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The purinoceptors of the guinea-pig isolated taenia caeci

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Abstract

The guinea-pig taenia caeci contains both P_1 and P_2 purinoceptors mediating relaxation. The P_2 purinoceptors have been further characterized using an experimental approach designed to minimise complicating factors. In the presence of the adenosine uptake inhibitor *S*-(4-nitrobenzyl)-6-thioinosine (NBTI, 300 nM) and a pA_{100} concentration of the P_1 purinoceptor antagonist 8-sulphophenyltheophylline (140 μM), the potency order of agonists was: 2-methylthio-ATP $\gg$ adenosine 5′-triphosphate (ATP) = α,β-methylene ATP > β,γ-methylene ATP $\gg$ uridine 5′-triphosphate. Suramin antagonized ATP ($pA_2 = 5.52 \pm 0.17$, Schild plot slope = 0.67 ± 0.08) and 2-methylthio-ATP ($pA_2 = 5.78 \pm 0.30$, Schild plot slope = 1.37 ± 0.39) while responses to 5′-*N*-ethylcarboxamidoadenosine (NECA) were unaffected. The findings suggest that suramin, while it is selective for P_2 relative to P_1 purinoceptors, is not a true competitive antagonist. Pyridoxalphosphate-6-azophenyl-2′,4′-disulphonic acid (PPADS) antagonized ATP in isolated guinea-pig vas deferens, but had no effect on responses to ATP in guinea-pig taenia caeci indicating it is selective for P_{2X} relative to P_{2Y} purinoceptors.

Keywords: P_1 purinoceptor; P_{2Y} purinoceptor; Taenia caeci, guinea-pig; Suramin; Adenosine 5′-triphosphate; PPADS (pyridoxalphosphate-6-azophenyl-2′,4′-disulphonic acid)

1. Introduction

It has been known for many years that adenosine, adenosine 5′-triphosphate (ATP) and their derivatives can contract and relax various smooth muscle preparations and can influence other biological processes such as platelet aggregation, neurotransmission and cardiac function (Gordon, 1986). For a detailed review of purinoceptor classification and nomenclature see Fredholm et al. (1994).

The trypanocidal drug suramin appears to be an antagonist at P_2 purinoceptors, displaying inhibitory activity equally at both P_{2X} and P_{2Y} purinoceptors (Dunn and Blakeley, 1988; Den Hertog et al., 1989a,b; Hoyle et al., 1990; Leff et al., 1990; Von Kügelgen et al., 1990). The interaction of suramin with P_1 purinoceptors has not been studied. Pyridoxalphosphate-6-azophenyl-2′,4′-disulphonic acid (PPADS) has been reported to be an antagonist at P_{2X} purinoceptors of rabbit vas deferens (Lambrecht et al., 1992), rabbit urinary bladder (Ziganshin et al., 1993), guinea-pig vas deferens (McLaren et al., 1994) and rabbit isolated blood vessels where PPADS appears to show some selectivity for P_{2X} as opposed to P_{2Y} purinoceptors (Ziganshin et al., 1994).

The purpose of the present study was to measure the properties of agonists and antagonists at the P_{2Y} purinoceptor under experimental conditions where confounding factors which affect agonist potency, such as the presence of multiple receptor types and agonist uptake mechanisms, were minimised (O'Connor et al., 1990; Matharu and Hollingsworth, 1992). The guinea-pig taenia caeci contains a mixture of purinoceptors, P_1 (Burnstock et al., 1984) and P_{2Y} (Burnstock and Kennedy, 1985) both mediating relaxation. The P_1 purinoceptor was characterized first using adenosine and the selective P_1 purinoceptor agonist 5′-*N*-ethylcarboxamidoadenosine (NECA) (Kennedy, 1990) in order to define concentrations of modifying agents for use in studies of the P_2 purinoceptor. Potentiation of

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adenosine by the selective uptake inhibitor *S*-(4-nitrobenzyl)-6-thioinosine (NBTI) (Clanachan et al., 1987) was studied to determine the concentration of NBTI which produced maximum inhibition of adenosine uptake, allowing the true potency of adenosine at the P_1 purinoceptor to be determined. The selective P_1 purinoceptor antagonist 8-sulphophenyltheophylline (Gustafsson, 1984; Collis et al., 1987; Hourani et al., 1991) was also examined to obtain a concentration of 8-sulphophenyltheophylline which would produce 100-fold inhibition of P_1 purinoceptor-mediated responses. These concentrations of NBTI and 8-sulphophenyltheophylline were then present when the potency order for ATP and its analogues for P_{2Y} purinoceptor-induced relaxation was determined. It has been reported that the potency of ATP and analogues can be affected by inhibition of adenosine uptake in this tissue (Maguire and Satchell, 1979). Also, it is known that this tissue possesses ectonucleotidases which rapidly metabolise ATP and its analogues to adenosine (Welford et al., 1986). However, the contribution, if any, of metabolized adenosine acting at P_1 purinoceptors to the relaxant action of ATP and its analogues in this tissue has not been determined. The use of 8-sulphophenyltheophylline and NBTI in this manner allowed functional responses mediated via the P_{2Y} purinoceptor only to be studied, giving the true potency order for ATP and analogues. Cyclo-oxygenase activity was inhibited by the use of indomethacin (Vane, 1971) to prevent spasm due to prostanoid release. Studies of the P_2 purinoceptor antagonists, suramin and PPADS, were carried out in order to determine their activity at the P_2 purinoceptor and the nature of the antagonism. Preliminary results have been presented to the British Pharmacological Society (Kelley and Hollingsworth, 1993) and the Montreal meeting of IUPHAR (Kelley and Hollingsworth, 1994).

