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ATP and β,γ-methylene ATP produce relaxation of guinea-pig isolated trachealis muscle via actions at P_1 purinoceptors

Angela S. Piper, Michael Hollingsworth *

Smooth Muscle Pharmacology Group, School of Biological Sciences, G38 Stopford Building, University of Manchester, Oxford Road, Manchester M13 9PT, UK

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Abstract

Adenosine 5′-triphosphate (ATP), β,γ-methylene ATP and α,β-methylene ATP produced relaxation of carbachol-precontracted isolated trachealis muscle from the guinea-pig in the presence of indomethacin (2.8 μM) and the adenosine uptake inhibitor *S*-(4-nitrobenzyl)-6-thioinosine (NBTI; 300 nM). The potency order for ATP and analogues was: β,γ-methylene ATP = ATP > α,β-methylene ATP = uridine 5′-triphosphate (UTP) = 2-methylthio ATP. Adenosine and 5′-*N*-ethylcarboxamidoadenosine (NECA) also caused relaxation. Relaxations to ATP, β,γ-methylene ATP, adenosine and NECA were not inhibited by the P_2 purinoceptor antagonist suramin (100 μM), but were inhibited by the P_1 purinoceptor antagonist 8-sulphophenyltheophylline (140 μM). NBTI significantly potentiated adenosine and ATP but not β,γ-methylene ATP or NECA. The data are compatible with the idea that β,γ-methylene ATP could interact directly with P_1 purinoceptors while ATP acts indirectly at P_1 purinoceptors via conversion to adenosine.

Keywords: P_1 purinoceptor; P_2 purinoceptor; Trachealis muscle, guinea-pig, isolated; Suramin; 8-Sulphophenyltheophylline; Adenosine 5′-triphosphate

1. Introduction

Cell surface receptors for adenosine and ATP (P_1 and P_2 purinoceptors, respectively; Burnstock, 1978) have been described in a wide variety of both neuronal and non-neuronal tissues. P_1 purinoceptors have been subdivided into A_1, A_2 and A_3 subtypes based on different agonist potency orders, antagonist selectivity and receptor coupling to second messenger systems (Daziel and Westfall, 1994). P_2 purinoceptors have been divided into P_{2X}, P_{2Y}, P_{2U}, P_{2T} and P_{2Z} subtypes based mainly on different agonist potency orders (Fredholm et al., 1994).

Guinea-pig isolated trachealis muscle is believed to contain a mixture of P_1 and P_2 purinoceptors. P_1 purinoceptors of the A_2 subtype mediate relaxation (Brown and Collis, 1982; Losinski and Alexander, 1995) and P_2 purinoceptors mediate spasm (Fedan et al., 1993a). However, it has also been shown that ATP and analogues can produce relaxation of guinea-pig isolated trachealis muscle (Christie and Satchell, 1980; Welford and Anderson, 1988; Fedan et al., 1993b). Maguire and Satchell (1979) and Christie and Satchell (1980) suggested that ATP must first be metabolized by ecto-nucleotidases present on the surface of trachealis muscle cells, and it was the resultant adenosine, acting at P_1 purinoceptors, that caused relaxation. Dephosphorylation of ATP and related P_2 purinoceptor agonists to adenosine by ecto-ATPases present on the surface of most cell types is a well-characterized phenomenon which complicates purinoceptor classification (for review, see Ziganshin et al., 1994). It was suggested later, based mainly on studies of the enzymic breakdown of ATP and related analogues, that ATP was able to relax guinea-pig isolated trachealis muscle directly, without the need for conversion to adenosine (Welford and Anderson, 1988). Two further studies of guinea-pig (Fedan et al., 1993b) and rabbit (Aksoy and Kelsen, 1994) isolated trachealis muscle preparations, suggested the presence of relaxant P_2 purinoceptors in these tissues, but no attempt was made to characterize the receptor sub-type.

