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Purinoceptors mediating relaxation and spasm in the rat gastric fundus

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1 The relaxant and spasmogenic effects of purines and analogues were studied in longitudinal strips of rat gastric fundus to characterize the purinoceptors involved. Classification was studied by use of agonist potency orders and of antagonists in circumstances where the influence of confounding factors was reduced. In general tone was raised by carbachol (0.1 μM).

2 Adenosine produced relaxation and was potentiated by nitrobenzylthioinosine (NBTI, 0.3 and 30 μM), an adenosine-uptake inhibitor. 8-Sulphophenyl-theophylline (8-SPT, 30 μM), a selective P_1-purinoceptor antagonist, antagonized adenosine and 5'-N-ethylcarboxamidoadenosine (NECA), a selective agonist at P_1-purinoceptors.

3 At resting tone, adenosine 5'-triphosphate (ATP) induced a small, phasic relaxation followed by a maintained spasm. When tone was raised by carbachol, ATP induced a larger relaxation followed by a smaller spasm. NBTI did not potentiate ATP, nor did 8-SPT antagonize ATP, suggesting that ATP does not act directly or indirectly at P_1-purinoceptors.

4 With raised tone, and in the presence of indomethacin (10 μM) and 8-SPT (30 μM), 2-methylthio ATP (2-MeSATP) and ATP produced relaxations followed by spasms while α,β-methylene ATP (α,β-MeATP) induced only relaxation; all responses were concentration-dependent. The compounds had similar slopes and maxima for relaxation and spasm. The rank orders of potency were 2-MeSATP ≫ α,β-MeATP > ATP for relaxation and 2-MeSATP ≫ ATP for spasm.

5 With raised tone, and in the presence of indomethacin and 8-SPT, desensitization to α,β-MeATP (100 μM) completely and only slightly suppressed responses to ATP and 2-MeSATP, respectively, as relaxants but had no effect on relaxant responses to adenosine. The magnitude of the spasms to ATP and 2-MeSATP was considerably increased by desensitization with α,β-MeATP but the spasm to KCl was not affected.

6 With raised tone, and in the presence of indomethacin and 8-SPT, reactive blue 2 (10 μM) non-selectively antagonized ATP, 2-MeATP, α,β-MeATP, adenosine and isoprenaline as relaxants. Reactive blue 2 prevented the spasms to ATP and 2-MeSATP but not spasm to KCl.

7 With raised tone, and in the presence of indomethacin, suramin (100 μM) antagonized ATP, but not adenosine, as relaxants and antagonized ATP, but not KCl, as spasmogens.

8 It is proposed that adenosine is susceptible to nucleoside-specific uptake and acts predominantly via a P_1-purinoceptor and also by a non-P_1-purinoceptor mechanism. ATP- and α,β-MeATP-induced relaxations probably occur via a P_{2X}-purinoceptor. The anomalous nature of the 2-MeSATP-induced relaxation suggests it acts both via a P_{2X}-purinoceptor and an additional mechanism. A P_{2Y}-purinoceptor is most likely to be involved in the spasms to ATP and 2-MeSATP. Therefore, the functional nature of the responses mediated by P_{2X}- and P_{2Y}-purinoceptors, relaxation and spasm respectively, are opposite to those seen in most smooth muscles.

Keywords: P_{2X}-purinoceptor; P_{2Y}-purinoceptor; P_1-purinoceptor; adenosine uptake; rat gastric fundus

Introduction

Adenosine, adenosine 5'-triphosphate (ATP) and analogues have long been known to induce relaxation and spasm of smooth muscles via an extracellular site of action. Burnstock (1978) suggested that these effects were elicited via two classes of receptors, designated P_1-purinoceptors and P_2-purinoceptors. At the P_1-purinoceptor the rank order of agonist potency is adenosine > ATP and methylxanthines are selective antagonists, whereas at the P_2-purinoceptor the agonist potency order is ATP > adenosine but here there are currently no potent selective antagonists. It has become clear that the P_2-purinoceptor is not homogeneous. Burnstock & Kennedy (1985) proposed a subdivision of the P_2-purinoceptor into P_{2X}- and P_{2Y}-subtypes. At the P_{2X}-purinoceptor the agonist potency order is α,β-methylene ATP (α,β-MeATP) > ATP = 2-methylthioATP (2-MeSATP), while it is 2-MeSATP ≫ ATP > α,β-MeATP at the P_{2Y}-purinoceptor. It has been suggested that α,β-MeATP is a selective desensitizing agent at P_{2X}-purinoceptors. Usually P_{2X}-purinoceptors mediate spasm while P_{2Y}-purinoceptors mediate relaxation of smooth muscle. In general, studies since 1985 have supported this subdivision of P_2-purinoceptors (Kennedy, 1990).

