (1–20 Hz, P>0.05, Fig. 4).

Initial exposure to αβmethylene ATP (10 μM) elicited a contractile response. The magnitude of this initial response to αβmethylene ATP (10 μM) ranged from 0.12 to 0.39 g across six experiments. Without washout, subsequent additions of the drug failed to elicit a contractile response. αβmethylene ATP (10 μM) caused reductions of 21–28% in electrical field stimulation-induced responses as indicated by AUC values at higher frequencies (10–20 Hz, respectively, P<0.05, n=6, Fig. 5). Moreover, αβmethylene ATP (10 μM) displayed the ability to further attenuate contractile responses to electrical field stimulation over a frequency range of 5–20 Hz in the presence of prazosin (0.3 μM; P<0.05, Fig. 5). This inhibition of residual nerve-mediated contractions ranged from 27% to 33% with maximum inhibition achieved at 5 Hz (P<0.05, Fig. 5). Preincubation with prazosin (0.3 μM) reduced the AUC of the contractile response to electrical field stimulation (Fig. 5), although no change in the peak height of electrical field stimulation-induced contractions was observed (Fig. 3). Representative traces indicate preservation of the initial portion of the response; however, the subsequent plateau was attenuated. The presence of both αβmethylene ATP

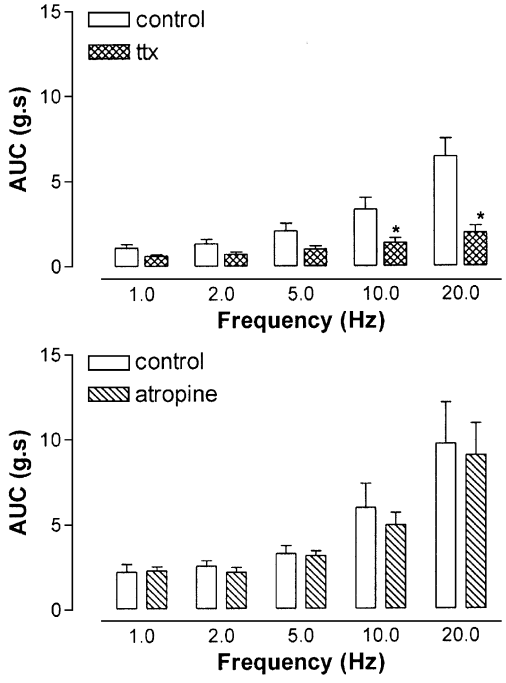

Fig. 4. Mean contractile responses to electrical field stimulation represented by area under the curve (AUC; 1–20 Hz, 0.5 ms, 50 V for 10 s) following administration of: (open bars) no drug, (crosshatched bars) tetrodotoxin (1 μM) or (diagonally striped) atropine (10 μM). Each column represents the mean±S.E.M. of 6 experiments. *Significant difference from control response (*P<0.05; two-way repeated measure ANOVA, followed by Bonferroni posttests for multiple comparisons).

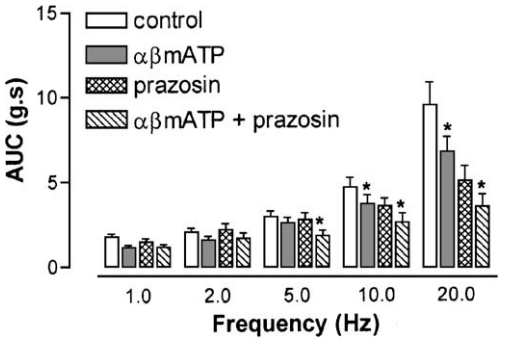

Fig. 5. Mean contractile responses to electrical field stimulation represented by the area under the curve (AUC; 1–20 Hz, 0.5 ms, 50 V for 10 s) following administration of: (open bars) no drug, (checkered bars) αβmethylene ATP (10 μM), (crosshatched) prazosin (0.3 μM) or (diagonally striped) αβmethylene ATP (10 μM), and prazosin (0.3 μM). Control tissues were paired with those treated with αβmethylene ATP. Tissues treated with prazosin were paired with those treated with a combination of prazosin and αβmethylene ATP. *Significant difference from the corresponding paired response (*P<0.05; two-way repeated measure ANOVA, followed by Bonferroni posttests for multiple comparisons).

