British Journal of Pharmacology (1996) 118, 537-542

Evidence for two distinct P_2-purinoceptors subserving contraction of the rat anococcygeus smooth muscle

[1]Alicja T. Najbar, C.G. Li & Michael J. Rand

Pharmacology Research Unit, Department of Medical Laboratory Science, RMIT University, GPO Box 2476V, Melbourne 3001, Vic., Australia

1 The effects of the P_2-purinoceptor agonists, adenosine 5'-triphosphate (ATP), α,β-methylene ATP (α,β-MeATP), β,γ-methylene ATP (β,γ-MeATP), L-β,γ-methylene ATP (L-β,γ-MeATP), adenosine-5'-O-(2-thiodiphosphate) (ADPβS), and 2-methylthio ATP (2-MeSATP) were investigated on the isometric tension of the rat anococcygeus muscle.

2 Non-cumulative additions of ATP (100-1500 μM), α,β-MeATP (1-300 μM), β,γ-MeATP (10-300 μM), L-β,γ-MeATP (3-100 μM) and ADPβS (1-100 μM) produced concentration-dependent contractions, whereas 2-MeSATP (1-100 μM) had no effect. The rank order of potency was α,β-MeATP > L-β,γ-MeATP ≥ ADPβS > β,γ-MeATP >> ATP>2-MeSATP.

3 Contractions to cumulative additions of ATP, α,β-MeATP, β,γ-MeATP and L-β,γ-MeATP were subject to desensitization whilst those to ADPβS were unaffected.

4 Contractions to ATP, α,β-MeATP, β,γ-MeATP and ADPβS were abolished by the non-selective P_{2X}/P_{2Y}-purinoceptor antagonist, suramin (100 μM). In contrast, contractions to ATP, α,β-MeATP and β,γ-MeATP were not affected by the non-selective P_1-purinoceptor antagonist 8-(*p*-sulphophenyl)-theophylline (8SPT, 30 μM). Blockade of P_{2X}-purinoceptors with the selective P_{2X}-purinoceptor antagonist pyridoxal-phosphate-6-azophenyl-2',4'-disulphonic acid (PPADS, 10 μM) or desensitization with L-β,γ-MeATP (10 μM) abolished contractions to α,β-MeATP, but enhanced those to ADPβS. The P_{2Y}-purinoceptor antagonist, reactive blue 2 (RB2, 100 μM) enhanced contractions to ATP and α,β-MeATP but abolished those to ADPβS.

5 Simultaneous addition of α,β-MeATP and ADPβS produced an additive contraction.

6 The findings suggest that in the rat anococcygeus, smooth muscle cells are endowed with two distinct P_2-purinoceptors which subserve contractions: a P_{2X}-purinoceptor activated by ATP and its analogues, and another type of P_2-purinoceptor activated by ADPβS.

Keywords: P_{2X}-purinoceptors; P_{2Y}-purinoceptors; ATP/ADP analogues; suramin; reactive blue 2; PPADS; 8SPT; anococcygeus muscle (rat).

Introduction

Adenine nucleotides and nucleosides, such as ATP and adenosine, exert their pharmacological actions by activating purinoceptors. Purinoceptors were originally divided into two main classes, P_1 and P_2 (Burnstock, 1978). P_2-purinoceptors present on smooth muscle have been further divided into P_{2X} and P_{2Y} subtypes, primarily based upon the relative rank order of potency of ATP and its analogues (Burnstock & Kennedy, 1985; Kennedy, 1990). It is well known that responses produced by activation of P_{2X}, but not P_{2Y}-purinoceptors, are desensitized upon repeated exposure to or in the continued presence of P_{2X}-purinoceptor agonists, such as α,β-methylene ATP (α,β-MeATP) and L-β,γ-methylene ATP (L-β,γ-MeATP) (Burnstock & Kennedy, 1985; Kennedy, 1990). According to the original classification, the rank order of agonist potency at P_{2X}-purinoceptors is α,β-MeATP ≥ β,γ-methylene ATP (β,γ-MeATP), L-β,γ-MeATP > ATP ≥ 2-methylthio ATP (2-MeSATP), adenosine-5'-O-(2-thiodiphosphate) (ADPβS), whilst at P_{2Y}-purinoceptors the potency order is 2-MeSATP > ADPβS > ATP >> α,β-MeATP ≥ β,γ-MeATP, L-β,γ-MeATP (Burnstock & Kennedy, 1985; Burnstock, 1991).

Previously, it has been reported that ATP produces contractions of the rat anococcygeus muscle, whereas adenosine is without effect, indicating the presence of P_2-purinoceptors on smooth muscle (Stone, 1983). More recently, we showed that the stable analogue of ATP, α,β-MeATP also produced contractions of the rat anococcygeus muscle (Najbar *et al.*, 1994) and was considerably more potent than ATP, suggesting an action at P_{2X}-purinoceptors; however, the subtype of the receptor involved was not fully characterized. Thus, the aim of the present study was to characterize the purinoceptor subtypes subserving contraction of the rat anococcygeus muscle.