The identification of the purinoceptor(s) present in a particular smooth muscle is complicated by several factors; such as the presence of more than one receptor possibly mediating the same functional effect, the rapid metabolism of some agonists to other active compounds, the uptake of some agonists into the tissue by active transport processes, the lack of selectivity of many agonists for specific receptors and the lack of potent, selective antagonists. It has been suggested that reactive blue 2 is a moderately selective antagonist at P_{2Y}-purinoceptors at low but not high concentrations without

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effects at P_{2X}-purinoceptors or other receptors (Burnstock & Warland, 1987; Hopwood & Burnstock, 1987; Taylor *et al.*, 1989). Furthermore, the trypanocidal drug suramin has been reported to be a selective antagonist at P_{2X}- and P_{2Y}-purinoceptors relative to other receptors (Dunn & Blakeley, 1988; Den Hertog *et al.*, 1989; Hoyle *et al.*, 1990; Leff *et al.*, 1990; Von Kügelgen *et al.*, 1990).

There are observations in some smooth muscles of the gastrointestinal tract which are not entirely consistent with the P_{2X}-/P_{2Y}-purinoceptor classification or the proposition that P_{2X}-purinoceptors always mediate spasm and the P_{2Y}-purinoceptors always mediate relaxation. Wiklund & Gustafsson (1988) found that ATP and analogues produced spasm of guinea-pig isolated ileum with a potency order compatible with an action at P_{2Y}-purinoceptors but the compounds were not antagonized by reactive blue 2. More recently, Bailey & Hourani (1990) have suggested that the rat colon muscalaris mucosae contains P_{2Y}-purinoceptors which mediate spasm. In the rat gastric fundus, ATP produced a biphasic effect, relaxation followed by spasm (Burnstock *et al.*, 1970; Lefebvre & Burnstock, 1990). Moreover, 2-MeSATP also produced a biphasic effect but α,β-MeATP and adenosine only induced relaxation. The rank order of potency for relaxation was 2-MeSATP > α,β-MeATP > ATP > adenosine which does not correspond with either P_{2X}- or P_{2Y}-purinoceptors. Also, ATP, as a relaxant, was antagonized by reactive blue 2, but not by suramin and the effect of ATP was reduced by desensitization with α,β-MeATP. Thus, Lefebvre & Burnstock (1990) concluded that the subtype mediating relaxation may differ from either the P_{2X}- and or the P_{2Y}-purinoceptor. They did not conduct a full analysis of the purinoceptor mediating spasm to ATP but suggested that it was rebound following the relaxation and involved prostaglandin generation as the spasm was reduced by indomethacin.

The purpose of this study was to clarify the nature of the purinoceptors in the isolated rat gastric fundus where the current classification is problematic. The approach was functional by using the rank order of potency of agonists. Experiments were performed both with agonists alone and in circumstances where confounding factors should be reduced (Kenakin, 1987; O'Connor *et al.*, 1990), that is, in the presence of an antagonist at P_1-purinoceptors (8-sulphophenyltheophylline, 8-SPT; Gustafsson, 1984), an inhibitor of adenosine uptake (nitrobenzylthioinosine, NBTI; Clanachan *et al.*, 1987; Jarvis, 1987), a cyclo-oxygenase inhibitor (indomethacin; Vane, 1971) and/or a desensitizing agent at P_{2X}-purinoceptors (α,β-MeATP; Burnstock & Kennedy, 1985). 5'-N-ethylcarboxaminoadenosine (NECA) was used as a stable and selective agonist at P_1-purinoceptors (Van Calker *et al.*, 1979). In addition, studies were conducted using the purported selective antagonists reactive blue 2 and suramin.