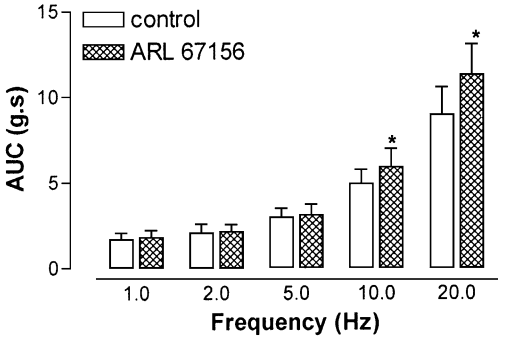

Fig. 6. Mean contractile responses to electrical field stimulation represented by area under the curve (AUC; 1–20 Hz, 0.5 ms, 50 V for 10 s) following administration of: (open bars) no drug or (crosshatched bars) ARL 67156 (100 μM). Each column represents the mean±S.E.M. of 6 experiments. *Significant difference from the control response (*P<0.05; two-way repeated measure ANOVA, followed by Bonferroni posttests for multiple comparisons).

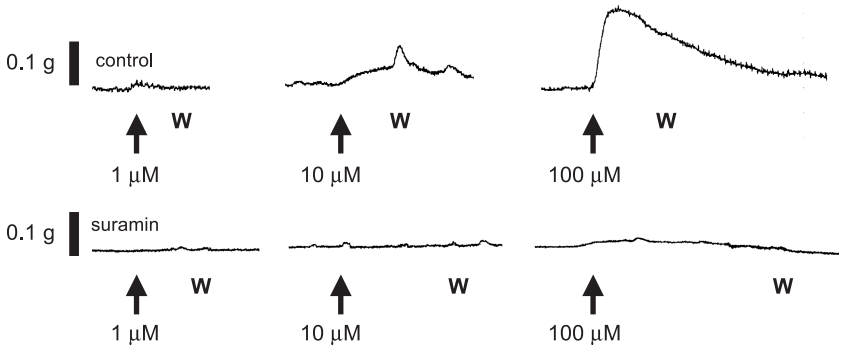

Fig. 7. Traces illustrating the effects of βγmethylene ATP (1-100 μM) on unstimulated preparations of the guinea pig prostate in the absence and presence of suramin (100 μM). (↑) Addition of specific concentrations of βγmethylene ATP. W=washout.

(10 μM) and prazosin (0.3 μM) largely blunted responses of both initial and sustained components of the contraction (Fig. 3).

The ATPase inhibitor ARL 67156 (100 μM) significantly potentiated electrical field stimulation-induced contractile responses at 10 and 20 Hz by 16% and 29%, respectively (P<0.01, Fig. 6).

3.2.2. P2-receptor classification

Administration of exogenous purine agonists to unstimulated guinea pig prostate preparations resulted in concentration-dependent contractile responses (Fig. 7). Adenosine and AMP were inactive. αβmethylene ATP (0.3–100 μM) generated concentration-dependent contractile responses with an EC_{50} value of 0.5±0.02 μM. Mean log concentration–response curves for each purine analogue are shown in Fig. 8.

The rank order of potency as measured by contractile force produced in the guinea pig prostate was: αβmethylene ATP>2methylthio ATP=βγmethylene ATP>ADP=ATP. Mean negative log $EC_{0.05\ g}$ values as obtained from fitted regression lines and potencies relative to ATP are shown in Table 1. Preincubation with suramin (100 μM) attenuated contractile responses elicited by 2methylthio ATP (apparent pK_B<0.65 μM) and βγmethylene ATP (apparent pK_B<1.47 μM). Preincubation with αβmethylene ATP (10 μM) had no effect on contractile responses to ADP (P>0.05; n=4; Fig. 9).

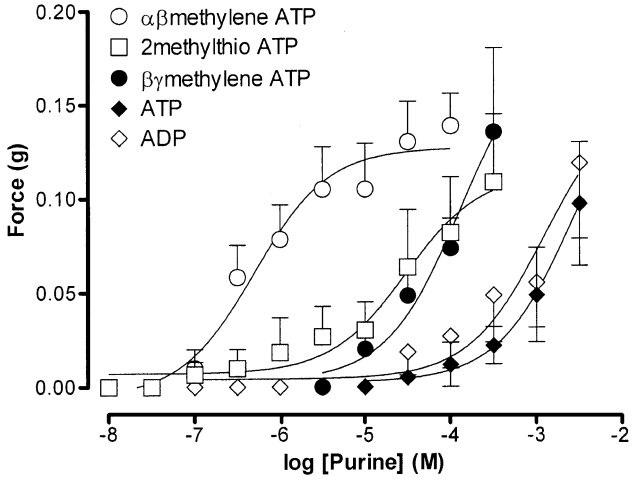

Fig. 8. Mean log concentration–response curves for ATP, ADP, βγmethylene ATP, 2methylthio ATP and αβmethylene ATP on unstimulated guinea pig prostatic preparations. Results are expressed as mean peak force developed to each concentration of agonist. Each point represents the mean±S.E.M. of 6 experiments.