Methods

Male Sprague-Dawley rats (240-400 g) were killed by decapitation and the anococcygeus muscles were isolated as described previously (Najbar *et al.*, 1994). Each muscle was mounted for isometric recording under a resting tension of 1 g in an organ bath containing 6 ml of physiological salt solution (PSS) of the following composition (mM): NaCl 118; KCl 4.7 $NaHCO_3$ 25, $MgSO_4$ 0.45, KH_2PO_4 1.03, $CaCl_2$ 2.5, D-(+)-glucose 11.1, disodium edetate 0.067 and ascorbic acid 0.14. The PSS was gassed with 95% O_2 and 5% CO_2 and maintained at 37°C. The tissue was allowed to equilibrate for 30 min before any experimental procedures were commenced. During the equilibration period, the PSS in the organ bath was replaced every 10 min.

When agonist drugs were added non-cumulatively, the tissue was exposed to each concentration for a period of 5-8 min and a 20 min period with repeated washings was allowed between applications: it had been previously determined that this procedure prevent desensitization. When agonist drugs were added cumulatively, the concentration was increased at 3-5 min intervals. In a separate series of experiments, a paired design was employed to test the effects of the antagonist drugs: after determining the effect of an agonist drug at a submaximal

[1] Author for correspondence.

concentration, the tissue was washed at least 3 times with fresh PSS and a period of at least 20 min was allowed for recovery of the tissue; an antagonist drug was then added and the effect of the agonist drug used previously was determined again. Corresponding time controls for the agonists in the absence of antagonists were performed on another preparation. The effect of an antagonist drug on a whole concentration-response curve to a given agonist drug could not be tested in the same preparation since desensitization occurred on cumulative addition of most of the agonist drugs, whereas with the non-cumulative addition procedure, the tissue did not survive the long period of the experiment required.

Pre-testing exposure times and concentrations for the antagonist drugs were: 10 min for 8-(*p*-sulphophenyl)-theophylline (8SPT, 30 μM), which effectively blocks the P_1-purinoceptors present on the noradrenergic nerves in this tissue (Najbar *et al.*, 1994); although it has been reported that suramin equilibrates with P_2-purinoceptors very slowly (Dunn & Blakely 1988; Leff *et al.*, 1990; Hoyle *et al.*, 1990), in preliminary experiments it was found that a 30 min pre-exposure to 100 μM suramin was sufficient to abolish contractile responses produced by α,β-MeATP (10 μM) in this tissue; 60 min for pyridoxal-phosphate-6-azophenyl-2'-4'-disulphonic acid (PPADS, 10 μM), which is also reported to be a slowly equilibrating antagonist in other tissues (Lambrecht *et al.*, 1992; Ziganshin *et al.*, 1994b) and 20–30 min for reactive blue 2 (RB2, 50 and 100 μM), which blocks P_{2Y}-purinoceptor-mediated responses in other tissues (Choo, 1981; Burnstock & Warland, 1987). When used as a desensitizing agonist, L-β,γ-MeATP was applied either at a single dose (10 μM) or in two cumulative doses (5 μM each, 7.5 min apart); with both procedures total pre-exposure time was 15 min and it was established that desensitization of the P_{2X}-purinoceptor occurred.

In another series of experiments, contractions to a submaximal concentration of each of α,β-MeATP and ADPβS were obtained which produced contractions of similar sizes in each preparation; subsequently, the two agonist drugs were added simultaneously to the organ bath to assess whether they produced an additive effect.

Drugs

The following drugs were used: ATP disodium salt, α,β-MeATP lithium salt, β,γ-MeATP sodium salt, ADPβS trilithium salt and (−)-noradrenaline bitartrate were obtained from Sigma (St. Louis, MO, U.S.A.); L-β,γ-MeATP tetrasodium salt, 2-MeSATP tetrasodium salt, PPADS tetrasodium salt and 8SPT were obtained from Research Biochemicals Inc. (Natick, MA, USA); suramin was a gift from Bayer (Leverkusen, Germany); RB2 was obtained from Aldrich Chemical Company Inc. (Milwaukee, WIS, U.S.A.).

All drugs, except 8SPT were dissolved in distilled water and diluted in PSS. 8SPT was dissolved in dimethylsulphoxide (DMSO, Ajax Chemicals, Sydney, NSW, Australia) and diluted in PSS. The final concentrations of DMSO in the organ bath was 0.05%. DMSO had no effect on the contractions to any of the agonist drugs used.

Statistical analysis of results

Data are expressed as means ± standard error of means (s.e. mean); *n* indicates the number of experiments. Increases in tension induced by agonist drugs were measured in grams (g). The statistical significance of differences between means was determined by performing a one-way or two-way analyses of variance. Where significant differences within groups were identified, planned comparisons (paired or unpaired Student's *t* test, based on pooled variance estimates) were performed to test for differences between pre-determined pairs of means. Probability levels less than 0.05 were considered significant.

Results

Effects of agonist drugs

Non-cumulative additions of ATP, α,β-MeATP, β,γ-MeATP, L-β,γ-MeATP and ADPβS produced concentration-dependent contractions of the rat anococcygeus muscle, as shown for ATP, α,β-MeATP and ADPβS in Figure 1 and for all the agonists in Figure 2. 2-MeSATP (1–30 μM) had no effect on the resting tension ($n=5$) and caused weak contractions (0.1–0.5 g) only at high concentrations (100 or 300 μM, $n=5$) (not shown). The rank order of agonist potency was α,β-MeATP > L-β,γ-MeATP $\geqslant$ ADPβS > β,γ-MeATP >> ATP > 2-MeSATP. In corresponding time control experiments, contractions to a single application of each agonist drug were reproducible with no evidence of desensitization. The contractions produced by L-β,γ-MeATP and β,γ-MeATP (records not shown) were similar in nature to those produced by α,β-MeATP (Figure 1b): contractions to all concentrations were immediate in onset; with lower concentrations the contractions were transient, whereas with higher concentrations they remained sustained until washout of the drugs. The contractions produced by ADPβS also partly resembled those produced by α,β-MeATP; however, the later sustained phase of the contraction was observed with *all* concentrations of ADPβS (Figure 1c). Contractions produced by ATP were different in nature from those produced by the other analogues: they were slower in onset at the lower concentrations, becoming more immediate in onset at higher concentrations, and were generally more sustained (Figure 1a).