2. Materials and methods

2.1. Tissue preparation

Male or female tricolour guinea-pigs (350–800 g) were stunned and bled. Taenia caeci were dissected free of the caecum and placed in a physiological salt solution (PSS) of the following composition (mM): NaCl 118, KCl 4.75, $CaCl_2 \cdot 6H_2O$ 2.55, $MgSO_4 \cdot 7H_2O$ 1.2, KH_2PO_4 1.19, $NaHCO_3$ 25 and glucose 11. Strips of caeci 1.5–2 cm in length were mounted for isometric tension recording in 10 or 20 ml tissue baths, gassed with 95% O_2 and 5% CO_2 and maintained at 37° C. The preparations were initially placed under a resting tension of 1 g, left to equilibrate for 1 h and washed at 15 min intervals. Vasa deferentia were removed from male tricolour guinea-pigs, divided into epididymal and prostatic segments and mounted for isometric recording. Indomethacin (1 μM) was present in the PSS during all experiments.

2.2. Non-cumulative agonist concentration-effect curves (guinea-pig taenia caeci)

Tone was produced by the addition of carbachol (50 or 100 nM) to the PSS. 5 min after each carbachol addition a single concentration of a relaxant agonist was added and left in contact with the tissue for 3 min before washing. A 10 min period was left between successive carbachol additions in order to prevent possible desensitisation to relaxants. A concentration-effect curve to ATP and analogues was obtained by successive carbachol additions with 2-fold increases in concentration of relaxant. After equilibration with antagonists or other modifying agents for 30–90 min, the concentration-effect curve to the relaxant was then repeated. Experiments were designed such that each test tissue was matched with a vehicle-treated tissue from the same animal.

2.3. Studies of antagonism by 8-sulphophenyltheophylline

After the construction of a control concentration-effect curve to either adenosine or NECA, four taenia obtained from the same animal were exposed to the P_1 purinoceptor antagonist 8-sulphophenyltheophylline (3 μM, 30 μM or 300 μM) or vehicle, in a concentration equivalent to that used with 8-sulphophenyltheophylline (300 μM), and the agonist concentration-effect curve repeated after 30 min. NBTI (300 nM) was present in the PSS throughout.

2.4. Determination of agonist potency at the P_2 purinoceptor

Tissues were exposed throughout to NBTI (300 nM) and 8-sulphophenyltheophylline (140 μM). After at least 25 min equilibration, a concentration-effect curve to ATP was constructed. After washing and a further 30 min, a concentration-effect curve was constructed to either α,β-methylene ATP, β,γ-methylene ATP, 2-methylthio-ATP or UTP.

2.5. Studies of antagonism by suramin or PPADS

Tissues were exposed throughout to NBTI (300 nM) and 8-sulphophenyltheophylline (140 μM). After an initial concentration-effect curve was obtained to either 2-methylthio-ATP or ATP, four taenia from the same animal were exposed to either suramin (10 μM, 100 μM or 1 mM) or PPADS (1 μM, 3 μM or 10 μM). As suramin has been reported to require 90 min to reach equilibrium (Leff et al., 1990), tissues were incu-

bated with suramin for 90 min before construction of a second agonist concentration-effect curve. Tissues were incubated with PPADS for 30 min. Time-matched control tissues were exposed to the vehicle for suramin or PPADS as appropriate.

2.6. Studies of antagonism by PPADS (guinea-pig vas deferens)

After obtaining a constant response to repeated exposures to KCl (50 mM), a non-cumulative ATP concentration-effect curve was constructed in each tissue. Increasing ATP concentrations were added at 15 min intervals. Once the spasm to ATP had reached a peak (within 1 min), tissues were washed to avoid possible desensitization. The four preparations obtained from each animal were exposed to either PPADS (1, 3 or 10 μM), or the equivalent vehicle, for 30 min before construction of a second concentration-effect curve to ATP.

2.7. Drugs and solutions

Drugs used in these studies were: indomethacin (Sigma), carbachol (Sigma), NBTI (Research Biochemicals), 8-sulphophenyltheophylline (Research Biochemicals), adenosine (hemisulphate salt, Sigma), NECA (Research Biochemicals), isoprenaline (hydrochloride salt, Sigma), ATP (sodium salt, Sigma), α,β-methylene ATP (lithium salt, Sigma), β,γ-methylene ATP (sodium salt, Sigma), 2-methylthio-ATP (tetrasodium salt, Research Biochemicals), UTP (sodium salt, Sigma), suramin (Bayer) and PPADS (Cooksons). Indomethacin was prepared at a concentration of 10 mM in 95% ethanol, while NBTI (1 mM) was dissolved in 100% dimethylsulphoxide (Sigma). Stock solutions of NECA and isoprenaline (each 10 mM) were prepared in 0.1 M HCl, with subsequent dilutions in 0.9% saline. Stock solutions of all other compounds were dissolved in distilled water and diluted when necessary using 0.9% saline.

