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Purinoceptor subtypes mediating contraction and relaxation of marmoset urinary bladder smooth muscle

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1 The effects of adenosine triphosphate (ATP), adenosine diphosphate (ADP), α,β-methylene-ATP (α,β-MeATP) and 2-methylthio-ATP (2-MeSATP) on longitudinally orientated smooth muscle strips from marmoset urinary bladder were investigated by use of standard organ bath techniques.

2 After being mounted in superfusion organ baths, 66.7% ($n=249$) of marmoset detrusor smooth muscle strips developed spontaneous tone, 48.2% of all strips examined developed tone equivalent to greater than 0.1 g mg^{-1} of tissue and were subsequently utilized in the present investigation.

3 On exposure to ATP, muscle strips exhibited a biphasic response, a rapid and transient contraction followed by a more prolonged relaxation. Both responses were found to be concentration-dependent. ADP and 2-MeSATP elicited a similar response (contraction followed by relaxation), whereas application of α,β-MeATP only produced a contraction. The potency order for each effect was α,β-MeATP > > 2-MeSATP ⩾ ATP > ADP (contractile response) and ATP = 2-MeSATP ⩾ ADP > > α,β-MeATP (relaxational response).

4 Desensitization with α,β-MeATP (10 μM) abolished the contractile phase of the response to ATP, but had no effect on the level of relaxation evoked by this agonist. On the other hand, the G-protein inactivator, GDPβS (100 μM) abolished only the relaxation response to ATP. Suramin (general P2 antagonist, 100 μM) shifted both the contractile and relaxation ATP concentration-response curves to the right, whereas cibacron blue (P2Y antagonist, 10 μM) only antagonized the relaxation response to ATP. In contrast, the adenosine receptor antagonist, 8-phenyltheophylline (10 μM), had no effect on the relaxation response curve to ATP.

5 Incubation with tetrodotoxin (TTX, 3 μM) or depolarization of the muscle strip with 40 mM K^+ Krebs failed to abolish the relaxation to ATP. In addition, neither N_ω-nitro-L-arginine (L-NOARG, 10 μM) nor methylene blue (10 μM) had any effect on the relaxation response curve. However, tos-phe-chloromethylketone (TPCK, 3 μM), an inhibitor of cyclicAMP-dependent protein kinase A (PKA), significantly ($P<0.01$) shifted the curve for the ATP-induced relaxation to the right.

6 It is proposed that marmoset detrusor smooth muscle contains two receptors for ATP, a classical P2X-type receptor mediating smooth muscle contraction, and a P2Y (G-protein linked) receptor mediating smooth muscle relaxation. The results also indicate that the ATP-evoked relaxation may occur through the activation of cyclicAMP-dependent PKA.

Keywords: ATP; marmoset urinary bladder; smooth muscle contraction and relaxation; P2 receptors; suramin; cibacron blue 3GA; cyclicAMP

Introduction

It has long been known that parasympathetic nerve stimulated contraction of the bladders of several mammalian species contains an atropine-resistant component (Langely & Anderson, 1985). Various evidence has accrued to suggest that the mediator of this effect in a number of small mammals is neuronally released adenosine 5′-triphosphate (ATP) acting at P2X receptors on detrusor smooth muscle cells (White, 1988; Anderson, 1993). Such evidence includes the finding that atropine-resistant, nerve-induced contractions can be mimicked by the application of ATP, and that both can be blocked by quinidine (Burnstock, 1972). Additionally, α,β-MeATP, which is known to activate and desensitize P2X receptors, has been shown to abolish the ATP, as well as a proportion of the nerve-induced contractions in guinea-pig urinary bladder (Kasakov & Burnstock, 1983).

Receptors for ATP on smooth muscles have been subclassified as P2X (contraction mediating) and P2Y (relaxation mediating). Traditionally, P2 receptor subtypes have been differentiated pharmacologically on the basis of the agonist potency order at each (Burnstock & Kennedy, 1985), although such potency orders have been shown to be partly dependent on the exonucleotidase enzyme activity when used in isolated tissue experiments (Kennedy & Leff, 1995). It has been suggested recently that receptors of the P2Y subtype may also be present on detrusor smooth muscle cells, in particular, in mouse and rat urinary bladder (Boland *et al*., 1993; Bolego *et al*., 1995), and that these receptors may mediate relaxation of the bladder musculature. In the present investigation, we have set out to explore the presence and mechanism of action of P2Y receptors in the urinary bladder of the marmoset (*Callithrix jacchus*) in order to extend the research carried out previously on rodent species to a primate. Moreover, we have routinely found that a percentage of marmoset urinary bladder smooth muscle strips develop a measure of spontaneous tone when mounted in an organ bath, providing an excellent model for the study of muscle relaxation which negates the need to raise tone artificially, as was previously necessary (Boland *et al*., 1993; Bolego *et al*., 1995). The presence of an inhibitory or relaxation-inducing system within the urinary bladder may provide an insight into the mechanism of compliance during bladder filling.

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Methods

Tissue preparation

Laboratory bred and maintained marmosets (*Callithrix jacchus*) of either sex and aged 9–12 months (mean weight 300 g) were obtained from another department within the university where they were killed by an anaesthetic overdose (sodium pentobarbitone, 0.25 ml kg^{-1} Euthatal, Rhone Merieux Ltd.). The bladder was removed and immediately placed in ice-cold (4°C) Krebs solution, before being cleared of external fat and connective tissue. The bladder body was then removed from a level above the ureters, opened along its anterior surface and pinned flat in a dissecting dish containing Krebs solution; subsequently, the mucosa was removed and strips of longitudinally orientated detrusor muscle measuring approximately $1 \times 1 \times 5$ mm were dissected from the bladder.