When ATP, α,β-MeATP, β,γ-MeATP and L-β,γ-MeATP were added cumulatively, desensitization to their contractions was evident, as shown in Figure 2 and Figures 3a and 3b. However, the desensitization occurring with ATP and β,γ-MeATP was less apparent at higher concentrations of these agonists and was not as marked as that occurring with α,β-MeATP or L-β,γ-MeATP. In contrast, cumulative addition of ADPβS did not result in desensitization as shown in Figures 2e and 3c. Note, that maximal effects of all agonists were not determined (due to expense of some of the agonists); thus, in Figure 2, the apparent differences in maximal effects of the agonists may not be real.

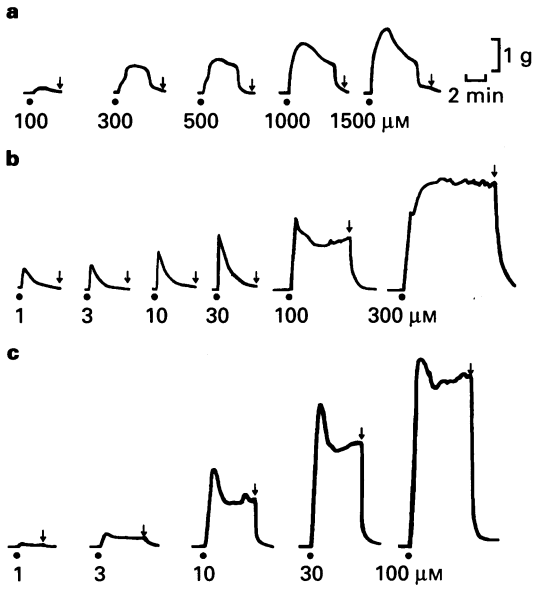

Figure 1 Recordings of the contractile effects of non-cumulative applications of **(a)** ATP, **(b)** α,β-MeATP and **(c)** ADPβS in rat anococcygeus muscle preparations; (●) indicates addition of each agonist drug and ↓ indicates washout of drug. Note, 20 min washout periods were allowed between successive applications of each agonist drug; thus, the time scale pertains only to the contraction phase. The time and tension scales apply to all records.

Effects of antagonist drugs on contractions produced by agonist drugs

In time control experiments, contractions to all agonists were reproducible, and did not differ significantly from initial (control) values ($P>0.05$; paired t tests), regardless of the time between the two applications of each agonist drug which ranged from 15 to 60 min. The resting tension of the anococcygeus muscle was not affected by DMSO (0.05%) or any of the antagonist drugs at the concentrations used.

The non-selective P_1-purinoceptor antagonist, 8SPT (30 μM) had no significant effect on contractions produced by