2.8. Statistical analysis

Data are expressed as mean ± standard error of the mean. The potency of each agonist was calculated as pD_2 ($= -\log_{10}$ M EC_{50}, where EC_{50} was the concentration of agonist that produced 50% inhibition of carbachol-induced spasm). pD_2 was determined by linear regression of % relaxation against $\log_{10}$ agonist concentration. Log_{10} concentration ratio (CR) values were calculated as the difference in pD_2 values be-

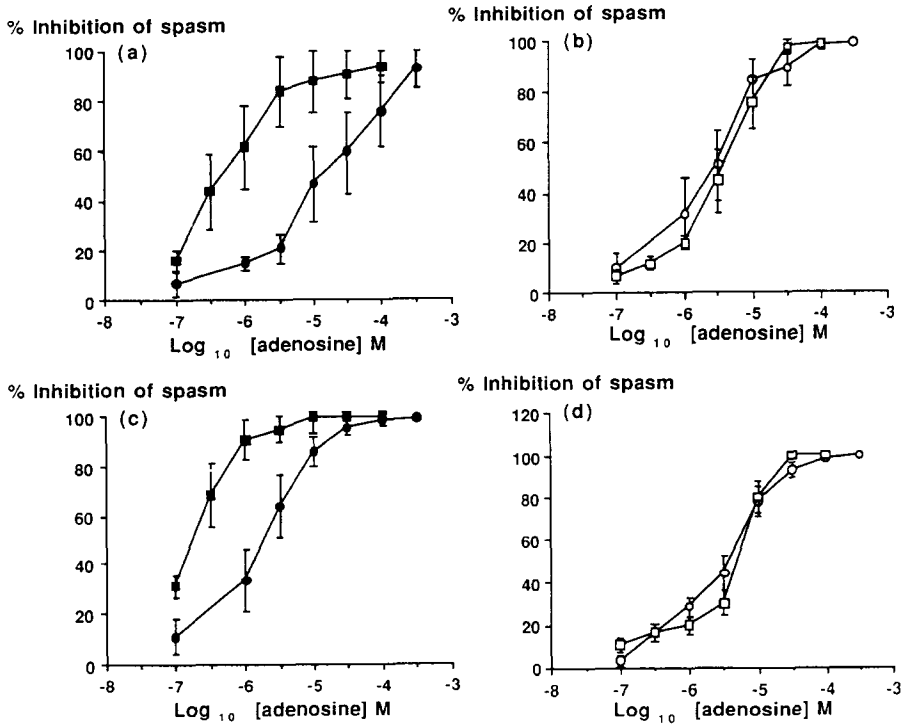

Fig. 1. Potentiation of adenosine by NBTI in isolated guinea-pig taenia caeci. Shown are (a) responses to adenosine in the absence (●) and in the presence of NBTI (300 nM, ■); (b) the equivalent time-matched control tissues in the absence (○) and in the presence of vehicle (□); (c) responses to adenosine in the absence (●) and presence of NBTI (30 μM, ■); (d) the equivalent time-matched control tissues in the absence (○) and in the presence of vehicle (□) after 30 min equilibration. Relaxant responses are expressed as the percentage inhibition of carbachol-induced spasm. Points represent mean values, with vertical lines showing the S.E.M. ($n = 6$).

tween the initial and second concentration-effect curves for each tissue. Statistical comparisons were made from this data using paired or unpaired Student's t-test as appropriate, with a probability level of $P < 0.05$ being considered significant. Where it was necessary to make multiple comparisons of pD_2 values, e.g. in antagonist studies, pD_2 values obtained during the second agonist concentration-effect curves were compared using analysis of variance. A supplementary Student's t-test was then carried out to define the boundaries around the control mean pD_2 outside of which any treated group mean pD_2 would be significant at $P < 0.05$ (Wardlaw, 1989).

Analysis of antagonism of ATP and analogues by 8-sulphophenyltheophylline, suramin and PPADS was carried out according to the method of Arunlakshana and Schild (1959). Schild plots of $\log_{10}$ (CR − 1) versus $\log_{10}$ M antagonist concentration were prepared for tissues obtained from each animal from which pA_2 and slopes were determined. If the mean slope of these plots was not significantly different from 1, the intercept on the ordinate was recalculated after constraining the slope to 1 and assumed to be the pK_B (Kenakin, 1993).

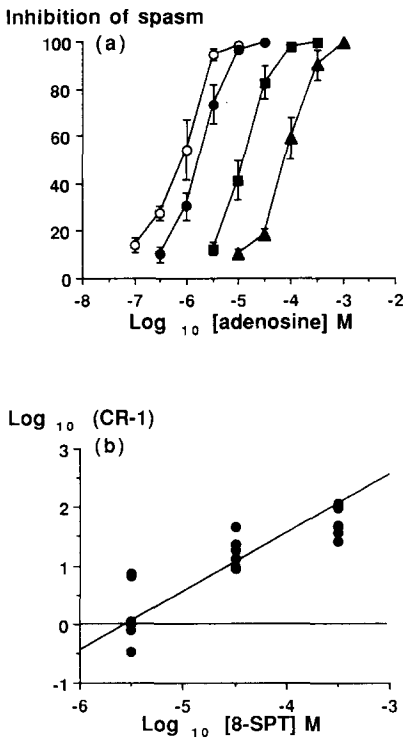

Fig. 2. Antagonism of adenosine by 8-sulphophenyltheophylline in isolated guinea-pig taenia caeci. Shown are (a) $\log_{10}$ concentration-effect curves to adenosine in the presence of vehicle (○), or 8-sulphophenyltheophylline (3 μM, ●, 30 μM, ■ and 300 μM, ▲) after 30 min equilibration and (b) the Schild plot derived from this data (slope constrained to 1). Relaxant responses are expressed as the percentage inhibition of carbachol-induced spasm. Points represent mean values, with vertical lines showing the S.E.M. ($n = 6$).