4. Discussion

These studies reveal that ATP contributes to neuromuscular transmission in the guinea pig prostate via the activation of the $P2X_1$-receptor subtype. The addition of specific purinoceptor blocking drugs to prostate preparations inhibited contractile responses to purinoceptor agonists and electrical field stimulation. Thus, ATP appears to be co-released with noradrenaline and both act postjunctionally to elicit a contractile response in the guinea pig prostate. Immunoreactivity studies further implicated the $P2X_1$-receptor in the pathway for ATP action. Its distribution was exclusive to the fibromuscular stroma. This was supported by the colocalization of $P2X_1$-receptor immunoreactivity with α-actin immunoreactivity, suggesting a role in

Table 1
Mean negative log $EC_{0.05\ g}$ values, potency and mean peak force obtained at $P2X_1$-receptors on guinea pig prostatic smooth muscle

Agonist	−log $EC_{0.05\ g}$ (M) (95% CL)	Potency ratio[a]	Mean peak force (g; mean±S.E.M.)
αβmethylene ATP	6.44 (6.0–6.88)	2754	0.138±0.02
2methylthio ATP	4.69 (3.96–5.42)	49	0.108±0.04[b]
βγmethylene ATP	4.34 (3.31–5.36)	22	0.253±0.04[b]
ADP	3.27 (2.51–4.03)	1.9	0.118±0.04[b]
ATP	3.0 (1.65–4.35)	1	0.097±0.03[b]

n=6 animals for each purine agonist.

[a] Potency ratio=antilog ((neg log $EC_{0.05\ g}$ value for purine analogue)−(neg log $EC_{0.05\ g}$ value for ATP)).

[b] Maximal contractile responses to βγmethylene ATP, 2methylthio ATP, ADP and ATP were not achieved; thus, peak contractile force is given as the maximum force produced at the highest concentration used.

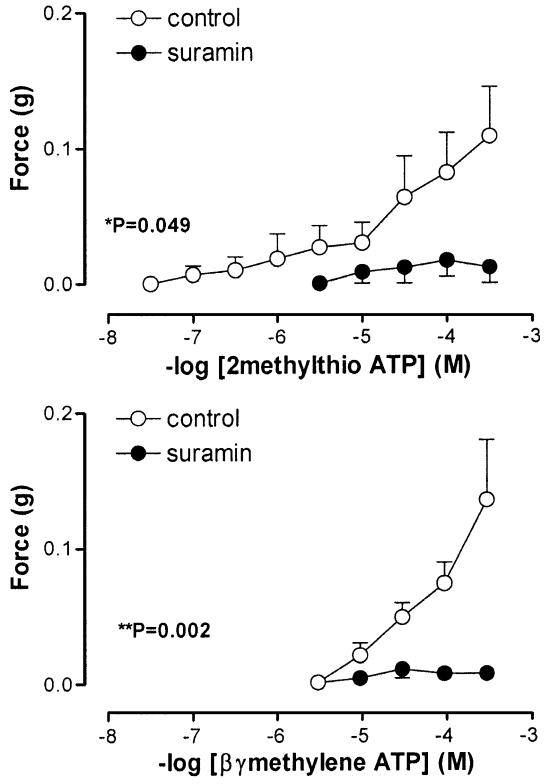

Fig. 9. Mean log concentration–response curves to 2methylthio ATP (upper panel) and βγmethylene ATP (lower panel) on unstimulated guinea pig prostatic preparations in the absence (○) and presence of suramin (●; 100 μM). Results are expressed as the mean peak force developed to each concentration of agonist. Each point represents the mean±S.E.M. of six experiments. *P*-values are for the concentration×treatment interaction of a repeated measure ANOVA and represent the difference in the concentration–response curves in the absence and presence of suramin. Significant difference: **P*<0.05; ***P*<0.005.

stromal contractility. Similar results have been obtained in the rat prostate gland where the $P2X_1$-receptor subtype was exclusively expressed in the fibromuscular stroma and colocalized with α-actin (Ventura et al., 2003). The presence of ecto 5′-nucleotidase (Konrad et al., 1998) and the expression of receptors for ATP (Janssens et al., 1996; Longhurst et al., 1996; Wasilenko et al., 1997) in the human prostate suggests that similar mechanisms may exist in humans.