Fine (5/0) silk ligatures were tied to each end of the strips, which were then mounted vertically for isometric recording of tension in 0.2 ml capacity superfusion organ baths (Brading & Sibley, 1983). Strips were continuously bathed with gas equilibrated (97% O_2 and 3% CO_2, maintaining pH at 7.4 ± 0.1) temperature controlled (37 ± 0.5°C) Krebs solution. Tension was measured with Pioden dynamometer UF1 force-displacement transducers (Pioden Controls Ltd., Canterbury, U.K.), and the responses amplified (Harvard Apparatus Ltd., Edenbridge, U.K.) and recorded on a Tekman 900 pen recorder (Tekman electronics, Leamington Spa, U.K.). An initial resting tension of 1 g was applied to the strips and drugs were only applied after an equilibration period of at least 60 min. Drugs were delivered to the organ baths in the perfusing solution by immersing the feeder tubes in Krebs solution containing the required final concentration of the drug, in this way the exposure times of the agonists could be accurately controlled. The exposure times used were those found to evoke routinely a maximal contraction (60 s for ATP, ADP and 2-MeSATP, and 20 s for α,β-MeATP and carbachol).

Concentration-response curves (CRCs) were constructed by addition of increasing drug concentrations in a non-cumulative manner, tension being allowed to return and remain at baseline for at least 20 min before application of the next concentration. Antagonists were applied to the strips for at least 30 min before CRCs were repeated in the presence of the antagonist. In the absence of an antagonist or enzyme inhibitor, or indeed after the addition of non-active drugs, repeated CRCs were found not to be significantly different from the primary CRC. When high molarity K^+ Krebs solution was used, normal Krebs was replaced with high K^+ Krebs and the strips allowed to equilibrate for 60 min in order to allow stabilization of the induced tension, drugs were subsequently applied in high K^+ solution.

Drugs and solutions

The composition of the Krebs solution was (mM): NaCl 120, KCl 5.9, $NaHCO_3$ 15.4, NaH_2PO_4 1.2, $CaCl_2$ 2.5, $MgCl_2$ 1.2 and glucose 11.5. High potassium Krebs solution (40 mM) was made by substituting KCl in place of an equimolar amount of NaCl in normal Krebs solution. Drugs used were adenosine diphosphate (ADP), adenosine triphosphate (ATP), α,β-methylene-ATP (α,β-MeATP), carbamylcholine chloride (carbachol), cibacron blue 3GA (marketed by Sigma as a replacement to reactive blue 2 with the knowledge that this molecule has the sulphonic acid group of the A-ring at the *ortho* position, unlike some other commercially available compounds which may have a mixture of isomers regarding this A-ring sulphonic group), guanosine 5′-0-(2-thiodiphosphate)(GDPβS), methylene blue, N_ω-nitro-L-arginine (L-NOARG), 8-phenyltheophylline (8-PT) and tetrodotoxin (TTX) all from Sigma, tos-phe-chloromethylketone (TPCK) from Bachem, 2-methylthio-ATP (2-MeSATP) from RBI, and suramin was a kind gift from Bayer. All drugs were prepared freshly on the day of experiment by dissolving directly in Krebs solution, except for 8-PT which was dissolved in 80% methanol containing 0.1 M NaOH and TPCK which was dissolved in ethanol, both were subsequently diluted in Krebs. The control CRCs for both 8-PT and TPCK were constructed in the presence of the respective solvent.

Data analysis

The data from experiments are expressed as mean ± s.e.mean, tissue responses being normalized by expression in g of tension produced mg^{-1} tissue (g mg^{-1}), and statistical analysis was performed by an ANOVA (two factor with replication) test. A probability of $P < 0.05$ was considered statistically significant. Drug potencies are expressed as EC_{50} values, determined by non-linear iterative fitting of the experimental data to curves according to the method of De Lean *et al.* (1978); in the case of ATP and ADP the maximum contraction and relaxation were taken as the response obtained with the highest concentration of the agonist used (0.01 M), even though the CRC had not reached a plateau at this concentration. In order to give a measure of the action of antagonists in this system, the apparent affinities of the antagonists for the receptor (apparent K_B values) were calculated from the antagonist-induced shift in the CRC from the equation of Furchgott (1972),

$$\log\,(DR - 1) = \log[B] - \log\,K_B$$

where DR (dose-ratio) is the ratio of the concentrations of agonist giving an equal response in the presence and absence of antagonist, respectively, and [B] is the concentration of that antagonist (the values obtained are also expressed as the $-\log K_B$ to allow greater comparison to values obtained from other studies).