3. Results

3.1. Studies of the P_1 purinoceptor

Effect of NBTI on responses to adenosine

The peak response to each concentration of adenosine occurred within approximately 60 s, however relaxation was not maintained in the continued presence of adenosine. Non-cumulative addition of adenosine in the presence of carbachol yielded a concentration-dependent relaxation with a pD_2 of 5.35 ± 0.15 ($n = 24$) and an E_{max} of 100% (Fig. 1). Adenosine was significantly potentiated by NBTI (300 nM and 30 μM, $\log_{10}$ CR = 1.33 ± 0.28, $n = 6$ and 0.78 ± 0.23, $n = 6$; $P < 0.01$) (Fig. 1). NBTI did not modify the spasm to carbachol (data not shown). There was no significant difference in the degree of potentiation ($P > 0.05$) by NBTI (300 nM or 30 μM), indicating that NBTI (300 nM) produced maximum inhibition of adenosine uptake over the range of adenosine concentrations used to construct the $\log_{10}$ concentration-effect curve.

Studies of antagonism of adenosine and NECA by 8-sulphophenyltheophylline

In the presence of NBTI (300 nM), increasing concentrations of 8-sulphophenyltheophylline produced progressive parallel rightward shifts in the adenosine $\log_{10}$ concentration-effect curve (Fig. 2a). As the mean slope of the resultant Schild plot was not significantly different from 1 (slope = 0.78 ± 0.10, $P > 0.05$), the slope was constrained to 1 for estimation of the pK_B (5.54 ± 0.10, $n = 6$, Fig. 2b).

The peak relaxation to each concentration of NECA was recorded after approximately 60 s, however relaxation was not maintained in the continued presence of NECA. When added non-cumulatively, in the presence of NBTI (300 nM), NECA produced a concentration-dependant inhibition of carbachol spasm with a pD_2 of 6.64 ± 0.14 ($n = 24$). As with adenosine, increasing concentrations of 8-sulphophenyltheophylline produced progressive parallel rightward displacements in the $\log_{10}$ concentration-effect curve to NECA with the slope of the Schild plot slope not significantly different from 1 (slope = 1.18 ± 0.09, $P > 0.05$) (Fig. 3a). A Schild plot of these data yielded a pK_B for 8-sulphophenyltheophylline against NECA of 5.56 ± 0.11 with the slope of the plot constrained to 1 (Fig. 3b). 8-Sulphophenyltheophylline (3, 30 and 300 μM) did not modify the spasm to carbachol (data not shown). There was no significant difference in the mean pK_B obtained for 8-sulphophenyltheophylline against adenosine and NECA ($P > 0.05$). The pA_{100} concentration of 8-sulphophenyltheophylline, calculated from

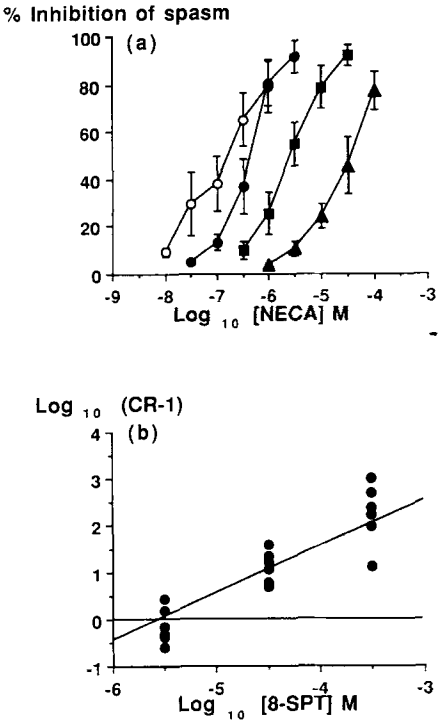

Fig. 3. Antagonism of NECA by 8-sulphophenyltheophylline in isolated guinea-pig taenia caeci. Shown are (a) $\log_{10}$ concentration-effect curves to NECA in the presence of vehicle (○) or 8-sulphophenyltheophylline (3 μM, ●, 30 μM, ■ and 300 μM, ▲) after 30 min equilibration and (b) the Schild plot derived from this data (slope constrained to 1). Relaxant responses are expressed as the percentage inhibition of carbachol-induced spasm. Points represent mean values, with vertical lines showing the S.E.M. ($n = 6$)

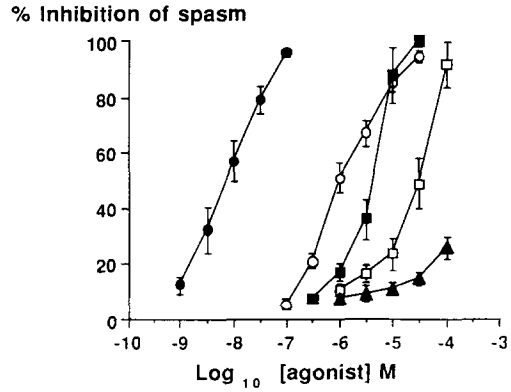

Fig. 4. Relaxant action of ATP and analogues in the isolated guinea-pig taenia caeci. (●) represents 2 methylthio-ATP, (○) ATP, (■) α,β methylene ATP, (□) β,γ methylene ATP and (▲) UTP. Relaxant responses are expressed as the percentage inhibition of carbachol-induced spasm. Points represent mean values, with vertical lines showing the S.E.M. ($n = 6$ except for ATP where $n = 24$).

the Schild plot for 8-sulphophenyltheophylline versus NECA, was 140 μM. This concentration of 8-sulphophenyltheophylline was used in subsequent experiments.

Effect of NBTI and 8-sulphophenyltheophylline on responses to isoprenaline

In order to assess the selectivity of both NBTI and 8-sulphophenyltheophylline, they were tested against isoprenaline-induced relaxation following the same protocol as before. Neither NBTI (300 nM) nor 8-sulphophenyltheophylline (300 μM) had any effect on responses to isoprenaline (Table 1).