The purpose of the present study was to characterize the mechanisms and purinoceptor type(s) involved in relaxation to ATP and analogues in guinea-pig trachealis muscle. This aim was achieved by (i) investigating the potency order of agonists under conditions where indirect mecha-

* Corresponding author. Tel.: (44) (161) 275-5489; fax: (44) (161) 275-5600; e-mail: mike.hollingsworth@man.ac.uk

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nisms involving metabolism to adenosine could be accounted for, and (ii) by the use of antagonists selective for particular purinoceptors. ATP and analogues can produce spasm of guinea-pig isolated trachealis muscle (Fedan et al., 1993a). However, this spasm was totally abolished by indomethacin, indicating prostanoid involvement (Vane, 1971), consequently indomethacin (2.8 μM) was present in all experiments. It has been shown previously that adenosine and ATP can be potentiated in guinea-pig isolated trachealis muscle by dipyridamole (Christie and Satchell, 1980; Welford and Anderson, 1988), possibly due to inhibition of adenosine uptake. Therefore, the effect of a maximal concentration (Piper and Hollingsworth, 1995) of the more selective adenosine uptake inhibitor *S*-(4-nitrobenzyl)-6-thioinosine (NBTI; Clanachan et al., 1987) on responses to ATP, adenosine and analogues was determined. Suramin was used as an antagonist selective for P_2 purinoceptors compared to P_1 purinoceptors (Piper and Hollingsworth, 1995). Suramin displays 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) and is also an antagonist at P_{2U} purinoceptors in some tissues (Fredholm et al., 1994). 8-Sulphophenyltheophylline was used as a selective P_1 purinoceptor antagonist (Gustafsson, 1984; Collis et al., 1987; Hourani et al., 1991) which does not discriminate between A_1 and A_2 adenosine receptors. Preliminary results have been presented to the British Pharmacological Society (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. The trachea was removed 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. The trachea was opened longitudinally diametrically opposite the trachealis muscle and divided into segments three to four cartilaginous rings wide. The tissue segments were mounted for isometric tension recording in 10 or 20 ml tissue baths where they were 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 and left to equilibrate for 1 h, washing at 15 min intervals. Indomethacin (2.8 μM) was present in the PSS throughout to inhibit prostanoid formation (Vane, 1971).

2.2. Cumulative agonist concentration-effect curves

Tone was produced by the addition of carbachol (300 nM) to the PSS. 30 min after carbachol addition, a cumulative agonist concentration-effect curve was constructed with two-fold increases in concentration of relaxant. Agonist concentration increments were made at 2 min intervals. To determine the maximum relaxation possible an EC_{100} concentration of aminophylline (1 mM) was added after completion of each agonist concentration-effect curve. Relaxant responses to each agonist concentration were expressed as a percentage of the aminophylline-induced relaxation. After washing and equilibration with antagonists or other modifying agents for 30–90 min, the concentration-effect curve to the relaxant was repeated. Experiments were designed such that each test tissue was matched with a vehicle-treated tissue from the same animal.

2.3. Determination of agonist potency at P_2 purinoceptors

Tissues were exposed throughout to NBTI (300 nM), a concentration which produced maximum potentiation of adenosine in guinea-pig taenia caeci (Piper and Hollingsworth, 1995). After at least 25 min equilibration, a cumulative concentration-effect curve to ATP was constructed against carbachol (300 nM)-induced spasm. After washing and a further 30 min, carbachol (300 nM) was added again to the tissues and 30 min later a concentration-effect curve was constructed to either α,β-methylene ATP, β,γ-methylene ATP, 2-methylthio ATP or UTP.

2.4. Studies of antagonism by suramin

Tissues were exposed throughout to NBTI (300 nM). After an initial concentration-effect curve was obtained to either adenosine, 5′-*N*-ethylcarboxamidoadenosine (NECA), β,γ-methylene ATP or ATP, trachealis muscle preparations were exposed to either suramin (100 μM) or vehicle in time-matched control tissues. As suramin (100 μM) has been reported to require 90 min to reach equilibrium (Leff et al., 1990), tissues were incubated with suramin for 90 min before construction of a second agonist concentration-effect curve in its presence.