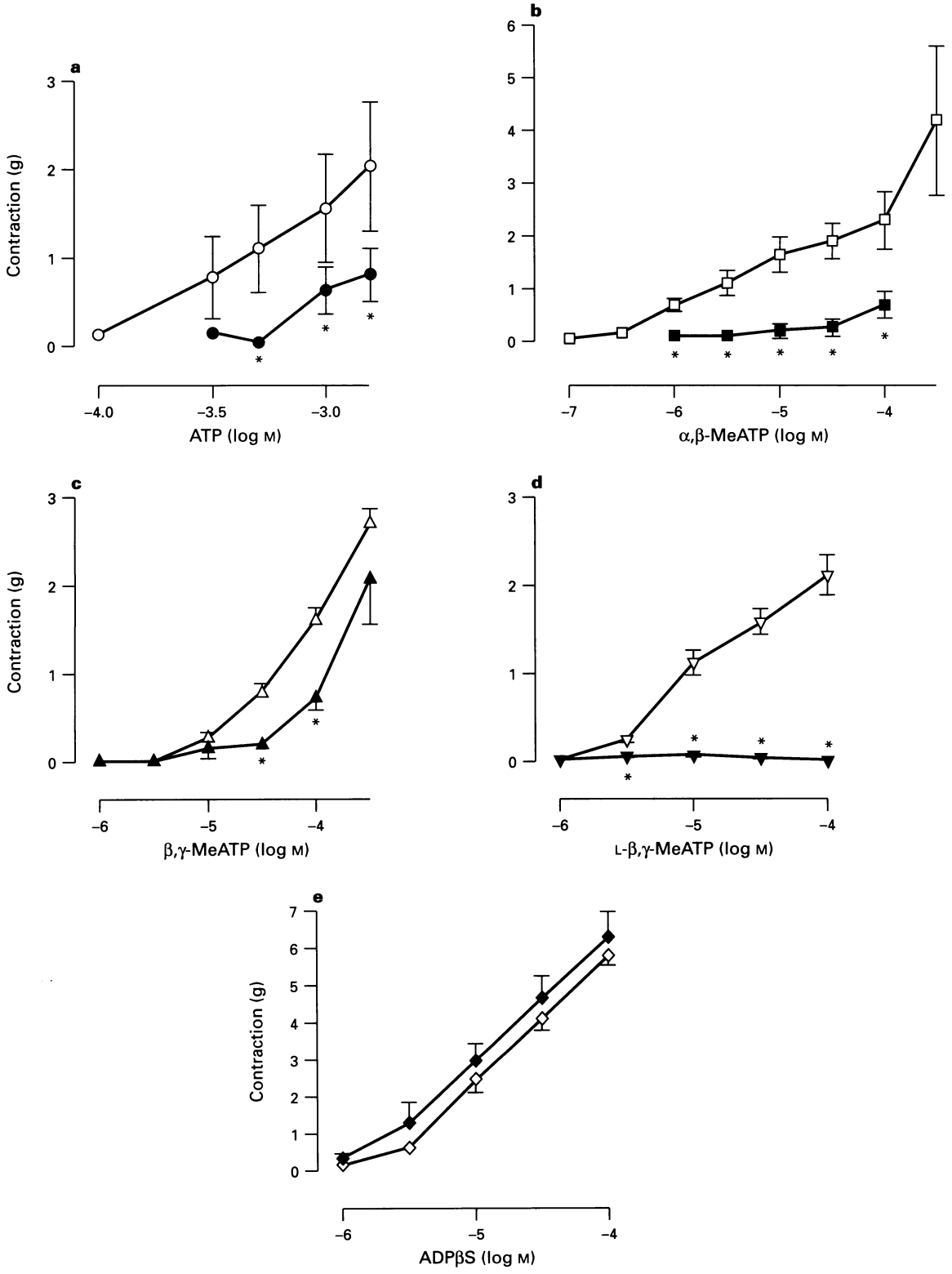

Figure 2 Effects of **(a)** ATP (100–1500 μM), **(b)** α,β-MeATP (0.1–300 μM), **(c)** β,γ-MeATP (1–300 μM), **(d)** L-β,γ-MeATP (1–100 μM) and **(e)** ADPβS (1–100 μM), applied non-cumulatively (open symbols) or cumulatively (closed symbols), in rat anococcygeus muscle preparations. Each value represents the mean ± s.e.mean (g); $n=3-6$ preparations. For each agonist drug, significant differences between cumulative and non-cumulative additions are indicated by asterisks (*$P<0.05$).

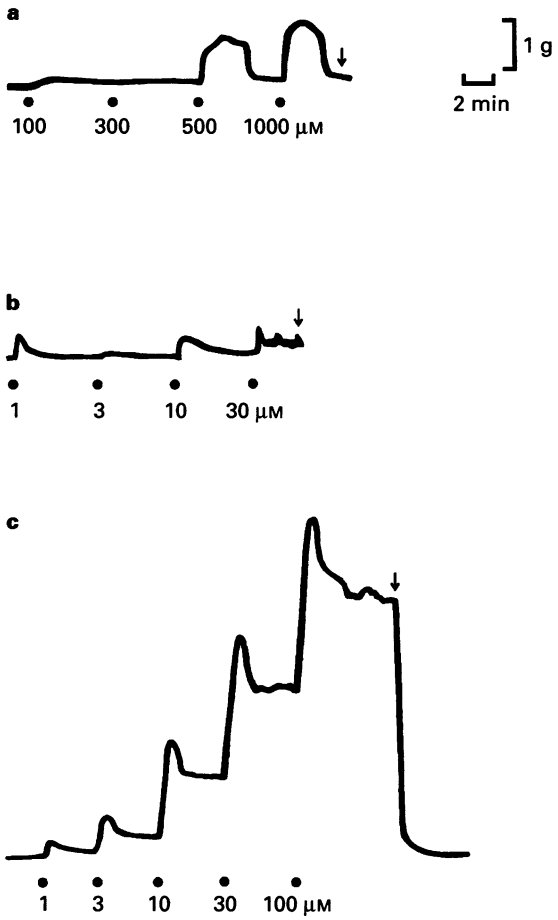

Figure 3 Recordings of the effects of cumulative applications of **(a)** ATP, **(b)** α,β-MeATP and **(c)** ADPβS in rat anococcygeus muscle preparations. Records **(a)**, **(b)** and **(c)** were taken, respectively, from the same anococcygeus muscle preparation as those in Figure 1. (●) Indicates addition of each agonist drug and ↓ indicates washout of drug. The time and tension scales apply to all records.

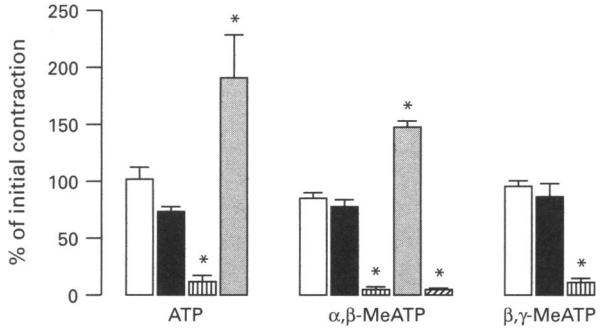

Figure 4 Effects of 8SPT (30 μM, solid columns), suramin (100 μM, vertically hatched columns), RB2 (100 μM, grey shaded columns) and PPADS (10 μM, diagonally hatched column) on contractions produced by ATP (500 μM), α,β-MeATP (10 μM) and β,γ-MeATP (100 μM) in rat anococcygeus muscle preparations. Open columns represent time controls for each agonist drug, in the absence of antagonist drugs. Results are expressed as % of the initial contraction. Each column represents the mean ± s.e.mean; $n = 3-6$ preparations. Significant differences from corresponding time controls are indicated by asterisks ($^*P < 0.05$).