3.2. Studies of the P_2 purinoceptor

Potency order at the P_2 purinoceptor

ATP produced its peak effect within 60 s, although relaxation was not maintained in the continued presence of ATP. Non-cumulative addition of ATP to tissues precontracted with carbachol (50 nM), in the presence of indomethacin (1 μM), NBTI (300 nM) and 8-sulphophenyltheophylline (140 μM), produced rapid, concentration-dependent inhibition of spasm with a pD_2 of 5.87 ± 0.10 ($n = 24$). Similar relaxant responses were recorded on non-cumulative addition of 2-methylthio-ATP, α,β-methylene ATP and β,γ-methylene ATP but UTP was almost inactive (Fig. 4). 2-Methylthio-ATP ($pD_2 = 8.09 \pm 0.11$, $n = 6$) was significantly more potent (relative potency = 78; $P < 0.05$) than ATP (potency taken as 1), while α,β-methylene ATP ($_pD_2 = 5.36 \pm 0.10$, $n = 6$) was equipotent (relative potency = 0.4; $P > 0.05$) with ATP. β,γ-methylene ATP ($_pD_2 = 4.53 \pm 0.12$, $n = 6$) and UTP ($pD_2 < 4$, $n = 6$) were

Table 1
Effect of addition of NBTI (300 nM) or 8-sulphophenyltheophylline (300 μM), or the appropriate vehicle (distilled water and DMSO, respectively) in time-matched controls, on relaxation to isoprenaline

	Test		Time-matched controls	
	Initial curve	Second curve	Initial curve	Second curve
NBTI	8.1 ± 0.15	8.1 ± 0.10	8.0 ± 0.16	7.9 ± 0.14
8-Sulphophenyltheophylline	8.1 ± 0.22	7.8 ± 0.12	8.0 ± 0.12	7.9 ± 0.12

Data are pD_2 values as means ± S.E.M., $n = 6$. $pD_2 = -\log EC_{50}$ where EC_{50} was the concentration of isoprenaline that produced 50% inhibition of carbachol-induced spasm. The physiological salt solution contained indomethacin (1 μM) throughout.

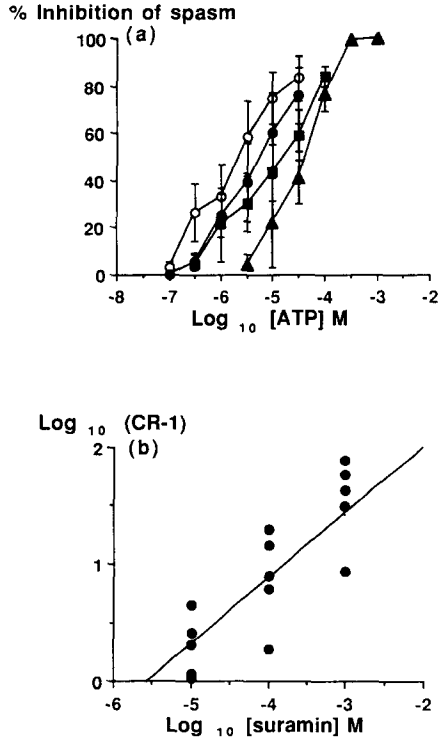

Fig. 5. Antagonism of ATP by suramin in isolated guinea-pig taenia caeci. Shown are (a) the $\log_{10}$ concentration-effect curves to ATP in the presence of vehicle (○), and suramin (10 μM, ●, 100 μM, ■ and 1 mM, ▲) after 90 min equilibration and (b) the Schild plot derived from this data (see text). Relaxant responses are expressed as the percentage inhibition of carbachol-induced spasm. Points represent mean values, with vertical lines showing the S.E.M. ($n = 6$).

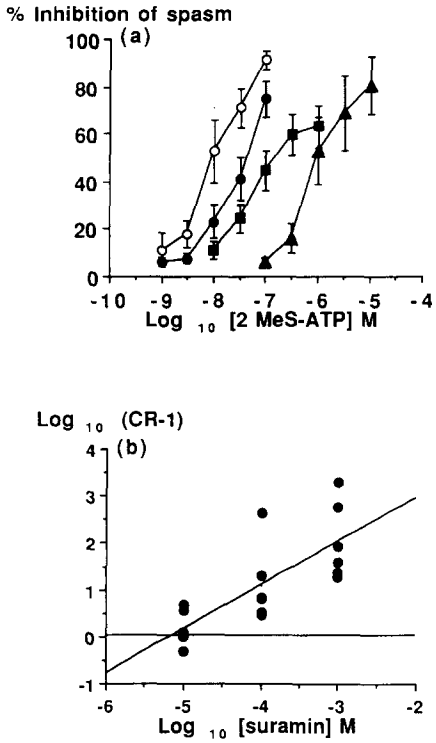

Fig. 6. Antagonism of 2 methylthio-ATP by suramin in isolated guinea-pig taenia caeci. Shown are (a) the $\log_{10}$ concentration-effect curves to 2 methylthio-ATP in the presence of vehicle (○) or suramin (10 μM, ●, 100 μM, ■ and 1 mM, ▲) after 90 min equilibration and (b) the Schild plot derived from this data (see text). Relaxant responses are expressed as the percentage inhibition of carbachol-induced spasm. Points represent mean values, with vertical lines showing the S.E.M. ($n = 6$)

significantly less potent than ATP (relative potency 0.05 [$P < 0.05$] and < 0.01 [$P < 0.05$] respectively) UTP did not achieve 50% relaxation at the highest concentration used (100 μM).

Effect of NBTI (300 nM) on relaxation caused by ATP and analogues

Table 2 lists the pD_2 values obtained in the absence and presence of NBTI (300 nM) for ATP, 2-methylthio-ATP, α,β-methylene ATP and β,γ-methylene ATP in the presence of 8-sulphophenyltheophylline (140 μM). After 30 min incubation, NBTI (300 nM) produced no significant shift ($P > 0.05$) in the concentration-effect curves to these agonists.