Results

After application of the initial resting tension and subsequent equilibration, 66.7% of marmoset detrusor strips ($n = 249$) developed a measure of spontaneous tone. Of these, 48.2% of strips developed tone greater than 0.1 g mg^{-1} and subsequently developed a biphasic response on exposure to ATP, a rapid and transient contraction succeeded by a substantial and longer lasting relaxation (Figure 1a). Both the contractile and relaxant phases of the ATP response were found to be dose-dependent (Figure 1b and c), the contractile phase showing a maximum of 0.41 ± 0.03 g mg^{-1} and an EC_{50} value of 350 ± 124 μM, while the relaxation phase indicated a maximum of 0.24 ± 0.01 g mg^{-1} and an EC_{50} value of 480 ± 83 μM ($n = 24$). Similar responses were found to occur on exposure to ADP and 2-MeSATP (Figure 1b and c), while α,β-MeATP produced only a rapid contraction, although at higher concentrations or longer exposure times this agonist abolished spontaneous activity for a short period after contraction. The maximum contraction and EC_{50} values obtained with α,β-MeATP were 0.42 ± 0.02 g mg^{-1} and 4.5 ± 1.2 μM, respectively ($n = 24$). The agonist potency orders for the two different responses were α,β-MeATP >> 2-MeSATP ⩾ ATP > ADP (contraction), and ATP = 2-MeSATP ⩾ ADP >> α,β-MeATP

(relaxation). For comparison, the maximum contraction obtained with carbachol (100 μM) was 1.06 ± 0.05 g mg^{-1} ($n = 36$).

In order to discount the possible role of adenosine formed through the degradation of ATP by exonucleotidases present in the muscle on the relaxation mechanism in marmoset detrusor strips, the effects of the adenosine receptor antagonist, 8-PT (10 μM), on the ATP induced relaxation were examined. Exposure to 8-PT (10 μM) and subsequent re-application of ATP indicated that this compound had no effect on the ATP CRC (Figure 2). Furthermore, it was found that GDPβS (100 μM), a molecule known to stabilize G-proteins in their inactive state, almost completely abolished the relaxation to ATP (1 mM), while exerting no effect on the contractile phase of the response (Figure 3a). Although α,β-MeATP had only meagre direct relaxant effects, the desensitizing effect of this compound was investigated on both phases of the ATP response; desensitization with α,β-MeATP completely abolished the contractile response to ATP, but had no appreciable effect on the maximum relaxation observed with ATP (Figure 3b). However, desensitization with α,β-MeATP did cause a slight decrease in the time course of the relaxation, reducing the time taken for the tension to return to baseline levels.

Suramin (100 μM), a non-selective P2 receptor antagonist, significantly shifted the CRC for both the contractile ($P < 0.05$) and relaxant ($P < 0.01$) responses to ATP to the right (Figure 4a and b). From the shift in the CRC, an apparent K_B of 12.6 μM ($-\log K_B$ 4.9) was calculated for suramin acting on

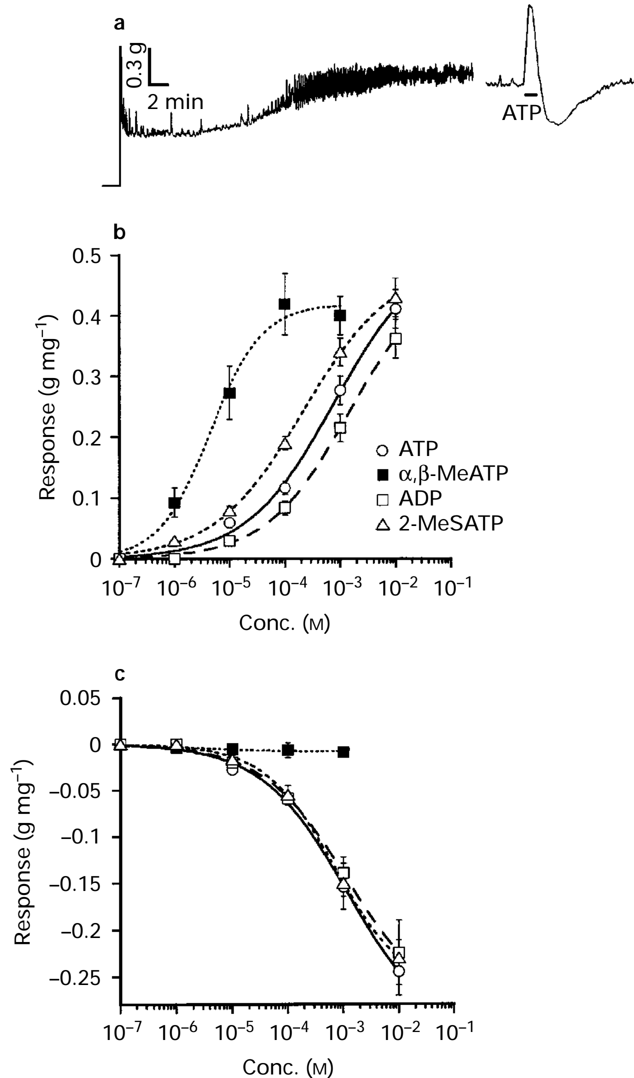

Figure 1 ATP induces a concentration-dependent biphasic response in marmoset detrusor strips. Typical tracing (a) showing the development of spontaneous tone in a marmoset detrusor strip and its subsequent response to ATP (0.01 M, 60 s application). Graphs of tension (g mg^{-1}) against concentration (M) for the contractile (b) and relaxation (c) responses of marmoset detrusor smooth muscle strips to ATP ($n = 24$), ADP ($n = 18$), α,β-MeATP ($n = 24$) and 2-MeSATP ($n = 12$). Values represent the mean and vertical lines show s.e.mean.