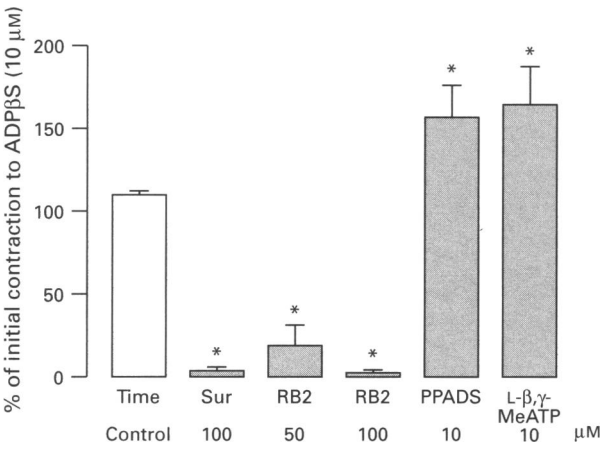

Figure 5 Effects of suramin (Sur, 100 μM), RB2 (50 μM and 100 μM), PPADS (10 μM) and desensitization with L-β,γ-MeATP (10 μM) on the contraction produced by ADPβS (10 μM) in rat anococcygeus muscle preparations. The open column represents the time control to the contraction produced by ADPβS (10 μM) in the absence of antagonist drugs, and shaded columns indicate the contraction in the presence of antagonist drugs. Results are expressed as a % of the initial contraction. Each column represents the mean ± s.e.mean; $n = 4-9$ preparations. Significant differences from control are indicated by asterisks ($^*P < 0.05$).

ATP (500 μM), α,β-MeATP (10 μM) or β,γ-MeATP (100 μM), whereas the non-selective P_{2X}/P_{2Y}-purinoceptor antagonist suramin (100 μM) virtually abolished contractions to all these agonist drugs. The selective P_{2X}-purinoceptor antagonist, PPADS (10 μM) also abolished contractions to α,β-MeATP. In contrast, the selective P_{2Y}-purinoceptor antagonist RB2 (100 μM) significantly enhanced the contractions produced by ATP (500 μM) and α,β-MeATP (10 μM) (Figure 4). The contractions to ADPβS (10 μM) were also greatly reduced or abolished by suramin (100 μM). However, in contrast to the other agonist drugs, contractions to ADPβS were also significantly reduced or abolished by RB2 (50 and 100 μM), and were enhanced by PPADS (10 μM) and desensitization with L-β,γ-MeATP (10 μM) (Figure 5).

Since the selectivity of RB2 for the P_{2Y}-purinoceptor has been questioned (Kennedy, 1990; Bültmann & Starke, 1994; Kennedy & Leff, 1995), we tested the effect of 100 μM RB2 on contractions to a submaximal concentration of noradrenaline (3 μM). In the presence of RB2, the contractions to noradrenaline were 95 ± 5% ($n = 3$) of initial contractions, while in time control experiments, the contractions were 100 ± 5% ($n = 4$) of initial contractions ($P > 0.05$, unpaired t tests).

Effect of a simultaneous addition of α,β-MeATP and ADPβS

In concentrations in which α,β-MeATP (10 μM) and ADPβS (6–20 μM) produced contractions of similar sizes (1.8 ± 0.2 g and 1.8 ± 0.3 g, respectively, $n = 6$, $P > 0.05$, paired t tests), simultaneous addition of the agonists produced a contraction of 3.8 ± 0.4 g, which was equal to the algebraic sum of the contractions produced by each drug alone, 3.6 ± 0.5 g ($n = 6$, $P > 0.05$, paired t tests).

Discussion

The present study shows for the first time, that a number of ATP analogues produce concentration-dependent contractions of the resting tension of the rat anococcygeus muscle, confirming the presence of P_2-purinoceptors on the smooth muscle. The rank order of agonist potency was α,β-MeATP > L-β,γ-MeATP ⩾ ADPβS > β,γ-MeATP >> ATP > 2-MeSATP. This rank order of agonist potency does not correspond to either the P_{2X}- or the P_{2Y}-purinoceptor subtypes, as originally classified (Burnstock & Kennedy, 1985; Burnstock, 1991). However, since some purinoceptor agonists, such as ATP and 2-MeSATP may be susceptible to considerable de-