2.5. Studies of antagonism by 8-sulphophenyltheophylline

NBTI (300 nM) was present in the PSS throughout. After the construction of a control cumulative concentration-effect curve to either adenosine, NECA, β,γ-methylene ATP or ATP, tissues were exposed to the P_1 purinoceptor antagonist 8-sulphophenyltheophylline (140 μM) or vehicle for 30 min and the agonist concentration-effect curve repeated in its presence.

2.6. Potentiation of agonists by NBTI

After the construction of a control concentration-effect curve to either adenosine, NECA, β,γ-methylene ATP or ATP, tissues were exposed to NBTI (300 nM) or the equivalent vehicle for 30 min and the agonist concentration-effect curve repeated.

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), 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) and suramin (Bayer). Indomethacin was prepared at a concentration of 10 mM in 95% ethanol, while NBTI (1 mM) was dissolved in 100% dimethylsulphoxide (Sigma). A stock solution of NECA (10 mM) was prepared in 0.1 M HCl, with subsequent dilutions in 0.9% saline. Stock solutions of all other compounds were prepared in distilled water and diluted when necessary using 0.9% saline.

2.8. Statistical analysis

Data is expressed as mean ± S.E.M. The potency of each agonist was calculated as $-\log_{10} EC_{30}$, where EC_{30} was the concentration of agonist that produced 30% of the maximum relaxation recorded to aminophylline (1 mM). EC_{30}, rather than EC_{50}, was used as the maximum relaxation to most agonists was less than 50% of the aminophylline maximum. $-\log_{10} EC_{30}$ was determined by linear regression of % relaxation against $\log_{10}$ agonist concentration. $\log_{10}$ concentration ratio (CR) values were calculated as the difference in $-\log_{10} EC_{30}$ values between 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 (Wardlaw, 1989).

3. Results

3.1. Potency order for ATP and analogues

ATP, when added cumulatively, in the presence of indomethacin (2.8 μM) and NBTI (300 nM), produced relaxation of guinea-pig isolated trachealis muscle precontracted with carbachol. The $-\log_{10} EC_{30}$ of ATP was 4.3 ± 0.0 ($n = 21$) and the maximum relaxation was 51.9 ± 3.6% of the relaxation to aminophylline (1 mM, Fig. 1). The only analogue that produced marked relaxation was β,γ-methylene ATP with a $-\log_{10} EC_{30}$ of 4.3 ± 0.1 ($n = 6$) and a maximum relaxation of 33.9 ± 4.4% (Fig. 1). α,β-Methylene ATP produced a small relaxation at the highest concentration tested (100 μM; maximum relaxation = 13.9 ± 2.9%, $n = 6$), while UTP and 2-methylthio ATP produced maximal relaxations of 4.3 ± 1.5% (100 μM, $n = 6$) and 2.6 ± 0.9% (1 μM, $n = 6$), respectively. The potency order was therefore: β,γ-methylene ATP = ATP > α,β-methylene ATP = UTP = 2-methylthio ATP (Fig. 1).

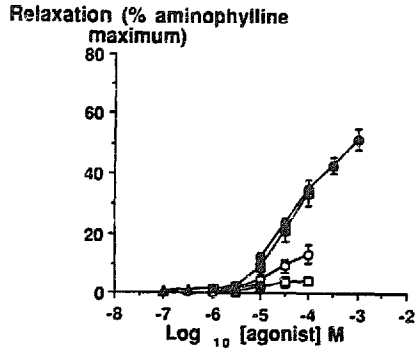

Fig. 1. ATP- and ATP analogue-induced relaxation of guinea-pig isolated trachealis muscle precontracted with carbachol (300 nM). (●) represents ATP, (■) β,γ-methylene ATP, (▲) α,β-methylene ATP, (○) UTP and (□) 2-methylthio ATP. Relaxant responses are expressed as the percentage of aminophylline (1 mM)-induced relaxation. Points represent mean values, with vertical lines showing the S.E.M. ($n = 6$, except for ATP where $n = 23$).