Methods

Rat gastric fundus

Male Sprague Dawley or Allen and Hanburys rats (150–600 g) were killed by a blow to the head and exsanguination. The stomach was removed and two strips of longitudinal fundal smooth muscle were prepared as described by Vane (1957). The tissues were suspended under isometric conditions in 20 ml organ baths containing a physiological salt solution (PSS) of the following composition (mM): Na^+ 143.0, K^+ 5.9, Mg^{2+} 1.2, Ca^{2+} 2.55, $H_2PO_4^-$ 1.2, SO_4^{2-} 1.2, Cl^- 128.0, HCO_3^- 25.0 and glucose 11.0. The PSS was maintained at 37°C and gassed with 95% O_2/5% CO_2. Tissues were placed under an initial tension of 1 g and allowed to equilibrate for 1 h.

In most experiments tissue tone was raised by carbachol (0.1 μM; Lefebrve & Burnstock, 1990). The carbachol contact time was 14 min. After the tissues had been exposed to carbachol (0.1 μM) for 10 min, the agonist (for example, ATP) was added and left in contact for 4 min. The tissues were washed to remove carbachol and agonist and left for 6 min before re-exposure to carbachol (0.1 μM) and agonist. In this manner non-cumulative concentration-response curves for agonists were obtained by stepwise increments of 0.5 or 1.0 $\log_{10}$ M concentrations.

Preliminary studies

Some preliminary experiments were conducted to establish the conditions to be used in the main study.

Tone induced by carbachol Two cumulative concentration-response curves for carbachol were constructed to determine a concentration that would produce a submaximal spasm of about an EC_{60}. The two curves were constructed 1 h apart. The amplitude of the spasms were expressed as a percentage of the maximum spasm obtained in each curve.

Effect of ATP at different tone levels Each tissue was initially challenged with carbachol (1 μM; an EC_{100}). Non-cumulative concentration-response curves were obtained to ATP at resting tension and, 1 h later, when tone was raised by carbachol (0.1 μM). The responses to ATP in this set of experiments were expressed as a % of the spasm to carbachol (1 μM) (but see later).

Influence of indomethacin on the responses to ATP A study was conducted to assess the involvement of the products of cyclo-oxygenase in the actions of ATP. Concentration-effect curves were constructed to ATP, with tone raised by carbachol (0.1 μM), before and after incubation with indomethacin (10 μM) for 45 min. Time-matched control tissues were exposed to the vehicle for indomethacin.

P_1-purinoceptors, adenosine uptake and the relaxation to adenosine and ATP

8-SPT (30 μM) was included in the PSS in most experiments to eliminate agonist-induced relaxations mediated directly or indirectly via P_1-purinoceptors. Concentration-response curves were constructed to adenosine, NECA or ATP in the absence and in the presence, after 45 min incubation, of 8-SPT (30 μM). To determine the importance of adenosine uptake on the potencies of adenosine or ATP, non-cumulative concentration-response curves were constructed to the compounds in the absence and presence of NBTI (0.3 and 30 μM; 45 min incubation). Indomethacin (10 μM) was included in the PSS throughout. Time-matched control tissues were exposed to the vehicle for 8-SPT or NBTI as appropriate.

All subsequent experiments were conducted on tissues with spasm induced by carbachol (0.1 μM) and with the PSS containing indomethacin (10 μM) and 8-SPT (30 μM) throughout. 8-SPT was not present for the experiments with suramin.

Agonist potencies

A non-cumulative concentration-response curve was constructed to ATP with spasm induced by carbachol (0.1 μM). After 1 h, a concentration-response curve was constructed to ATP, 2-MeSATP, α,β-MeATP or adenosine, one agonist per tissue.