gradation by nucleotidases in some tissues, their true potency may be underestimated (Evans & Kennedy, 1994; Trezise *et al.*, 1994; Crack *et al.*, 1995; Kennedy & Leff, 1995). In addition, a differential rank order of agonist potency may be observed when heterogeneous purinoceptor populations exist in the same tissue (for example, Palea *et al.*, 1994; Knight & Burnstock, 1995). Thus, P_2-purinoceptors are more adequately classified by use of antagonists, rather than rank order of agonist potency. In the present study, evidence obtained with antagonist profiles suggests that P_{2X}-purinoceptors are present in the rat anococcygeus muscle which subserve contraction. This is based on the findings that contractions produced by ATP, α,β-MeATP and β,γ-MeATP were blocked by the non-selective P_{2X}/P_{2Y}-purinoceptor antagonist suramin (Dunn & Blakely, 1988; Leff *et al.*, 1990), but were not affected by the P_1-purinoceptor antagonist 8SPT (Bruns *et al.*, 1986); furthermore, contractions to ATP and α,β-MeATP were not reduced by the P_{2Y}-purinoceptor antagonist RB2 (Choo, 1981; Burnstock & Warland, 1987), but those to α,β-MeATP were abolished by the selective P_{2X}-purinoceptor antagonist PPADS (Lambrecht *et al.*, 1992; Ziganshin *et al.*, 1994b). The presence of P_{2X}-purinoceptors is further confirmed by the manifestation of desensitization to contractions produced by cumulative additions of ATP, α,β-MeATP, β,γ-MeATP and L-β,γ-MeATP. The reason for less marked desensitization occurring with ATP and β,γ-MeATP than with α,β-MeATP and L-β,γ-MeATP, is probably due to the fact that the former agonists are more susceptible to degradation by nucleotidases than the latter agonists (Welford *et al.*, 1986; Cusack, 1993).

In contrast to the contractions produced by α,β-MeATP (and ATP), contractions to ADPβS, although blocked by suramin, showed the opposite sensitivity to the P_2 purinoceptor subtype selective antagonists. Thus, RB2 abolished contractions to ADPβS but did not reduce contractions to α,β-MeATP (and ATP), while PPADS abolished contractions to α,β-MeATP but did not reduce contractions to ADPβS. Furthermore, unlike contractions to α,β-MeATP and the other purinoceptor agonists, contractions to ADPβS were not subject to desensitization and, in addition, were not reduced by desensitization of the P_{2X}-purinoceptor with L-β,γ-MeATP. Moreover, the simultaneous addition of ADPβS and α,β-MeATP produced an additive effect. These observations suggest that ADPβS produces its contraction by activation of a receptor which is different from P_{2X}-purinoceptors.

There are several possible mechanisms underlying contractions produced by ADPβS in the anococcygeus muscle. Firstly, since RB2 abolished contractions to ADPβS, it may act on a P_{2Y}-like purinoceptor. The concentration of RB2 used was selective as it did not affect responses to noradrenaline. However, it is not clear whether there are P_{2Y}-purinoceptors subserving contraction in this tissue as 2-MeSATP and ATP were considerably less potent than ADPβS. It is possible that the low potency of 2-MeSATP and ATP was due to their degradation (see above), whereas ADPβS is relatively resistant to degradation (Welford *et al.*, 1986; Cusack, 1993). In preliminary studies, we found that contractions to ATP were enhanced after blockade of 5′-nucleotidase with α,β-methylene adenosine 5′-diphosphonate, suggesting that nucleotidase activity in this tissue may impede the contractile action of ATP. However, even after preventing the breakdown of ATP, it was still considerably less potent than ADPβS. Furthermore, since contractions to ATP were subject to desensitization and were not reduced by RB2 (see above), ATP was probably acting on P_{2X}-purinoceptors. Overall, it is unlikely that P_{2Y}-purinoceptors subserving contractions to ATP are present in the rat anococcygeus muscle.

Alternatively, since it was recently proposed that there are further subtypes of the P_{2Y}-purinoceptor (Abbracchio & Burnstock, 1994), it is possible that in the rat anococcygeus muscle ADPβS acts on a subtype of the P_{2Y}-purinoceptor which is insensitive to ATP (and 2-MeSATP). ADPβS is a potent agonist at one of these subtypes, formerly known as the P_{2D}-purinoceptor, where the rank order of agonist potency is generally diadenosine tetraphosphate (Ap_4A) > ADPβS > α,β-MeATP > 2-MeSATP (Castro *et al.*, 1992; Pintor *et al.*, 1993; Fredholm *et al.*, 1994). Indeed, Ap_4A produced concentration-dependent contractions of the rat anococcygeus muscle. However, the rank order of agonist potency was α,β-MeATP > ADPβS > Ap_4A (Najbar, Li and Rand unpublished observations). Although the possibility exists that the low potency of Ap_4A was due to its degradation, there are no reports that suramin and RB2 antagonize this receptor subtype. Thus, it is unlikely that in the rat anococcygeus muscle ADPβS acts on P_{2D}-purinoceptors. Further investigation is required to examine the possibility that ADPβS acts on another subtype of the P_{2Y}-purinoceptor.

Alternatively, ADPβS could be acting on a novel subtype of the P_2-purinoceptor, not described previously. A recent study found that in the human bladder, ADPβS produced contractions by activation of a novel purinoceptor, which was different from the P_{2X}- or the P_{2Y}-subtypes (Palea *et al.*, 1994). The rank order of agonist potency was ADPβS = α,β-MeATP > 2-MeSATP $\geqslant$ ATP, which is similar to that obtained in the present study; however, in contrast to the findings of the present study, contractions to ADPβS were not reduced by RB2. It is not clear whether this is due to the low concentration of RB2 used (10 μM). ADPβS (1 – 100 μM) also produced contractions in the rat bladder, through receptors which were different from P_{2X}- or P_{2Y} subtypes (Suzuki & Kokobun, 1994; Bolego & Abbracchio, 1995), but which were blocked by RB2 (also known as cibacron blue 3GA).