3.2. Effect of suramin on relaxation to ATP, β,γ-methylene ATP, adenosine and NECA

Suramin (100 μM), in the presence of indomethacin (2.8 μM) and NBTI (300 nM), had no effect on relaxant responses to ATP and β,γ-methylene ATP (Fig. 2a,b). The potencies of ATP and β,γ-methylene ATP were similarly unchanged in time-matched vehicle-treated control tissues [ATP: initial $-\log_{10} EC_{30} = 4.1 \pm 0.3$, + vehicle: $-\log_{10} EC_{30} = 4.2 \pm 0.1$, $n = 6$, $P > 0.05$; β,γ-methylene ATP: initial $-\log_{10} EC_{30} = 4.2 \pm 0.1$, + vehicle: $-\log_{10} EC_{30} = 4.3 \pm 0.1$, $n = 6$, $P > 0.05$]. Suramin had no effect on the relaxant action of adenosine or its analogue NECA (Fig. 2c,d). The vehicle for suramin also had no effect on responses to adenosine and NECA [adenosine: initial $-\log_{10} EC_{30} = 4.3 \pm 0.2$, + vehicle: $-\log_{10} EC_{30} = 4.5 \pm 0.2$, $n = 4$, $P > 0.05$; NECA: initial $-\log_{10} EC_{30} = 5.2 \pm 0.3$, + vehicle: $-\log_{10} EC_{30} = 5.3 \pm 0.4$, $n = 4$, $P > 0.05$].

3.3. Effect of 8-sulphophenyltheophylline on relaxation to ATP, β,γ-methylene ATP, adenosine and NECA

8-Sulphophenyltheophylline (140 μM), in the presence of indomethacin (2.8 μM) and NBTI (300 nM), significantly reduced relaxant responses to ATP and β,γ-methylene ATP (Fig. 3a,b). Full concentration-effect curves to each agonist could not be constructed in the presence of 8-sulphophenyltheophylline due to limited solubility of the compounds. The vehicle for 8-sulphophenyltheophylline had no effect on relaxant responses [ATP: initial $-\log_{10} EC_{30} = 4.3 \pm 0.2$, + vehicle: $-\log_{10} EC_{30} = 4.3 \pm 0.0$, $n = 5$, $P > 0.05$; β,γ-methylene ATP: initial $-\log_{10} EC_{30} = 4.3 \pm 0.1$, + vehicle: $-\log_{10} EC_{30} = 4.2 \pm 0.1$, $n = 5$,