Influence of desensitization to α,β-methylene ATP

Non-cumulative concentration-response curves were constructed to ATP, 2-MeSATP, adenosine or potassium chloride (KCl) with spasm induced by carbachol (0.1 μM). After 15 min, tissues were exposed to carbachol (0.1 μM) and 10 min later α,β-MeATP (100 μM) added and left in contact with the tissue for 1 h. Initially the α,β-MeATP produced a pronounced relaxation but carbachol-induced tone partially or fully recovered. After 1 h, when necessary, further carba-

chol (0.05–0.1 μM) was added to return spasm to the original level. A second concentration-response curve was constructed to ATP, 2-MeSATP, adenosine or KCl in the continued presence of α,β-MeATP (100 μM). Time-matched control tissues were exposed to the vehicle for α,β-MeATP.

Influence of reactive blue 2 or suramin

Non-cumulative concentration-response curves were constructed to ATP, 2-MeSATP, α,β-MeATP, adenosine, KCl or isoprenaline (reactive blue 2 experiments) or to ATP, adenosine or KCl (suramin experiments). Tissues were subsequently exposed to reactive blue 2 (10 μM; 45 min) or to suramin (100 μM, 90 min; Leff *et al.*, 1990) before a second concentration-response curve was constructed to the appropriate agonist. Time-matched control tissues were exposed to the vehicles for reactive blue 2 or suramin.

Statistical analysis

The response (relaxation or spasm) to each concentration of agonist was expressed as a % of the tone induced by carbachol (0.1 μM). For each tissue strip linear regression of % response versus $-\log_{10}$ M concentration of agonist was conducted, only with data from responses which lay between 20 and 80% of the maximum. From these regressions, the concentrations producing 50% of maximum response to the agonist were calculated and hence pD_2 derived for each agonist on each tissue strip. Differences between the first and second concentration-response curves were expressed as the $\log_{10}$ concentration ratio (log CR). Data were calculated as means ± s.e.mean. The significance of differences between group pD_2 or log CR were determined by paired or unpaired Student's t test, with $P<0.05$ considered to be significant. For illustrative purposes the responses to an agonist at specific drug concentrations from all tissues in the same experimental group were calculated as means ± s.e.mean.

Drugs and solutions

Stock solutions (all of 10 mM) of carbamylcholine chloride (carbachol, Sigma), ATP (disodium salt, Sigma), adenosine (hemisulphate salt, Sigma), 2-MeSATP (Research Biochemicals Inc.), α,β-MeATP (dilithium salt, Sigma), 8-SPT (Research Biochemicals Inc.), reactive blue 2 (cibacron blue 3GA, Sigma) and suramin (Bayer) were made in distilled water. Agonist solutions or solvent in controls were added to the organ baths in volumes usually not exceeding 1 ml. Indomethacin (Sigma) was dissolved initially in 10% sodium bicarbonate solution and subsequently added to the PSS. NECA (Glaxo Group Research) and isoprenaline (Sigma) were dissolved in 0.1 N hydrochloric acid with further dilutions in distilled water. NBTI (Sigma) was dissolved in 0.1 M sodium hydroxide solution with further dilutions in distilled water.

Results

Preliminary studies

Tone induced by carbachol Carbachol, added cumulatively, caused a concentration-dependent spasm. The initial and second concentration-response curves did not differ significantly from each other in terms of position, slope or maximum. Carbachol (0.1 μM) produced 64.3 ± 4.9% (first curve) and 59.3 ± 3.6% (second curves, $n=8$) of the maximum spasm to carbachol.

Effects of ATP at different tone levels Under both resting tone and with tone raised by carbachol (0.1 μM), ATP (0.1 μM–1 mM) induced an initial phasic, concentration-dependent relaxation followed by a sustained, concentration-dependent spasm. At resting tension the relaxation was small and the spasm large, while the converse was seen in the presence of raised tone but the potencies of ATP in these situations did not differ. The pD_2 values of ATP, as a relaxant, at resting and raised tone were 5.31 ± 0.15 and 5.26 ± 0.18 ($n=6$) respectively. The pD_2 values of ATP, as a spasmogen, at resting and raised tone were 5.19 ± 0.23 and 5.09 ± 0.30 ($n=6$) respectively.