Antagonism of ATP and analogues by suramin

Fig. 5 illustrates the effects of increasing concentrations of suramin, incubated for 90 min, on ATP concentration-effect curves in the presence of 8-sulphophenyltheophylline (140 μM) and NBTI (300 nM). At concentrations higher than 10 μM, suramin produced a significant displacement in the ATP concentration-effect curve (vehicle: $\log_{10}$ CR = 0.18 ± 0.19;

Table 2
Effect of NBTI (300 nM), or its vehicle (0.03% DMSO) in time-matched controls, on relaxation to ATP and analogues

Compound	Test		Time-matched controls	
	Initial curve	Second curve	Initial curve	Second curve
ATP	5.6 ± 0.32	5.5 ± 0.18	4.7 ± 0.41	4.9 ± 0.43
α,β-Methylene ATP	5.4 ± 0.04	5.4 ± 0.02	5.3 ± 0.08	5.4 ± 0.09
2-Methylthio-ATP	7.8 ± 0.41	7.7 ± 0.20	7.6 ± 0.36	7.8 ± 0.26
β,γ-Methylene ATP	4.4 ± 0.19	4.3 ± 0.27	4.3 ± 0.17	4.3 ± 0.09

Data are pD_2 values as means ± S.E.M.; $n = 5$–6. $pD_2 = -\log EC_{50}$ where EC_{50} was the concentration of agonist that produced 50% inhibition of carbachol-induced spasm. The physiological salt solution contained indomethacin (1 μM) throughout.

10 μM: $\log_{10}$ CR = 0.29 ± 0.12 [$P > 0.05$]; 100 μM: $\log_{10}$ CR = 1.0 ± 0.13 [$P < 0.05$]; 1 mM: $\log_{10}$ CR = 1.43 ± 0.19 [$P < 0.01$]) (Fig. 5a). Schild analysis of this data yielded the plot shown in Fig. 5b. The mean slope of the Schild plot for suramin versus ATP was significantly less than 1 (mean slope = 0.67 ± 0.08, $n = 6$). The pA_2 for suramin against ATP was 5.52 ± 0.17.

Suramin antagonized 2-methylthio-ATP under the same conditions (Fig. 6a). At concentrations higher than 10 μM there was a significant displacement of the 2-methylthio-ATP concentration-effect curve (vehicle: $\log_{10}$ CR = −0.10 ± 0.11; 10 μM: $\log_{10}$ CR = 0.25 ± 0.17 [$P > 0.05$]; 100 μM: $\log_{10}$ CR = 1.16 ± 0.31 [$P < 0.05$]; 1 mM: $\log_{10}$ CR = 2.04 ± 0.33 [$P < 0.01$]). Schild analysis of this data yielded the plot shown by Fig. 6b. The mean slope of the Schild plot for suramin versus 2-methylthio-ATP was not significantly different from 1 (mean slope = 1.37 ± 0.39, $n = 6$). Despite the slope not being significantly different from 1, the slope of the Schild plot was not constrained to 1, as it was not clear if suramin was behaving as a competitive antagonist. The pA_2 for suramin against 2-methylthio ATP was 5.10 ± 0.31.

Suramin (100 μM, 330 μM and 1 mM) had no effect on responses to NECA (100 μM: $\log_{10}$ CR = 0.5 ± 0.4; 330 μM: $\log_{10}$ CR = 0.4 ± 0.4; 1 mM: $\log_{10}$ CR = 0.28 ± 0.5 [all $P > 0.05$]). Suramin (10 μM to 1 mM) did not modify the spasm to carbachol (data not shown).

Antagonism of ATP by PPADS

PPADS (1, 3 and 10 μM) produced significant depressions in the spasmogenic responses to all concentrations of ATP in isolated guinea-pig vas deferens (Fig. 7a) (1 μM: % reduction = 80.8 ± 10.8; 3 μM: % reduction = 91.0 ± 6.3; 10 μM: % reduction = 95.2 ± 3.1) compared to vehicle treated tissues (% reduction = 3.4 ± 9.1). However, PPADS had no effect on responses to ATP in isolated guinea-pig taenia caeci (Fig. 7b) (1 μM: $\log_{10}$ CR = 0.18 ± 0.10; 3 μM: $\log_{10}$ CR = 0.06 ± 0.10; 10 μM: $\log_{10}$ CR = 0.24 ± 0.24 [all $P > 0.05$]).