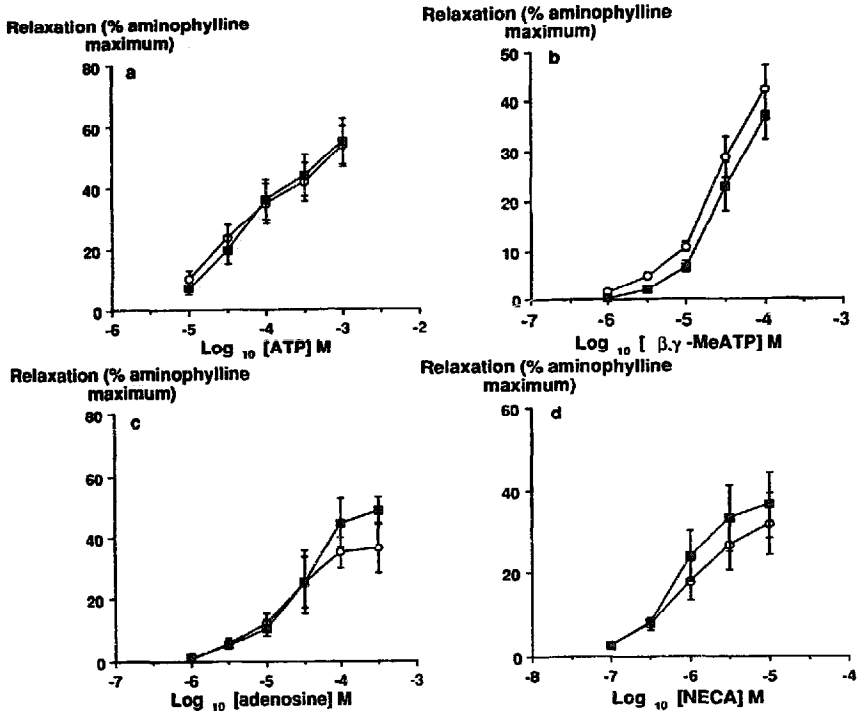

Fig. 2. Lack of effect of suramin on relaxation to ATP, β,γ-methylene ATP, adenosine and NECA in carbachol (300 nM)-precontracted guinea-pig isolated trachealis muscle. Shown are responses to (a) ATP, (b) β,γ-methylene ATP (β,γ Me ATP), (c) adenosine and (d) NECA in the absence (○) and in the presence of suramin (100 μM, ■) after 90 min equilibration. Relaxant responses are expressed as the percentage of aminophylline (1 mM)-induced relaxation. Points represent mean values, with vertical lines showing the S.E.M. ($n = 4$–6).

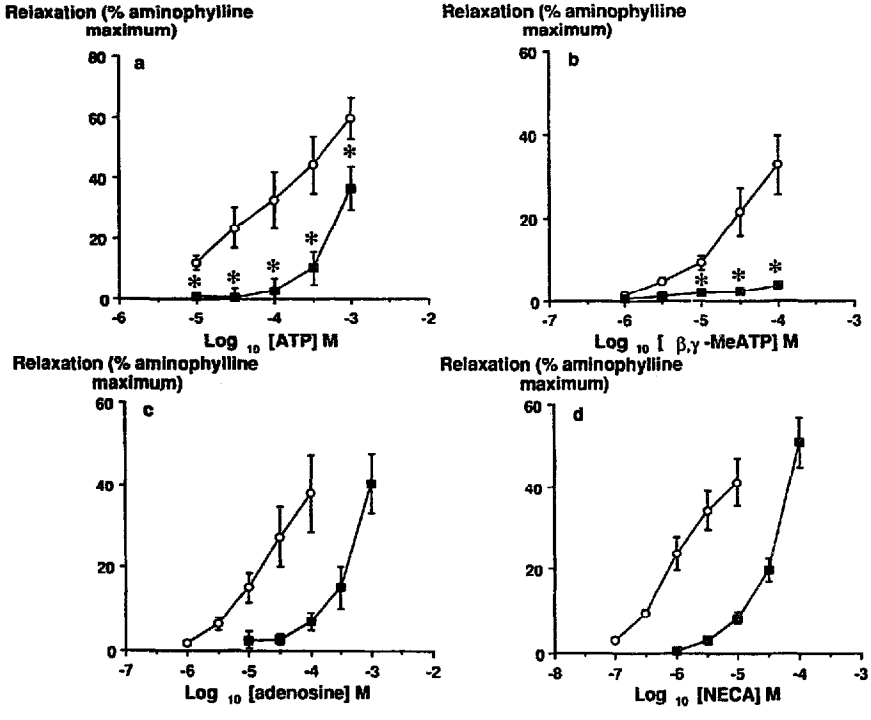

Fig. 3. Effect of 8-sulphophenyltheophylline on relaxation to ATP, β,γ-methylene ATP, adenosine and NECA in carbachol (300 nM)-precontracted guinea-pig isolated trachealis muscle. Shown are responses to (a) ATP, (b) β,γ-methylene ATP (β,γ Me ATP), (c) adenosine and (d) NECA in the absence (○) and in the presence of 8-sulphophenyltheophylline (140 μM, ■) after 60 min equilibration. Relaxant responses are expressed as the percentage of aminophylline (1 mM)-induced relaxation. Points represent mean values, with vertical lines showing the S.E.M. ($n = 4$–6). * Significant difference from initial curve ($P < 0.05$, paired t-test).