Influence of indomethacin on the responses to ATP ATP, as a relaxant, was slightly but significantly ($P<0.05$) potentiated (log CR = 0.30 ± 0.04, $n=6$) by indomethacin (10 μM). The spasm to ATP was nearly abolished by indomethacin (10 μM). The vehicle for indomethacin did not modify responses to ATP. Indomethacin (10 μM) did not modify the spasm to carbachol (0.1 μM).

P_1-purinoceptors, adenosine uptake and relaxation to adenosine and ATP

Adenosine (1 μM–3 mM) caused a maintained and concentration-dependent relaxation. 8-SPT (30 μM), with indomethacin (10 μM) present throughout, antagonized adenosine (log CR = 0.67 ± 0.11, $P<0.05$, $n=6$), although the shift in the concentration-response curve was greater at the foot then the top of the curve. The pD_2 values for adenosine in the absence and presence of 8-SPT (30 μM) were 4.19 ± 0.05 and 3.51 ± 0.19 ($n=6$) respectively.

NBTI (0.3 and 30 μM) potentiated adenosine (log CR = 0.36 ± 0.12 and 0.71 ± 0.08 respectively, $n=6$, $P<0.05$). In the presence throughout of NBTI (30 μM) and indomethacin (10 μM), 8-SPT (30 μM) produced a parallel rightward displacement of the concentration-effect curve to adenosine without reduction in the maximum response. Accounting for a small rightward shift in time-matched controls, the log CR of adenosine due to 8-SPT was 1.26 ± 0.18 ($n=6$).

NECA (0.1–100 μM) caused a maintained and concentration-dependent relaxation of similar time course to that of adenosine. 8-SPT (30 μM), in the presence of indomethacin (10 μM) throughout, caused a parallel, rightward displacement of the concentration-effect curve to NECA without reduction in the maximum response (pD_2 in absence of 8-SPT = 6.15 ± 0.07, pD_2 in presence of 8-SPT = 4.12 ± 0.13, log CR = 1.99 ± 0.13, $P<0.01$, $n=6$).

NBTI (30 μM) did not modify the potency of ATP as a relaxant (pD_2 in absence of NBTI = 5.84 ± 0.28, pD_2 in the presence of NBTI = 5.90 ± 0.15, $P>0.05$, $n=6$). Relaxations produced by ATP were not modified by 8-SPT (30 μM; pD_2 in the absence of 8-SPT = 5.99 ± 0.12, in the presence of 8-SPT = 6.04 ± 0.11, $P>0.05$, $n=6$).

Neither NBTI nor 8-SPT modified the spasm to carbachol (0.1 μM).

Agonist potencies

In the presence of indomethacin (10 μM) and 8-SPT (30 μM), ATP (0.1 μM–1 mM) and 2-MeSATP (1 nM–30 μM) induced concentration-dependent relaxations, followed by small, concentration-dependent spasms while α,β-MeATP (10 nM–0.1 μM) and adenosine (1 μM–3 mM) induced concentration-dependent relaxations only. All compounds produced relaxation log concentration-effect curves which were sigmoidal in nature and had similar maxima (84–87% of the carbachol spasm; Figure 1a). The rank order of potencies for relaxation, with pD_2 values and mean potencies relative to ATP, was 2-MeSATP (7.76 ± 0.13, 59, $n=5$) ≫ α,β-MeATP (6.53 ± 0.03, 3.5, $n=5$) > ATP (5.99 ± 0.14, 1, $n=6$) ≫ adenosine (3.78 ± 0.06, 0.006, $n=6$).

2-MeSATP was more potent (mean 55 fold) than ATP as a spasmogen (pD_2 = 6.99 ± 0.28 and 5.25 ± 0.3, $n=5$ and 6 respectively) but both compounds exhibited similar maxima, about 40% of the carbachol spasm (Figure 1b).