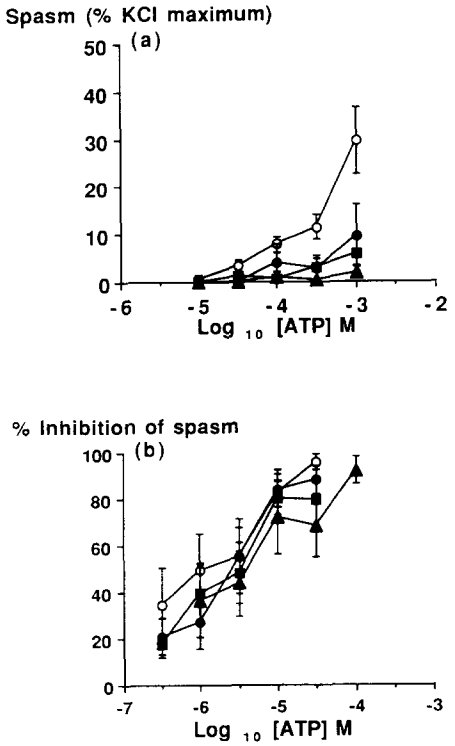

Fig. 7. Antagonism of ATP by PPADS. (a) Shown in (a) are the $\log_{10}$ concentration-effect curves to ATP in isolated guinea-pig vas deferens in the presence of vehicle (○) or PPADS (1 μM, ●, 3 μM, ■ and 10 μM, ▲) after 30 min equilibration. All tension developments are expressed as a percentage of the maximum spasm induced by KCl (50 mM). Points represent mean values, with vertical lines showing the S.E.M. ($n = 6$). Shown in (b) are the $\log_{10}$ concentration-effect curves to ATP in isolated guinea-pig taenia caeci in the presence of vehicle (○) or PPADS (1 μM, ●, 3 μM, ■ and 10 μM, ▲) after 30 min equilibration. Relaxant responses are expressed as the percentage inhibition of carbachol-induced spasm. Points represent mean values, with vertical lines showing the S.E.M. ($n = 6$).

4. Discussion

In the present study, the P_{2Y} purinoceptor in the taenia caeci was characterized under conditions where confounding factors were reduced (i.e. agonist uptake and prostanoid synthesis inhibited and P_1 purinoceptors blocked). The potency order for ATP and analogues in the guinea-pig taenia caeci was: 2-methylthio-ATP ≫ ATP = α,β-methylene ATP > β,γ-methylene ATP ≫ UTP. Suramin antagonized ATP and 2-methylthio ATP, but it may not be a true competitive antagonist at the P_{2Y} purinoceptor of the taenia caeci. PPADS did not antagonize ATP at P_{2Y} purinoceptors in the guinea-pig taenia caeci, but did antagonize ATP at P_{2X} purinoceptors in guinea-pig vas deferens, indicating that it may have some selectivity for the P_{2X} purinoceptor.

4.1. Studies of the P_1 purinoceptor

In isolated guinea-pig taenia caeci, non-cumulative addition of adenosine in the presence of carbachol yielded a concentration-dependent relaxation with a mean pD_2 of 5.35. Relaxant responses to both adenosine and NECA were not maintained in the continued presence of each agonist, in contrast to the maintained responses observed by Brown and Burnstock (1981). In the present study, a higher concentration of carbachol (approximately the EC_{70} concentration) was used which might account for the difference in duration of relaxant responses.

In the present study, the potency of NECA in the presence of NBTI (mean $pD_2 = 6.64$) was similar to

published values in the absence of NBTI in taenia caeci (6.33; Burnstock et al., 1984). Also, it has been shown that NECA was not potentiated by dipyridamole (Burnstock et al., 1984). Together these data suggest that NECA is not a substrate for adenosine uptake in this tissue.

Potentiation of adenosine by NBTI

Adenosine uptake is envisaged as the first step in removal of adenosine from the biophase around the cell surface receptors. The adenosine transport system is saturable and reversible (Clanachan et al., 1987). It may be inhibited by various agents such as dipyridamole and NBTI; indeed dipyridamole has been used in numerous studies as an inhibitor of adenosine uptake (Satchell and Burnstock, 1975; Maguire and Satchell, 1979; Burnstock et al., 1984; Hoyle and Edwards, 1992). However, dipyridamole can inhibit other intracellular enzymes such as adenosine deaminase and cyclic nucleotide phosphodiesterases (Maguire and Satchell, 1981). Also, NBTI has been shown to have a much higher affinity than dipyridamole for adenosine transport sites in guinea-pig cardiac myocytes (Clanachan et al., 1987). Therefore, we have used the more selective tool NBTI for the first time in this tissue.

In this study, NBTI potentiated adenosine, presumably by inhibiting its uptake and removal from the biophase close to P_1 purinoceptors. It appeared that NBTI (300 nM) produced maximum potentiation of adenosine over the concentration range used to construct the adenosine concentration-effect curve. The K_D for NBTI binding to transport sites in guinea-pig cardiac myocytes is 1–3 nM (Clanachan et al., 1987), therefore it is not unreasonable to assume that maximum inhibition of uptake occurred at 300 nM. NBTI was specific for purinergic mechanisms as it had no effect on the isoprenaline concentration-response curve.

Antagonism of adenosine and NECA by 8-sulphophenyltheophylline

In the present study, the P_1 purinoceptor antagonist 8-sulphophenyltheophylline produced concentration-dependent parallel rightward shifts in concentration-effect curves to both adenosine and NECA with no depression of maximum response. The mean pK_B values recorded with both agonists (5.56 and 5.54, respectively) were not significantly different and for each agonist the Schild plot slope was close to 1. The data indicate that 8-sulphophenyltheophylline is a true competitive antagonist at P_1 purinoceptors in the guinea-pig taenia caeci. The pK_B values agree closely with published values in guinea-pig tissues: ileum: 5.65 (Gustafsson, 1984); atrium: 4.94; aorta: 5.05 (Collis et al., 1987) and taenia caeci: 5.13 (Hourani et al., 1991). It has previously been reported that the related P_1 purinoceptor antagonist phenyltheophylline can potentiate certain relaxants via inhibition of cyclic nucleotide phosphodiesterases (Gustafsson, 1984). In this study, no evidence of PDE inhibition was seen as 8-sulphophenyltheophylline did not potentiate isoprenaline or produce relaxation in its own right. Hence, 8-sulphophenyltheophylline may be a more useful tool than phenyltheophylline.