$P > 0.05$]. 8-Sulphophenyltheophylline (140 μM) produced parallel rightward shifts in the concentration-effect curves for adenosine and NECA with no significant difference in concentration ratio (adenosine: $\log_{10}$ CR = 1.25 ± 0.1, $n = 6$; NECA: $\log_{10}$ CR = 1.24 ± 0.2, $n = 6$; Fig. 3c,d). No change in the potency of adenosine or NECA was seen in time-matched vehicle-treated control tissues [adenosine: initial $-\log_{10}$ $EC_{30} = 4.2 \pm 0.2$, +vehicle: $-\log_{10}$ $EC_{30} = 4.2 \pm 0.1$, $n = 4$, $P > 0.05$; NECA: initial $-\log_{10}$ $EC_{30} = 5.6 \pm 0.2$, +vehicle: $-\log_{10}$ $EC_{30} = 5.7 \pm 0.2$, $n = 4$, $P > 0.05$].

3.4. Effect of NBTI on relaxation to ATP, β,γ-methylene ATP, adenosine and NECA

NBTI (300 nM) caused a significant increase in relaxant responses to ATP and a leftward shift in the concentration-effect curve to adenosine (Fig. 4a,c). However, NBTI (300 nM) had no effect on relaxations caused by β,γ-methylene ATP and NECA (Fig. 4b,d). The vehicle for NBTI had no effect on agonist potency [ATP: initial $-\log_{10}$ $EC_{30} = 3.0 \pm 0.2$, +vehicle: $-\log_{10}$ $EC_{30} = 3.2 \pm 0.3$ $n = 4$, $P > 0.05$; β,γ-methylene ATP: initial $-\log_{10}$ $EC_{30} = 4.2 \pm 0.1$, +vehicle: $-\log_{10}$ $EC_{30} = 4.3 \pm 0.1$ $n = 4$, $P > 0.05$; adenosine: initial $-\log_{10}$ $EC_{30} = 3.1 \pm 0.1$, +vehicle: $-\log_{10}$ $EC_{30} = 3.2 \pm 0.1$, $n = 4$, $P > 0.05$; NECA: initial $-\log_{10}$ $EC_{30} = 4.9 \pm 0.3$, +vehicle: $-\log_{10}$ $EC_{30} = 4.9 \pm 0.3$, $n = 4$, $P > 0.05$]

4. Discussion

This study has shown that of ATP and its related compounds, only ATP and β,γ-methylene ATP produced any substantial relaxation of guinea-pig isolated trachealis muscle. The P_2 purinoceptor antagonist suramin (100 μM) did not antagonize ATP or β,γ-methylene ATP, while the P_1 purinoceptor antagonist 8-sulphophenyltheophylline (140 μM) caused significant inhibition of relaxant responses to ATP and β,γ-methylene ATP. These data suggest that relaxation caused by ATP and β,γ-methylene ATP involves P_1 and not P_2 purinoceptors.

4.1. Are P_2 purinoceptors mediating relaxation present in guinea-pig isolated tracheal muscle?

The potency order for relaxation of guinea-pig isolated trachealis muscle [β,γ-methylene ATP = ATP > α,β-methylene ATP = UTP = 2 methylthio ATP] does not correspond to any previously described purinoceptor subtype. ATP, β,γ-methylene ATP and, to a lesser extent, α,β-methylene ATP produced relaxation of guinea-pig isolated trachealis muscle, while UTP and 2-methylthio ATP were almost inactive. In an isolated tracheal preparation from the guinea-pig Clark et al. (1980) also demonstrated that ATP and β,γ-methylene ATP were equipotent in causing relaxation, while Fedan et al. (1993b) showed β,γ-methylene ATP to be more potent than ATP in superfused