Another finding of the present study is the interaction between two different purinoceptors activated by α,β-MeATP and ADPβS. Thus, blockade of the receptor for ADPβS with RB2 potentiated contractions to α,β-MeATP (and ATP), whereas blockade of the P_{2X}-purinoceptor with PPADS or desensitization with L-β,γ-MeATP potentiated contractions to ADPβS. Hence, although the two agonists appear to act through different sites at the receptor level, they may influence each other's actions at the post-receptor level. Whether the P_{2X}-purinoceptor and the purinoceptor for ADPβS are linked to different effector systems remains to be established. Alternatively, the potentiation of the contractions to ATP, α,β-MeATP and ADPβS by the respective agents suggests that these agonists may have also acted on purinoceptors subserving relaxation, and this would counteract their contractile effects. However, this is unlikely since in precontracted rat anococcygeus muscles ATP produced weak relaxations or small contractions (Najbar *et al.*, 1994), whereas α,β-MeATP and ADPβS produced contractions (Najbar, Li and Rand, unpublished observations).

It was recently reported, that the ecto-ATPase inhibitory property of suramin limits its use as a purinoceptor antagonist to studies with metabolically stable ATP analogues (Crack *et al.*, 1994). However, in the rat anococcygeus muscle, any ecto-ATPase inhibitory activity suramin may have had was not apparent, since suramin abolished contractions to ATP. The difference could be due either to a species or a tissue difference, since ecto-ATPases can have different activities in different tissues (Cusack, 1993; Ziganshin *et al.*, 1994a).

The contraction produced by ATP differed in nature from the contractions produced by the analogues. This difference could be due to the fact that ATP is rapidly degraded (as mentioned above) to ADP, AMP and adenosine; thus, the degradation products of ATP may contribute to its observed response. However, 5′-AMP and adenosine do not contract this tissue in concentrations up to 1 mM (Stone, 1983). In preliminary experiments, it was found that ADP in the concentration range of 0.1 – 1 mM, produced transient contractions of the rat anococcygeus muscle, which were similar to those produced by ATP; thus ADP could contribute to the contraction to ATP.

In conclusion, the findings of this study suggest that smooth muscle cells in the rat anococcygeus are endowed with two distinct P_2-purinoceptors which subserve contractions: P_{2X}-

purinoceptors activated by ATP and its analogues and another type of P_2-purinoceptor activated by ADPβS. Further investigation is required to determine whether ADPβS acts on a P_{2Y}-like receptor or on a novel subtype of the P_2-purinoceptor, as well as the mechanism of interaction between its receptor and the P_{2X}-purinoceptor.

This work was supported by a grant from the National Health & Medical Research Council. A.N. is in receipt of a Postgraduate Scholarship awarded by the Smoking & Health Research Foundation of Australia. We thank Bayer (Germany) for the generous gift of suramin. We are also grateful to Dr M.E. Fabiani for his useful comments and corrections of the manuscript.

References

ABBRACCHIO, M.P. & BURNSTOCK, G. (1994). Purinoceptors: are there families of P_{2X} and P_{2Y} purinoceptors? *Pharmacol. Ther.*, **64**, 445–475.

BOLEGO, C. & ABBRACCHIO, M.P. (1995). Effects of ADPβS and UTP on the rat urinary bladder smooth muscle. *Res Commun. Mol. Pathol. Pharmacol.*, **87**, 75–76.

BRUNS, R.F., LU, G.H. & PUGSLEY, T.A. (1986). Characterisation of the A_2 adenosine receptor labelled by [^{3}H]NECA in rat striatal membranes. *Mol. Pharmacol.*, **29**, 331–346.

BÜLTMANN, R. & STARKE, K. (1994). P_2-purinoceptor antagonists discriminate three contraction-mediating receptors for ATP in rat vas deferens. *Naunyn-Schmied. Arch. Pharmacol.*, **349**, 74–80.

BURNSTOCK, G. (1978). A basis for distinguishing two types of purinergic receptor. In *Cell Membrane Receptors for Drugs and Hormones: A Multidisciplinary Approach.* ed. Straub, R.W. & Bolis, L. pp. 107–118. New York: Raven Press.

BURNSTOCK, G. (1991). Distribution and roles of purinoceptors subtypes. *Nucleos. Nucleot.*, **10**, 917–930.

BURNSTOCK, G. & KENNEDY, C. (1985). Is there a basis for two types of P_2-purinoceptor? *Gen. Pharmacol.*, **16**, 433–440.

BURNSTOCK, G. & WARLAND, J.J.I. (1987). P_2 purinoceptors of two subtypes in the rabbit mesenteric artery: reactive blue 2 selectively inhibits responses mediated via P_{2Y} but not the P_{2X} purinoceptor. *Br. J. Pharmacol.*, **90**, 383–391.

CASTRO, E., PINTOR, J. & MIRAS-PORTUGAL, M.T. (1992). Ca^{2+} Stores mobilization by diadenosine tetraphosphate, Ap_4A, through a putative P_{2Y} purinoceptor in adrenal chromaffin cells. *Br. J. Pharmacol.*, **106**, 833–837.

CHOO, L.K. (1981). The effect of reactive blue, an antagonist of ATP, on isolated urinary bladders of guinea-pig and rat. *J. Pharm. Pharmacol.*, **33**, 248–250.

CRACK, B.E., BEUKERS, M.W., McKECHNIE, K.C.W., IJZERMAN, A.P. & LEFF, P. (1994). Pharmacological analysis of ecto-ATPase inhibition: evidence for combined enzyme inhibition and receptor antagonism in P_{2X}-purinoceptor ligands. *Br. J. Pharmacol.*, **113**, 1432–1438.