In addition to $P2X_1$-receptor immunoreactivity, we also observed immunoreactivity to the $P2X_4$- and $P2X_7$-receptors. $P2X_7$-receptor immunoreactivity was demonstrated throughout the stroma and epithelial segments of the guinea pig prostate. Stromal distribution of $P2X_7$-receptors was varicose, suggesting nerve fibre staining perhaps indicative of primary sensory neurotransmission involved in nociception (Burnstock and Wood, 1996). Our findings are also consistent with a recent study that demonstrated similarly distributed immunoreactivity to the $P2X_7$-receptor in the smooth muscle of the guinea pig vas deferens and urinary bladder (Menzies et al., 2003). The role of these receptors remains to be determined.

The administration of both αβmethylene ATP and the α_1-adrenoceptor antagonist prazosin to electrical field-stimulated tissues had an additive effect whereby responses from 5–20 Hz were consistently attenuated. Although reductions in contractile response were achieved with the addition of αβmethylene ATP and prazosin, a residual response remained evident. This may be attributed to direct smooth muscle stimulation as residual responses of similar magnitude were also seen in the presence of tetrodotoxin following electrical field stimulation. Furthermore, preincubation with the muscarinic antagonist atropine failed to attenuate responses across the entire range of frequencies tested, indicating that cholinergic transmission is not involved in mediating contraction in this species. Nevertheless, we have not proved that no other transmitters are involved, but we have shown ATP to be a neurotransmitter along with noradrenaline in the guinea pig prostate gland.

Alternatively, it is possible that ATP is not being released from nerves but from the epithelium of the prostate. Such release and subsequent contraction may be responsible for the tetrodotoxin-resistant portion of the contractile response to electrical field stimulation. ATP has been found to be released from the bladder (Andersson, 2002) and guinea pig ureter (Knight et al., 2002) in response to mechanical distortion or distension. This is possible but unlikely in the prostate as a combination of prazosin and αβmethylene ATP yielded a similar residual response to electrical field stimulation to that seen in the presence of tetrodotoxin.

Preincubation with αβmethylene ATP not only reduced the magnitude of responses to electrical field stimulation but also altered the shape of the responses compared to control. αβmethylene ATP alone preferentially blunted the initial segment of the contraction, suggesting that ATP is responsible for this initial component of the contraction. Moreover, electrical field stimulation-induced responses in the presence of the α_1-adrenoceptor antagonist prazosin did not display a difference in peak height. Although the initial component of this contraction is preserved, the subsequent plateau was significantly attenuated. This is analogous to what has been demonstrated within the rodent vas deferens by numerous studies whereby postjunctional antagonists to ATP and noradrenaline have preferentially inhibited the initial fast twitch and slow sustained tonic contraction of the biphasic contractile response, respectively (Meldrum and Burnstock, 1983; Ventura, 1998). As no difference in peak height of the contraction was seen with the addition of prazosin, this provided the rationale behind the use of AUC rather than peak height as a measure of the effects of these drugs on electrical field stimulation-induced contractions. Collectively, these results suggest that the initial portion of the response to electrical field stimulation is attributable mainly to $P2X_1$-receptors whereas noradrenaline is responsible for the slower, sustained contractile component via the activation of α_1-adrenoceptors.

The ATPase inhibitor, ARL 67156, significantly enhanced electrical field stimulation-induced contractile

responses at 10–20 Hz in prostatic preparations. ARL 67156 (previously known as FPL 67156) inhibits ecto-ATPase, the enzyme responsible for the sequential breakdown of ATP (Crack et al., 1995). A similar study reported an increase in height of electrical field stimulation-induced contractions in the guinea pig vas deferens following pre-treatment with ARL 67156 (Westfall et al., 1996). Thus, enzymatic degradation of ATP substantially affects the ability of neurally released ATP within the guinea pig prostate to produce contraction. This adds further evidence to ATP being involved in the nerve-mediated electrical field stimulation-induced contraction of the guinea pig prostate.