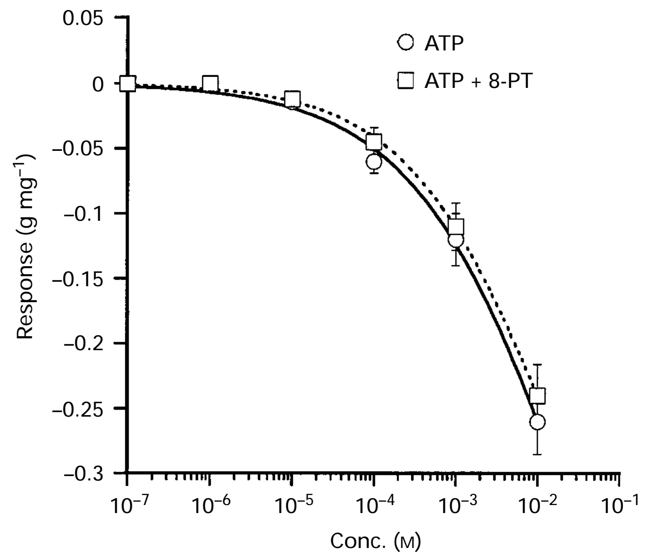

Figure 2 Graph of tension (g mg^{-1}) against concentration (M) for the relaxation response of marmoset detrusor smooth muscle strips to ATP in the absence and presence of the adenosine receptor antagonist 8-phenyltheophylline (8-PT; 10 μM). Control responses were performed in the presence of solvent ($n = 12$). Values represent the mean and vertical lines show s.e.mean.

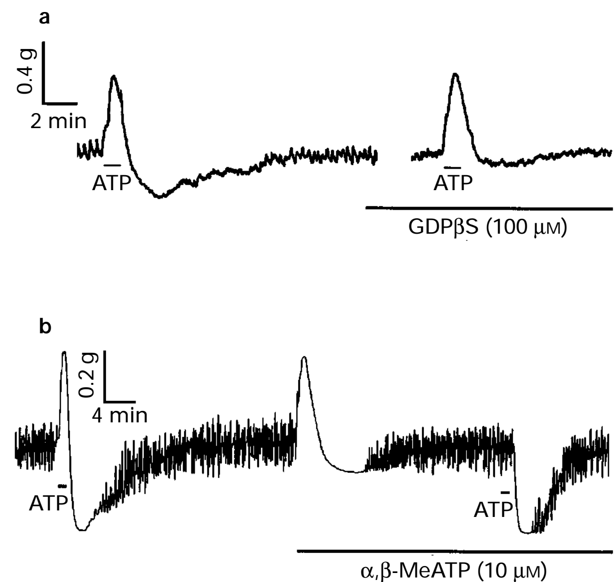

Figure 3 Representative tracing showing the response of a marmoset detrusor smooth muscle strip to ATP (1 mM, 60 s application) (a) in the absence and presence of the G-protein inactivator GDPβS (100 μM), strips being exposed to GDPβS for 20 min before being re-challenged with ATP, and (b) before and after desensitization of P2X receptors through prolonged exposure to α,β-MeATP (10 μM).

the contractile receptor. In the case of the ATP-mediated relaxation, the CRC for ATP in the presence of suramin appeared to indicate a non-competitive nature to the antagonism of this receptor; despite the obvious lack of validity for the calculation in the case of a non-competitive antagonist, the increase in the ATP concentration required to achieve a similar relaxation to that of the EC_{50} in the absence of suramin allowed an apparent K_B value of 8.1 μM ($-\log K_B$ 5.1) to be calculated. The relatively selective P2Y receptor antagonist cibacron blue 3GA (10 μM) had little effect on the contractile response to ATP (Figure 5a), but significantly ($P<0.01$) shifted the relaxant CRC to the right (Figure 5b), an apparent K_B of 3.4 μM ($-\log K_B$ 5.5) being calculated. A concentration of 10 μM cibacron blue 3GA was selected for use in the present study to increase its selectivity for P2Y receptors, it having been shown previously with reactive blue 2 that at concentrations greater than this P2X receptors are also antagonized (Bültmann & Starke, 1994).

The relaxation CRC was unaffected by the nitric oxide synthase inhibitor L-NOARG (10 μM, Figure 6a) and the guanylyl cyclase inhibitor methylene blue (10 μM, Figure 6b), but was significantly ($P<0.01$) shifted to the right in the presence of TPCK (3 μM, Figure 6c), an adenosine 3′ : 5′-cyclic monophosphate (cyclicAMP)-dependent protein kinase inhibitor. In addition, tetrodotoxin (TTX) did not diminish either the contractile or relaxation phases evoked by application of 1 mM ATP, suggesting a non-ganglionic site of action for this agonist (data not presented).

In order to investigate the presence of a putative relaxation-mediating ATP receptor in those marmoset detrusor smooth muscle strips which exhibited minimal spontaneous tone, the tension was raised artificially by the addition of 40 mM K^+ Krebs. After an initial sharp rise the tension fell and plateaued at a level which remained constant for a number of hours, existing above that of the original baseline tension and so allowing repeated agonist applications. The mean rise in