4.2. Studies of the P_2 purinoceptor: potency order of ATP and analogues and the effect of NBTI

The P_{2Y} purinoceptor was in part defined by the agonist potency order for relaxation of the guinea-pig taenia coli (Burnstock and Kennedy, 1985). However, in previous studies several factors which could affect agonist potency orders were operative. In the present study, with adenosine uptake prevented, prostanoid synthesis inhibited and effects mediated by P_1 purinoceptors prevented, the rank order of agonist potency and potencies relative to ATP were: 2-methylthio-ATP (78) $\gg$ ATP (1) = α,β-methylene ATP (0.4) > β,γ-methylene ATP (0.05) $\gg$ UTP (< 0.01).

A similar order was recorded by Maguire and Satchell (1979). Under our conditions, a truer reflection of agonist properties should be observed (Kenakin, 1993). It has been suggested that a component of the relaxant action of ATP in this tissue may be mediated via P_1 purinoceptors, either directly, or via its metabolites, including adenosine (Maguire and Satchell, 1979). Maguire and Satchell (1979) reported that ATP and β,γ-methylene ATP were significantly potentiated by dipyridamole (1 μM) in isolated guinea-pig taenia caeci. They postulated that these agonists were metabolised to adenosine and the adenosine was then potentiated by dipyridamole. In the current experiments it was unlikely that ATP or its analogues were acting at P_1 purinoceptors either directly or indirectly as 8-sulphophenyltheophylline was present. Also, there was no evidence of any potentiation of ATP and analogues by NBTI. These data suggest that, although metabolism does occur (Welford et al., 1986), any metabolites produced do not contribute to the relaxant effect of ATP or its analogues.

It has been shown for the first time that UTP has a low potency in this tissue which indicates that it is unlikely that P_{2U} purinoceptors (O'Connor, 1992) are present.

Antagonism of ATP and analogues by suramin

It has previously been reported that suramin can inhibit P_2 purinoceptor-mediated effects in this and other tissues (Dunn and Blakeley, 1988; Den Hertog et al., 1989a,b; Hoyle et al., 1990; Von Kügelgen et al., 1990) and displace [^{3}H]α,β-methylene ATP from high

affinity binding sites in various rat tissues (Michel and Humphrey, 1993). Suramin appears to be a competitive, if slowly equilibrating, antagonist at the P_{2X} purinoceptor of the rabbit ear artery (Leff et al., 1990). In the present study, suramin had no effect on responses to the P_1 purinoceptor agonist, NECA, indicating for the first time that suramin has no action at P_1 purinoceptors. In the guinea-pig taenia caeci, suramin was an antagonist of both ATP and 2-methylthio-ATP. However, in the case of ATP the slope of the resultant Schild plot was significantly less than 1 and for the suramin-2-methylthio-ATP interaction greater than 1. It has been reported that suramin is an inhibitor of the ectonucleotidase responsible for metabolism of ATP in the guinea-pig urinary bladder (Hourani and Chown, 1989). It is possible that inhibition of ATP metabolism by suramin, especially at high concentrations, may explain the steepness of the Schild plot slope recorded. However, it has been shown that in the guinea-pig taenia coli and urinary bladder that 2-methylthio-ATP is metabolised at the same rate as ATP (Welford et al., 1986, Welford et al., 1987) yet the Schild plot slopes for the two agonists were very different. Von Kügelgen et al. (1990) and Den Hertog et al. (1989a) both recorded non-parallel displacement of agonist $\log_{10}$ concentration-effect curves by suramin in mouse vas deferens and guinea-pig taenia caeci, respectively. These data, together with the findings of the present study, suggest that suramin is not a true competitive antagonist at P_{2Y} purinoceptors in the guinea-pig taenia caeci.

Antagonism of ATP and analogues by PPADS

In guinea-pig vas deferens ATP was a spasmogen, an effect mediated by P_{2X} purinoceptors (Burnstock and Kennedy, 1985), and ATP was antagonized by PPADS (1–10 μM). However, PPADS had no effect on P_{2Y} purinoceptor-mediated relaxation in guinea-pig taenia caeci. These data extend the report by Ziganshin et al. (1994) where PPADS had no effect on P_{2Y} purinoceptor-mediated relaxation in rabbit mesenteric artery, and suggest that PPADS may exhibit some selectivity for P_{2X} as opposed to P_{2Y} purinoceptors.

In conclusion, this study extends previous findings and provides more quantitative data on agonist potency order at P_2 purinoceptors in the guinea-pig taenia caeci under controlled conditions. There is no evidence for the existence of P_{2U} purinoceptors. The results show that NBTI is a potent, selective inhibitor of adenosine uptake. The P_1 purinoceptor antagonist 8-sulphophenyltheophylline (Gustafsson, 1984; Collis et al., 1987; Hourani et al., 1991) acted as a competitive antagonist of adenosine and NECA with a pK_B of around 5.5. As the presence of 8-sulphophenyltheophylline or NBTI did not affect the potency of ATP and analogues compared with previous studies (Satchell and Burnstock, 1975; Maguire and Satchell, 1979), it is unlikely that any action of ATP or its metabolites at P_1 purinoceptors contribute significantly to their relaxant responses. Suramin, although selective for P_{2Y} purinoceptors, may not be a true competitive antagonist. PPADS was shown to exhibit selectivity as an antagonist for P_{2X} compared to P_{2Y} purinoceptors.

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Note added in proof: Dudeck et al. (Naunyn-Schmied. Arch. Pharmacol. 1995, 351, 107) have shown that 4,4′-di-isothiocyanatostilbene-2,2′-disulphonic acid disodium (DIDS) produced marked antagonism of α,β-methylene ATP. 2-Methylthio-ATP and ATP were not antagonised by DIDS. They propose that α,β-methylene ATP acts at a different receptor from 2-methylthio-ATP and ATP. Our studies neither support or refute this conclusion.

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