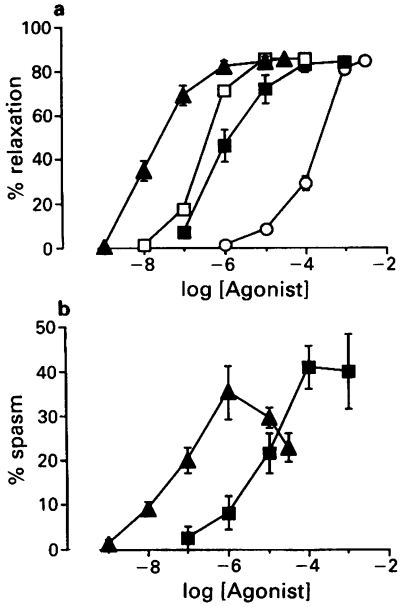

Figure 1 Non-cumulative log agonist concentration-effect curves for (a) relaxations to 2-methylthio ATP (2-MeSATP, ▲), α,β-methylene ATP (α,β-MeATP, □), ATP (■) and adenosine (○) and (b) spasms to 2-MeSATP (▲) and ATP (■) of the rat gastric fundus. Tone was induced with carbachol (0.1 μM) and tissues were incubated in indomethacin (10 μM) and 8-sulphophenyltheophylline (30 μM). The ordinate scale is the relaxation (a) or spasm (b) as a % of the tone to carbachol (0.1 μM). The abcissae are the $\log_{10}$ M concentrations of the agonist. Points represent the means and vertical lines the s.e.mean, $n = 5$–6.

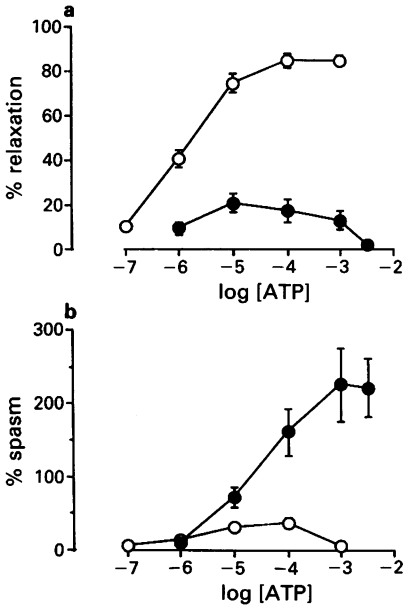

Figure 2 Non-cumulative log agonist concentration-effect curves to ATP for (a) relaxation and (b) spasm of the rat gastric fundus in the absence (○) and presence (●) of α,β-methylene ATP (α,β-MeATP, 100 μM). Tone was induced with carbachol (0.1 μM) and tissues were incubated in indomethacin (10 μM) and 8-sulphophenyltheophylline (30 μM). The ordinate scale is the relaxation (a) or spasm (b) as a % of the tone to carbachol (0.1 μM). The abcissae are the $\log_{10}$ M concentrations of ATP. Points represent the means and vertical lines the s.e.mean, $n = 5$.

Influence of desensitization to α,β-methylene ATP

Continuous exposure to a desensitizing concentration of α,β-MeATP (100 μM) in the presence of 8-SPT (30 μM) and indomethacin (10 μM) markedly reduced the relaxant response to ATP, which was now no longer concentration-dependent (Figure 2a). By contrast, the spasmogenic response to ATP was considerably enhanced during exposure to α,β-MeATP (100 μM, Figure 2b). It appeared that the potency of ATP as a spasmogen was decreased by α,β-MeATP (pD_2 in the absence of α,β-MeATP = 5.77 ± 0.20, pD_2 in the presence of α,β-MeATP = 4.58 ± 0.18, $P < 0.05$, $n = 5$), but the marked increase in maximum amplitude to ATP (from 37.8 ± 6.6% to 225.0 ± 49.6% of the carbachol-induced spasm) make such comparisons problematic. Responses to ATP were unchanged by the solvent for α,β-MeATP (distilled water).