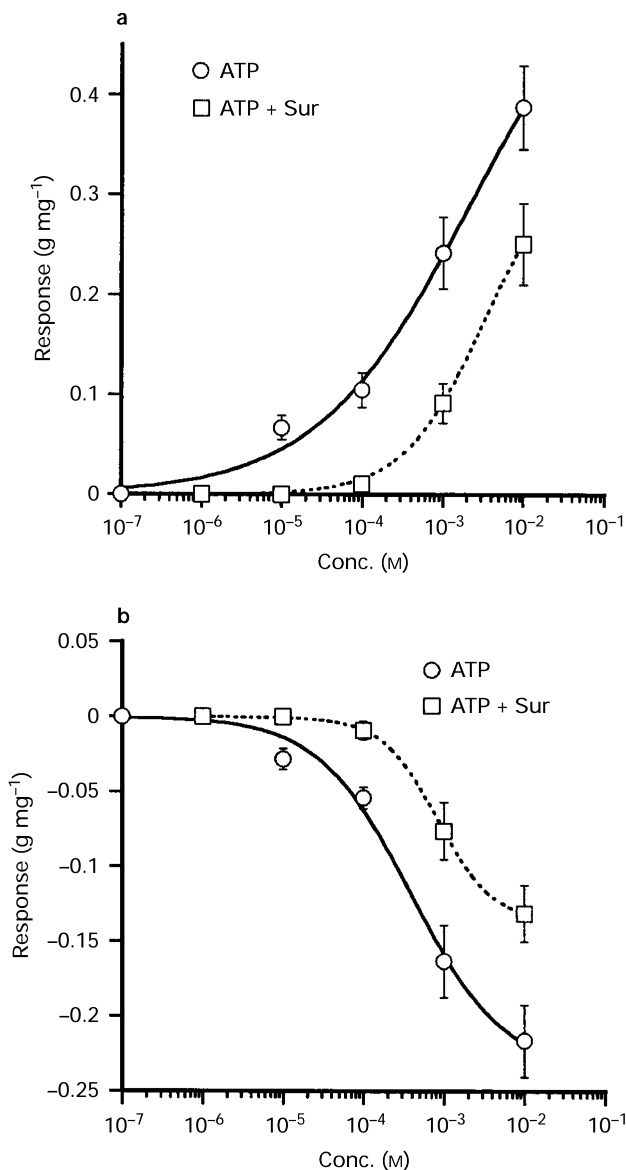

Figure 4 The effect of suramin on ATP-induced responses in marmoset bladder. Graphs of tension (g mg^{-1}) against concentration (M) for the contractile (a) and relaxation (b) responses of marmoset detrusor smooth muscle strips to ATP in the absence and presence of suramin (100 μM). Values represent the mean and vertical lines show s.e.mean ($n=18$).

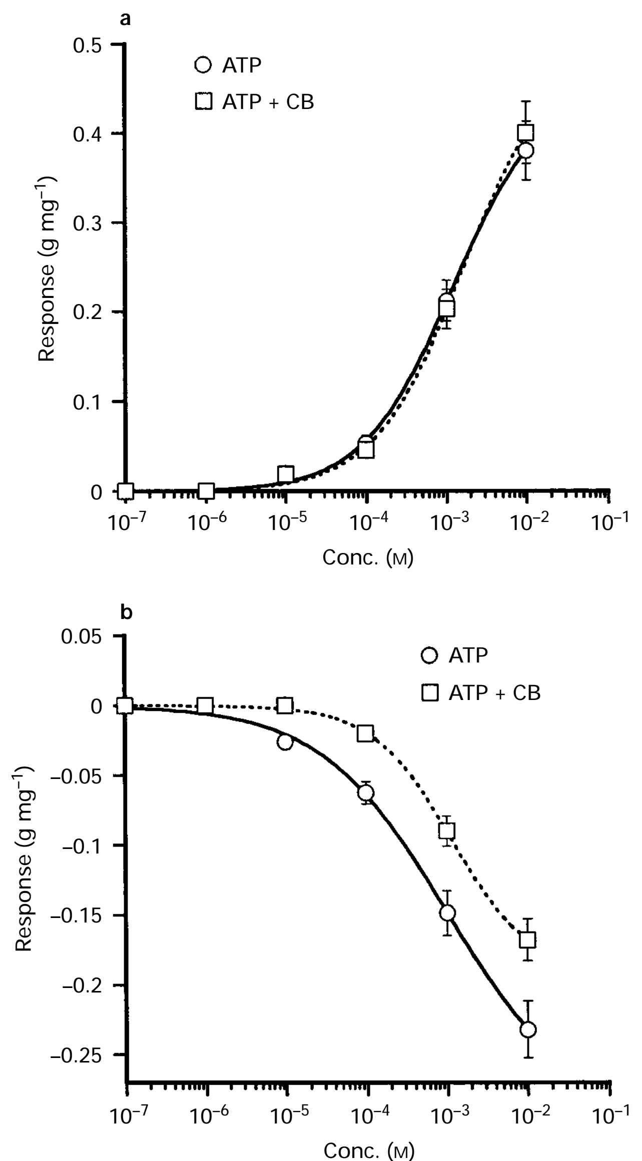

Figure 5 The effect of cibacron blue on ATP-induced responses in marmoset bladder. Graphs of tension (g mg^{-1}) against concentration (M) for the contractile (a) and relaxation (b) responses of marmoset detrusor smooth muscle strips to ATP in the absence and presence of cibacron blue (10 μM). Values represent the mean and vertical lines show s.e.mean ($n=18$).

tension from the original baseline to the tension observed during the plateau phase was calculated as 0.21 ± 0.07 g mg^{-1} ($n = 24$). Subsequently, exposure of the strip to ATP in these conditions resulted in a biphasic response similar to that seen in those strips with spontaneous tone (Figure 7a). Similar results were obtained when tension was raised artificially with carbachol (data not presented). Furthermore, application of 40 mM K^+ Krebs to strips already exhibiting spontaneous tone and a biphasic response to ATP resulted in a further increase in tone and an increase in the maximum relaxation on exposure to ATP (Figure 7b).