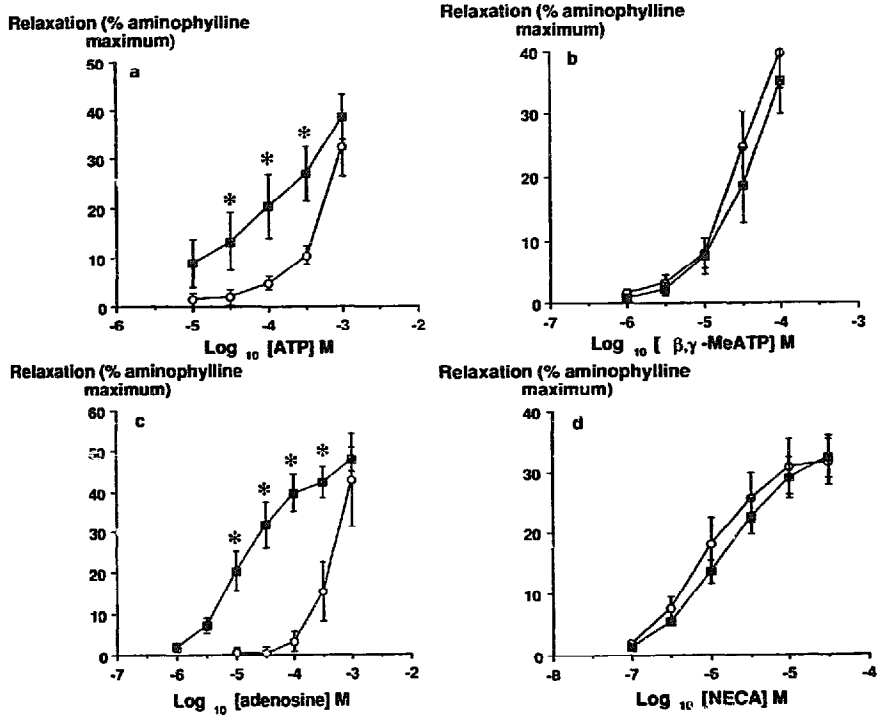

Fig. 4. Effect of NBTI on relaxation to ATP, β,γ-methylene ATP, adenosine and NECA in carbachol (300 nM)-precontracted guinea-pig isolated trachealis. Shown are responses to (a) ATP, (b) β,γ-methylene ATP (β,γ Me ATP), (c) adenosine and (d) NECA in the absence (○) and in the presence of NBTI (300 nM, ■) after 30 min equilibration. Relaxant responses are expressed as the percentage of aminophylline (1 mM)-induced relaxation. Points represent mean values, with vertical lines showing the S.E.M. ($n = 4$–5). * Significant difference from initial curve ($P < 0.05$, paired t-test).

guinea-pig trachea. The latter authors suggested that this anomalous potency order indicated the presence of a novel P_2 purinoceptor subtype. However, suramin, at a concentration that has been shown to cause antagonism at P_2 purinoceptors in other tissues (100 μM: Dunn and Blakeley, 1988; Den Hertog et al., 1989a,b; Hoyle et al., 1990; Leff et al., 1990; Von Kügelgen et al., 1990; Piper and Hollingsworth, 1995), did not have any effect on responses to ATP and β,γ-methylene ATP. Secondly, ATP and β,γ-methylene ATP were antagonized by the P_1 purinoceptor antagonist 8-sulphophenyltheophylline (140 μM), as were adenosine and NECA, suggesting that these agonists may produce relaxation via a similar mechanism, one involving P_1 purinoceptors. In the study of Fedan et al. (1993b) relaxant responses to ATP and β,γ-methylene ATP in intact superfused guinea-pig trachea were not inhibited by the P_1 purinoceptor antagonist 8-phenyltheophylline (1 μM). However, it has previously been reported that 8-phenyltheophylline can potentiate certain relaxants in guinea-pig isolated ileal smooth muscle and act as a relaxant in its own right via inhibition of cyclic nucleotide phosphodiesterases (Gustafsson, 1984). Therefore, the lack of effect of 8-phenyltheophylline in the study by Fedan et al. (1993b) may be a consequence of its antagonist and potentiating actions mutually cancelling. Due to its polar nature, 8-sulphophenyltheophylline cannot easily penetrate cells and thus is not an inhibitor of phosphodiesterase (Gustafsson, 1984). As expected, 8-sulphophenyltheophylline (140 μM) produced parallel rightward shifts in the concentration-effect curves to both adenosine and NECA. This concentration of 8-sulphophenyltheophylline produced about a 100-fold shift in the concentration-effect curves for adenosine and NECA acting as relaxants via A_1 adenosine receptors in the guinea-pig isolated taenia caeci (Piper and Hollingsworth, 1995). The rightward shifts in the concentration-effect curves for adenosine and NECA in the current experiments were of a similar order of magnitude (around 20-fold for both agonists).