Mean −log concentration–response curves to exogenous purines in the guinea pig prostate resulted in a rank order of potency: αβmethylene ATP>2methylthio ATP=βγ methylene ATP>ADP=ATP. This rank order of agonist potency in the guinea pig prostate follows a similar pattern to that seen in other smooth muscle preparations containing P2X-receptors such as the mesenteric artery of the rabbit (Burnstock and Warland, 1987), the cauda epididymis of the rat (Ventura and Pennefather, 1991) and the rat prostate gland (Ventura et al., 2003). In the present study, αβmethylene ATP was 2754 times more potent that ATP in producing contraction within the guinea pig prostate, as compared to 1500 times more potent in the rabbit mesenteric artery (Burnstock and Warland, 1987), 1150 times more potent in the rat cauda epididymis (Ventura and Pennefather, 1991) and 69 times more potent in the rat prostate gland (Ventura et al., 2003). Potency ratios for 2methylthio ATP and βγmethylene ATP followed a similar order of magnitude among these studies. The reason for these differences in potency may be tissue as well as species dependent. It has been suggested that the rate of catabolism of ATP between different tissues varies to a considerable extent, thus resulting in differences in potency ratios for the P2X-receptor (Ventura and Pennefather, 1991). Despite any tissue or species differences, the relative order of agonist potencies is very similar among these studies.

The purinoceptor responsible for mediating contraction in the guinea pig prostate being of the $P2X_1$-receptor subtype is further implied by the antagonist action of suramin and the tissue's high sensitivity to αβmethylene ATP. Apparent pK_B values for suramin correlate well with IC_{50} values previously reported (1–5 μM) for the $P2X_{1,2,3,5}$-receptor subtypes (Humphrey et al., 1998). Of these receptor subtypes, only the $P2X_1$- and $P2X_3$-receptor subtypes are highly sensitive to αβmethylene ATP (Humphrey et al., 1998). Confirmation of the P2X-receptor subtype mediating contraction in the guinea pig prostate was confirmed by immunohistological studies, which demonstrated the absence of the $P2X_3$-receptor in the guinea pig prostatic stroma yet with strong expression by the $P2X_1$-receptor.

Although contractile responses to ADP are equipotent to those initiated by ATP, these responses do not appear to be mediated by the $P2X_1$-receptor as in the case of ATP. Preincubation with suramin or αβmethylene ATP had no effect on the contractile responses elicited by this purine. A more extensive investigation by our laboratory is currently underway to determine the mode of action of ADP in the guinea pig prostate gland.

This study is the first to demonstrate the presence of $P2X_1$-receptors which mediate contraction and an ATP enzyme catabolism system within the fibromuscular stroma of the guinea pig prostate. Another finding of this study is that ATP is involved in neuromuscular transmission predominantly at higher frequencies within the guinea pig prostate. It is considered unusual for ATP to be a cotransmitter at high-frequency stimulation but similar effects have been demonstrated in the rat vas deferens and cauda epididymis in response to trains of electrical field stimulation (Ventura and Pennefather, 1991; Mallard et al., 1992; Ventura and Pennefather, 1994; Ventura, 1998). Alternatively, the lack of detectable ATP release at lower frequencies may simply be due to the considerable nonneurogenic component of contractile response seen at low frequencies. Nevertheless, this observation is different to purinergic neurotransmission in the rat prostate gland which occurs predominantly at lower frequencies (Ventura et al., 2003). Such species differences are relatively common in the prostate as a number of studies have reported similar differences whereby the actions of sensory neuropeptides on smooth muscle contractility in the rat and guinea pig vary considerably (Buljubasich et al., 1999; Ventura et al., 2000a,b).

Previous studies with human prostate have suggested that the response of the human prostate to electrical field stimulation is almost completely suppressed by α-adrenoceptor antagonists (Hedlund et al., 1985; Guh et al., 1995; Chueh et al., 1996). These studies were limited in that they used only high-frequency stimulation (>2 Hz). This study and our previous study using the rat prostate (Ventura et al., 2003) have shown the frequency-dependent nature of purinergic neurotransmission suggesting that a whole range of frequencies need to be tested before ATP can be dismissed as a transmitter. Furthermore, these studies also had the limitation of the tissue being obtained from surgery. Many prostate surgery techniques cause damage to the nerves innervating the tissue and nerves containing ATP may be particularly susceptible.

These findings may potentially play a significant role in improving the pharmacotherapy currently available for those suffering from benign prostatic hyperplasia. If ATP is also involved in neurotransmission in the human prostate, the development of selective inhibitors at this receptor subtype may provide an alternative to current treatments available for benign prostatic hyperplasia.

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