Subsequently, it was found that in these conditions the relaxant response to ATP was antagonized by cibacron blue 3GA and TPCK in a similar fashion to that seen in normal Krebs conditions (data not presented).

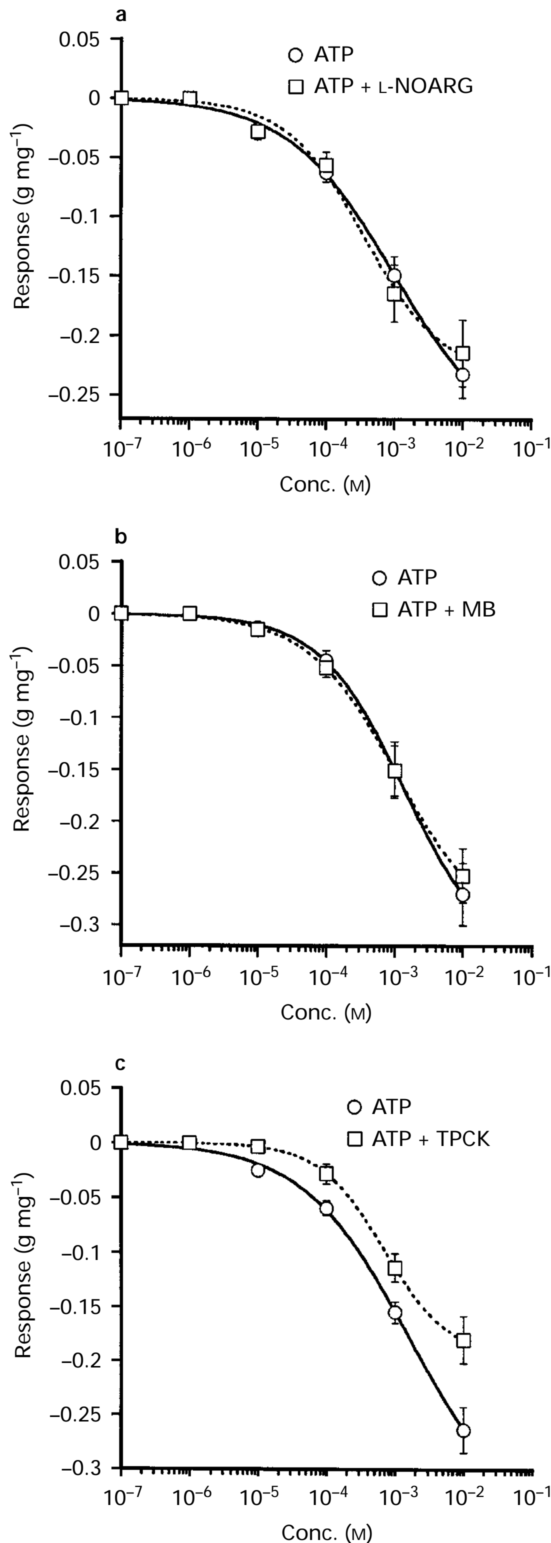

Figure 6 Graphs of tension (g mg^{-1}) against concentration (M) for the relaxation response of marmoset detrusor smooth muscle strips in the absence and presence of L-NOARG (10 μM) (a), methylene blue (MB; 10 μM) (b) and TPCK (3 μM) (c). Values represent the mean and vertical lines show s.e.mean ($n = 12$, 12 and 18, for (a), (b) and (c) respectively).

Discussion

Neuronal release of ATP, and in particular its co-transmission with acetylcholine, has been described in mammalian urinary bladder smooth muscle (Ambache & Aboo Zar, 1970; Burnstock *et al*., 1972; Kasakov & Burnstock, 1983), including that of the marmoset (Moss & Burnstock, 1985). Both neuronally released and bath applied ATP are thought to mediate their actions through the activation of P2X receptors on the membrane of the smooth muscle cells, opening non-selective cation channels and causing depolarization (Inoue & Brading, 1990; 1991; Andersson, 1993).

As regards marmoset detrusor smooth muscle, we have shown dose-dependent contractions in response to ATP, α,β-MeATP, 2-MeSATP and ADP, and that the ATP-evoked contraction is sensitive to desensitization with α,β-MeATP (Figures 1b and 3b). Furthermore, the contractile ATP CRC was shifted to the right by suramin (100 μM) but not by cibacron blue (10 μM) (Figures 4a and 5a); the apparent K_B calculated from this shift (apparent $-\log K_B$ 4.9) compares favourably with previous estimations for the action of suramin