4.2. How do ATP and β,γ-methylene ATP produce relaxation of guinea-pig trachealis muscle?

The adenosine uptake inhibitor NBTI potentiated adenosine, presumably by preventing its uptake and removal from the biophase surrounding P_1 purinoceptors. However, NBTI had no effect on the potency of NECA, in line with observations that NECA is not a substrate for the adenosine uptake process (Clanachan et al., 1987). NBTI also potentiated ATP, but not β,γ-methylene ATP. Potentiation of ATP by dipyridamole in guinea-pig isolated trachealis muscle has been noted in previous studies (Christie and Satchell, 1980; Clark et al., 1980; Welford and Anderson, 1988). However, care needs to be exercised in interpretation of studies using dipyridamole as it can inhibit other intracellular enzymes such as adenosine deaminase and cyclic nucleotide phosphodiesterases (Maguire and Satchell, 1979, 1981).

β,γ-Methylene ATP was not subject to enzymic degradation by guinea-pig trachealis muscle (Welford and Anderson, 1988). The authors interpretation of their data was that as β,γ-methylene ATP did not give rise to adenosine, ATP and its analogues could produce relaxation by direct interaction with receptors without being converted to adenosine. β,γ-Methylene ATP has been shown to be an agonist at P_1 purinoceptors in rat colon muscularis mucosae (Bailey and Hourani, 1990), rat duodenum (Hourani et al., 1991) and rat colon (Nicholls et al., 1992). It is, therefore, possible that β,γ-methylene ATP could produce relaxation of guinea-pig isolated trachealis muscle directly via relaxant P_1 purinoceptors. However, ATP is not believed to have substantial agonist activity at P_1 purinoceptors (Burnstock, 1978) and is, therefore, unlikely to produce relaxation via a direct action at the A_2 adenosine receptors present in this tissue. As the relaxant action of ATP was enhanced by adenosine uptake inhibition, it appears that either ATP is subject to the same uptake process as adenosine, which seems unlikely, or that adenosine is involved in the relaxant action of ATP. It has been shown when ATP is added to segments of guinea-pig isolated trachealis muscle in tissue baths the amount of adenosine present in the bulk phase is very low over the time period in which relaxation is recorded (Welford and Anderson, 1988). However, it is likely that the concentration of adenosine formed by ATP breakdown close to receptors is much higher. It is, therefore, possible that ATP could produce relaxation in guinea-pig isolated trachealis muscle via its metabolite adenosine acting at P_1 purinoceptors.

In conclusion, this study has shown that ATP and β,γ-methylene ATP can produce relaxations of isolated guinea-pig trachealis muscle. However, there was no evidence to suggest the presence of P_2 purinoceptors mediating these relaxations, rather that their relaxant actions are probably mediated via P_1 purinoceptors. It appears that ATP may produce relaxation by conversion to adenosine while β,γ-methylene ATP directly interacts with P_1 purinoceptors. These findings indicate that caution must be used in the interpretation of results obtained using ATP and close analogues as these compounds superficially may have the same effect, but via different mechanisms which do not necessarily involve P_2 purinoceptors.

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