The maximum relaxation to 2-MeSATP was reduced slightly but significantly (80.0 ± 1.9% to 59.8 ± 4.9% of the carbachol-induced spasm, $P < 0.05$, $n = 5$) during exposure to α,β-MeATP (100 μM) but the potency of 2-MeSATP was unaffected (pD_2 in the absence of α,β-MeATP = 7.71 ± 0.19, pD_2 in the presence of α,β-MeATP = 7.64 ± 0.11, $P > 0.05$, $n = 5$; Figure 3a). Like ATP, it appeared that the potency of 2-MeSATP as a spasmogen was decreased by α,β-MeATP (pD_2 in the absence of α,β-MeATP = 7.84 ± 0.15, pD_2 in the presence of α,β-MeATP = 7.28 ± 0.16, $P < 0.05$, $n = 5$), but there was an increase in the maximum amplitude to 2-MeSATP after α,β-MeATP (from 32.6 ± 4.3% to 63.0 ± 10.2% of the carbachol-induced spasm; Figure 3b). Responses to 2-MeSATP were unaffected by the solvent for α,β-MeATP (distilled water).

The potency of adenosine as a relaxant was unaffected by exposure to α,β-MeATP (100 μM; pD_2 in the absence of α,β-MeATP = 3.72 ± 0.11, pD_2 in the presence of α,β-MeATP = 3.76 ± 0.15, $n = 5$, $P > 0.05$) (Figure 4a) or the solvent for α,β-MeATP (distilled water).

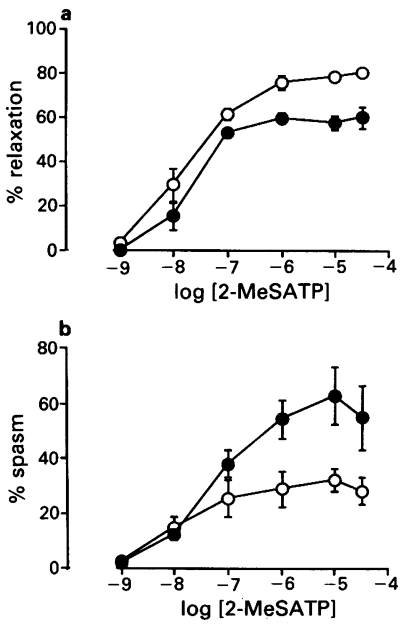

Figure 3 Non-cumulative log agonist concentration-effect curves to 2-methylthio ATP (2-MeSATP) for (a) relaxation and (b) spasm of the rat gastric fundus in the absence (○) and presence (●) of α,β-methylene ATP (α,β-MeATP, 100 μM). Tone was induced with carbachol (0.1 μM) and tissues were incubated in indomethacin (10 μM) and 8-sulphophenyltheophylline (30 μM). The ordinate scale is the relaxation (a) or spasm (b) as a % of the tone to carbachol (0.1 μM). The abcissae are the $\log_{10}$ M concentrations of 2-MeSATP. Points represent the means and vertical lines the s.e.mean, $n = 5$.

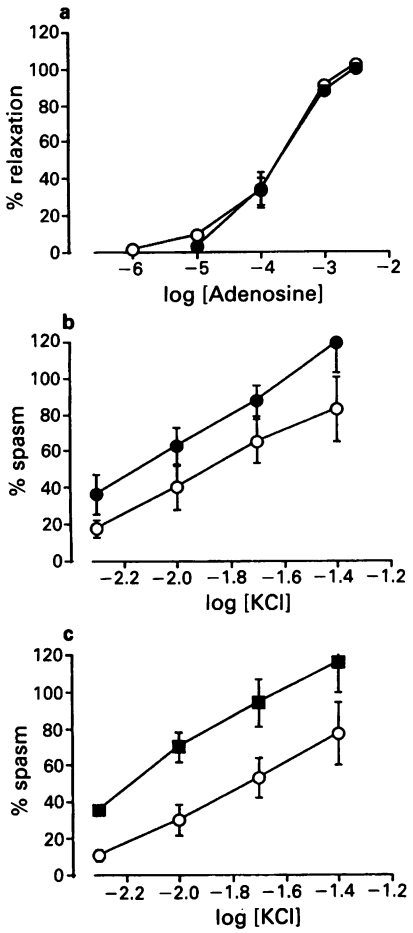

Figure 4 Non-cumulative log agonist concentration-effect curves in rat gastric fundus for (a) relaxation to adenosine and (b) spasm to KCl in the absence (○) and presence (●) of α,β-methylene ATP (α,β-MeATP, 100 μM) and (c) spasm to KCl in the absence (○) and presence (■) of solvent for α,β-MeATP. Tone was induced with carbachol (0.1 μM) and tissues were incubated in indomethacin (10 μM) and 8-sulphophenyltheophylline (30 μM). The ordinate scale is the relaxation (a) or spasm (b,c) as a % of the tone to carbachol (0.1 μM). The abcissae are the $\log_{10}$ M concentrations of adenosine or KCl. Points represent the means and vertical lines the s.e.mean, $n = 5$.