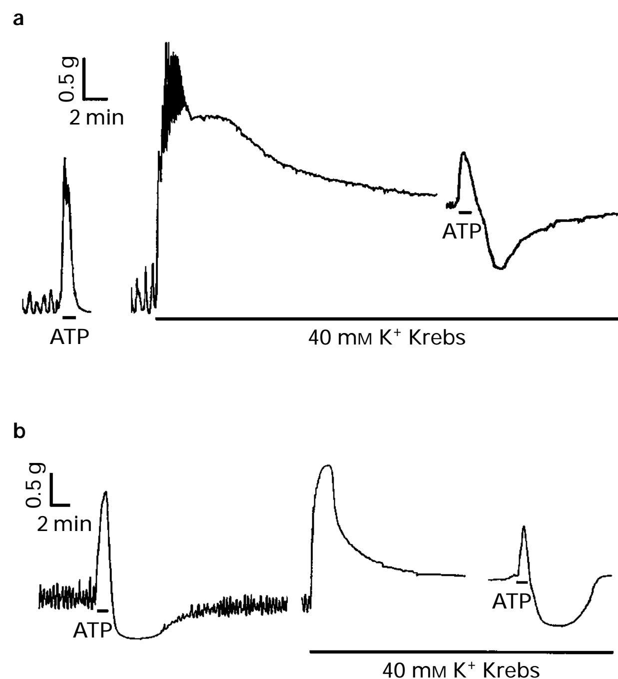

Figure 7 Original tracings showing the response of marmoset detrusor smooth muscle strips to ATP (1 mM, 60 s application) before and after tone was raised by incubation in 40 mM K^+ Krebs. (a) Detrusor strip for which no spontaneous tone or relaxation response to ATP was observed before the addition of 40 mM K^+. (b) Detrusor strip which exhibited both spontaneous tone and a relaxation response to ATP before the addition of 40 mM K^+.

at P2X receptors; a pA_2 of 4.7 has been found in guinea-pig urinary bladder (Hoyle *et al*., 1990), and a range of 4.6 to 5.0 has been quoted in a review by Burnstock (1996). As such, we can conclude that the contractile mechanism of ATP in marmoset urinary bladder smooth muscle appears similar to that of other small mammals, involving the activation of P2X receptors on the muscle membrane, as was concluded previously for the atropine-resistant component of the electrical field stimulated contraction in this species (Moss & Burnstock, 1985).

More recently, it has become apparent that this classical P2X receptor may not be the only P2 receptor subtype present in the urinary bladder. Two subtypes of contractile purinoceptors have been proposed to exist in rat (Suzuki & Kokubun, 1994), rabbit (Chen *et al*., 1992) and human (Palea *et al*., 1994) bladder, while multiple receptor subtypes have been suggested in feline urinary bladder (Theobald, 1992). As well as numerous contractile P2 receptors, it has previously been shown with mouse and rat urinary bladder smooth muscle that ATP is capable of producing a dose-dependent relaxation *in vitro* (Boland *et al*., 1993; Bolego *et al*., 1995). Although the ATP induced relaxation in rat bladder strips had formerly been ascribed to the breakdown of ATP to form adenosine and the subsequent action of this compound on P1 receptors (Suzuki & Kokubun, 1994), in the same study it was shown that ATP had no relaxant effect on dog detrusor strips. Interestingly, the ensuing study by Bolego *et al*. (1995) highlighted the complete lack of effect of the adenosine receptor antagonist 8-PT on the ATP-evoked relaxation in the rat, as was also noted by Boland *et al*. (1993) in the mouse. Despite the use of 8-PT in these investigations, it should be noted that ATP was left in contact with the tissue strips for over 5 min in the case of the rat study, and for greater than 15 min in the study of Boland *et al*. allowing considerable time for degradation to occur.

Currently, we have investigated the presence of similar receptors in urinary bladder smooth muscle from the marmoset using organ bath techniques. Ideally, this preparation negated the need to raise the tone of the muscle strips artificially *in vitro* before a relaxation to ATP could be observed, as was carried out in previous investigations (Boland *et al*., 1993; Bolego *et al*., 1995). Moreover, by utilizing the 0.2 ml superfusion organ baths of Brading and Sibley (1983) it is possible to limit accurately the exposure of muscle strips to an agonist, with continuous washing subsequent to exposure; as such, in the present study we have limited the exposure of ATP to 60 s in order to reduce the possibility of adenosine production.

The results of these experiments provide us with definitive evidence for the presence of a P2 receptor mediating relaxation of marmoset detrusor smooth muscle. The potency order of ATP = 2-MeSATP $\geqslant$ ADP $>>$ α,β-MeATP, the insensitivity of the relaxation response to desensitization with α,β-MeATP (Figure 3b), the antagonism by suramin (Figure 4b) and cibacron blue 3GA (Figure 5b) and lack of antagonism by 8-PT (Figure 2), and the significant effect of inactivating G-proteins within the system (GDPβS, Figure 3a) allow us to conclude that these receptors are P2Y-like in nature. Previous investigations on the action of suramin at P2Y receptors have provided a wide range of pA_2 values, 5.0 ($-\log K_B$, 100 μM) (Bültmann *et al*., 1996), 5.0 (Hoyle *et al*., 1990), 5.3 ($-\log K_B$, 100 μM) (Bültmann *et al*., 1996), 5.4 (Boyer *et al*., 1994) and 5.52 (Piper & Hollingsworth, 1995), the figure calculated from the shift in the CRC in the present study ($-\log K_B$ 5.1) agrees reasonably well with the lower values obtained. Similarly, the literature indicates the highly variable results obtained when calculating pA_2 values for the antagonism of P2Y receptors with cibacron blue 3GA/reactive blue 2, 4.7 ($-\log K_B$) (Fuder & Muth, 1993), 5.3 (von Kügelgen *et al*., 1994), 5.4 ($-\log K_B$) (Hoyle & Edwards, 1992), 5.44 and 5.71 (Houston *et al*., 1987) and 6.0 (Fieber & Adams, 1991). However, the majority of these constants are at around 5.5, agreeing strongly with the present finding ($-\log K_B$ 5.5).