CRACK, B.E., POLLARD, C.E., BEUKERS, M.W., ROBERTS, S.M., HUNT, S.F., INGALL, A.H., McKECHNIE, K.C.W., IJZERMAN, A.P. & LEFF, P. (1995). Pharmacological and biochemical analysis of FPL 67156, a novel, selective inhibitor of ecto-ATPase. *Br. J. Pharmacol.*, **114**, 475–481.

CUSACK, N.J. (1993). P_2-receptor: subclassification and structure-activity relationships. *Drug Dev. Res.*, **28**, 244–252.

DUNN, P.M. & BLAKELY, A.G.H. (1988). Suramin: a reversible P_2-purinoceptor antagonist in the mouse vas deferens. *Br. J. Pharmacol.*, **93**, 243–245.

EVANS, R.J. & KENNEDY, C. (1994). Characterisation of P_2-purinoceptors in the smooth muscle of the rat tail artery: a comparison between contractile and electrophysiological responses. *Br. J. Pharmacol*, **113**, 853–860.

FREDHOLM, B.B., ABBRACCHIO, M.P., BURNSTOCK, G., DALY, J.W., HARDEN, T.K., JACOBSON, K.A., LEFF, P. & WILLIAMS, M. (1994). Nomenclature and classification of purinoceptors. *Pharmacol. Rev.*, **46**, 143–156.

HOYLE, C.H.V., KNIGHT, G.E. & BURNSTOCK, G. (1990). Suramin antagonises responses to P_2-purinoceptor agonists and purinergic nerve stimulation in the guinea-pig urinary bladder and taenia coli. *Br. J. Pharmacol.*, **99**, 617–621.

KENNEDY, C. (1990). P_1- and P_2-purinoceptor subtypes – an update. *Arch. Int. Pharmacodyn.*, **303**, 30–50.

KENNEDY, C. & LEFF, P. (1995). How should P_{2X}-purinoceptors be classified pharmacologically? *Trends Pharmacol. Sci.*, **16**, 168–174.

KNIGHT, G.E. & BURNSTOCK, G. (1995). Responses of the aorta of the garter snake (*Thamnophis sirtalis parietalis*) to purines. *Br. J. Pharmacol.*, **114**, 41–48.

LAMBRECHT, G., FRIEBE, T., GRIMM, U., WINDSCHEIF, U., BUNGARDT, E., HILDEBRANDT, C., BÄUMERT, H.G., SPATZ-KÜMBEL, G. & MUTSCHLER, E. (1992). PPADS, a novel functionally selective antagonist of P_2 purinoceptor-mediated responses. *Eur. J. Pharmacol.*, **217**, 217–219.

LEFF, P., WOOD, B.E. & O'CONNOR, S.E. (1990). Suramin is a slowly equilibrating but competitive antagonist at P_{2X}-receptors in the rabbit isolated ear artery. *Br. J. Pharmacol.*, **101**, 645–649.

NAJBAR, A., LI, C.G. & RAND, M.J. (1994). Pharmacological actions of the coenzymes NAD(H) and NADP(H) on the rat anococcygeus muscle. *Clin. Exp. Pharmacol. Physiol.*, **21**, 9–20.

PALEA, S., CORSI, M., PIETRA, C., ARTIBANI, W., CALPISTA, A., GAVIRAGHI, G. & TRIST, D.G. (1994). ADPβS induces contraction of the human isolated urinary bladder through a purinoceptor subtype different from P_{2X} and P_{2Y}. *J. Pharmacol. Exp. Ther.*, **269**, 193–197.

PINTOR, J.M., DIAZ-REY, M.A. & MIRAS-PORTUGAL, M.T. (1993). Ap_4A and ADPβS binding to P_2 purinoceptors present on rat brain synaptic terminals. *Br. J. Pharmacol.*, **108**, 1094–1099.

STONE, T.W. (1983). Purine receptors in the rat anococcygeus muscle. *J. Physiol.*, **335**, 591–608.

SUZUKI, H. & KOKUBUN, S. (1994). Subtypes of purinoceptors in rat and dog urinary bladder smooth muscles. *Br. J. Pharmacol.*, **112**, 117–122.

TREZISE, D.J., KENNEDY, I. & HUMPHREY, P.P.A. (1994). Effects of divalent cations on the potency of ATP and related agonists in the rat isolated vagus nerve: implications for P_2 purinoceptor classification. *Br. J. Pharmacol.*, **113**, 463–470.

WELFORD, L.A., CUSACK, N.J. & HOURANI, S.M.O. (1986). ATP analogues and the guinea-pig taenia coli: a comparison of the structure-activity relationships of ectonucleotidases with those of the P_2 purinoceptor. *Eur. J. Pharmacol.*, **129**, 217–224.

ZIGANSHIN, A.U., HOYLE, C.H.V. & BURNSTOCK, G. (1994a). Ecto-enzymes and metabolism of extracellular ATP. *Drug Dev. Res.*, **32**, 134–146.

ZIGANSHIN, A.U., HOYLE, C.H.V., LAMBRECHT, G., MUTSCHLER, E., BÄUMERT, H.G. & BURNSTOCK, G. (1994b). Selective antagonism by PPADS at P_{2X}-purinoceptors in rabbit isolated blood vessels. *Br. J. Pharmacol.*, **111**, 923–929.

(Recieved September 18, 1995
Recieved January 16, 1996
Accepted February 9, 1996)