Although the use of various agonists to evoke tissue responses with the subsequent establishment of an agonist potency order has been the traditional method of defining P2 receptor subtypes (Burnstock & Kennedy, 1985), the effect of tissue exonucleotidase activity is now realized to be an important factor in masking the true agonist potency order. In particular, such activity has substantial affects on those responses evoked by agonists susceptible to breakdown (Kennedy & Leff, 1995). With this in mind more recent classification criteria have been suggested which rely mainly on structural data from cloned receptors (Fredholm *et al*., 1997). Despite the obvious clarity which such an approach offers the field it should also be taken into account that the functional effects of receptor activation within the tissue of interest remains important. As regards the present work, the receptors studied have been typed mainly on the lack of effect of adenosine receptor antagonists, and the effects of P2 receptor antagonists and G-protein inactivation, as was considered reasonable by previous investigators (Fredholm *et al*., 1997).

The G-protein linked second messenger system involved in the ATP-induced relaxation was also studied. Neither L-NOARG nor methylene blue had any effect on the ATP-mediated relaxation, refuting any possibility that the response was due to ATP-induced nitric oxide release, or that the second messenger system involved guanylyl cyclase activation and production of cyclicGMP. In the presence of TTX and after depolarization of the nerve fibres within the tissue, by exposure to 40 mM K^+ Krebs, the relaxation to ATP was unaffected, suggesting that this response was not mediated through the action of ATP on ganglionic receptors in the bladder wall or on the presynaptic membrane, causing subsequent release of an inhibitory transmitter, but more likely that the effect of ATP on the tissue was through a direct interaction of the bath-applied ATP with receptors on the muscle cells. The effect of TPCK (3 μM), which at this concentration has been shown to antagonize the effects of drugs known to act through the activation of cyclicAMP-dependent protein kinase A (PKA) (Jarvest *et al*., 1982; Van Haarstert *et al*., 1984; Parker-Botelho *et al*., 1988; Potter, 1990), suggests the involvement of cyclicAMP in activating PKA, which in turn causes phosphorylation and hence inactivation of myosin light chain kinase and so leads to muscle relaxation. Such a finding correlates well with the recent discovery that the ATP-induced relaxation in smooth muscle of the mouse vas deferens is mediated through a P2Y receptor coupled to a G-protein which activates adenylyl cyclase, so raising levels of cyclicAMP (Gailly *et al*., 1993). It is not possible to conclude from the present experiments whether the P2Y receptor in marmoset bladder smooth muscle also functions through activation of adenylyl cyclase, although this appears likely.

The presence of P2Y receptors on bladder smooth muscle cells has only recently come to light and the possible function of such receptors remains unknown. However, the presence of a relaxation mechanism in the urinary bladder has long been the grail of a number of scientists wishing to provide a neuronal basis for the control of bladder filling and accommodation. A non-adrenergic, non-cholinergic nerve mediated relaxation has been described in pig and human detrusor (Klarskov, 1987), although these findings have proven

difficult to reproduce. Presently, there is no evidence to suggest that the P2Y receptors detected in the marmoset, or those previously detected in mouse and rat urinary bladder (Boland *et al*., 1993; Bolego *et al*., 1995), are activated by neuronally released ATP; with this in mind a possible contribution of these receptors to a neuronal control mechanism of bladder filling must be questioned. It has been suggested previously that the P2Y receptors found to be present on rat bladder smooth muscle may be modulated by excitatory factors released from the epithelium (Bolego *et al*., 1995). This is not unlikely, particularly in the bladders of small mammals which are known to be reasonably thin walled. Such a theory also raises the question as to whether P2Y receptors could be activated by non-neuronally released ATP. In support of this, ATP has been shown to be released from a non-neuronal source in rabbit urinary bladder through the effects of electrical stimulation (Chaudhry *et al*., 1984; Ferguson *et al*., 1997), as well as through increases in muscle tension or changes in pH (Ferguson *et al*., 1997), and a low basal release of ATP has been measured in guinea-pig bladder (Burnstock *et al*., 1978). It is plausible that during intense contraction or high levels of tension in the bladder such release of ATP may act on P2Y receptors to dampen the contractile effect and protect the muscle cells against damage. Whether such an effect would prove important during bladder filling and accommodation is also interesting. Given the widespread presence of this receptor subtype in the bladder of a number of animal species, including a primate, the question arises as to whether this is also the case in human detrusor, which we are presently investigating. Furthermore, considering the finding that purinergic innervation may be present in the urinary bladders of patients suffering from interstitial cystitis (Palea *et al*., 1993), the possible contribution or effects of P2Y receptors in this and other bladder conditions which exhibit abnormal innervation deserves investigation, as does the potential of such receptors as possible pharmacological targets in the treatment of unstable bladder contractions.

In conclusion, we have shown for the first time the presence of two purinoceptor subtypes in the urinary bladder of the marmoset (*Callithrix jacchus*), one mediating contraction (P2X) and the other mediating relaxation (P2Y). The results presented suggest the mechanism of relaxation to be through the action of ATP on a G-protein coupled receptor which, subsequent to agonist binding, activates cyclicAMP production and in turn, cyclicAMP-dependent PKA. The widespread nature of relaxation mediating purinoceptors in the urinary bladders of a number of species is now becoming apparent, and the mechanisms and function of these receptors in health and disease deserves further investigation.

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