KCl (5–40 mM) produced a concentration-dependent spasm. A small increase in response to KCl occurred in the presence of α,β-MeATP compared to the initial curve (Figure 4b). In time-matched control tissues the responses in the second curve, in the presence of the solvent for α,β-MeATP (distilled water), were similarly greater in magnitude than in the first curve (Figure 4c). Therefore, α,β-MeATP had no effect on responses to KCl.

Influence of reactive blue 2

Reactive blue 2 (10 μM), in the presence of indomethacin (10 μM) and 8-SPT (30 μM) throughout, produced antagonism of ATP (14.5 fold), 2-MeSATP (13.8 fold), α,β-MeATP (3.9 fold), adenosine (3.6 fold) and isoprenaline (3.8 fold) as relaxants (Figure 5 and Table 1). For each of the agonists the log concentration-effect curves were displaced to the right in an approximately parallel manner with no reduction in the maximum response (except for a small reduction in the maximum response to ATP). The potencies of the relaxants were unchanged in solvent (distilled water), time-matched control tissues.

Reactive blue 2 (10 μM) virtually abolished the spasm to ATP and to 2-MeSATP (Figure 6a,b and Table 1). A small increase in response occurred to KCl in the presence of reactive blue 2 (10 μM), but a similar change was seen in time-matched controls (Figure 6c,d).

Neither reactive blue 2 nor its solvent (distilled water) affected the spasm to carbachol (0.1 μM).

Influence of suramin

Suramin (100 μM), in the presence of indomethacin (10 μM) throughout, produced a parallel rightward displacement of the concentration-effect curve to ATP (5.8 fold) as a relaxant with no change in the maximum response (Figure 7a and Table 2). The relaxant adenosine was not antagonized by suramin.

Suramin (100 μM) caused a rightward displacement of the log concentration-effect curve to ATP (4.3 fold) as a spasmogen with a slight reduction in the maximum response (pD_2 in absence of suramin = 5.59 ± 0.11, pD_2 in the presence of suramin = 4.96 ± 0.15, $P < 0.05$, Figure 7b and Table 2). A small increase in response occurred to KCl in the presence of suramin (100 μM), but a similar change was seen in time-matched controls, identical to that shown in Figures 4 and 6.

Neither suramin (100 μM) nor its solvent (distilled water) affected the spasm to carbachol (0.1 μM).

Discussion

This study has attempted to clarify the purinoceptors in the rat gastric fundus using functional criteria under conditions where some of the confounding problems have been reduced. The data suggest the presence of P_1-purinoceptors mediating relaxation and possibly the presence of P_{2X}- and P_{2Y}-purinoceptors mediating relaxation and spasm respectively.

Adenosine uptake and P_1-purinoceptors

NBTI is reported to be a selective inhibitor of adenosine uptake (Clanachan *et al.*, 1987; Jarvis, 1987). In the present study NBTI selectively potentiated adenosine without affecting responses to ATP. These data suggest that uptake of adenosine is a significant factor in terminating the action of adenosine which results in an underestimation of the true potency of adenosine. It is likely that NBTI (30 μM) had produced a complete block of adenosine uptake as this concentration only induced a 2.2 fold greater potentiation of adenosine than NBTI (0.3 μM).

8-SPT produced marked and selective antagonism of adenosine and NECA, as relaxants, without any antagonism of ATP. As NECA is purported to be a selective agonist at P_1-purinoceptors (van Calker *et al.*, 1979) and 8-SPT a selective antagonist at P_1-purinoceptors (Gustafsson, 1984), these results provide strong support for the idea that relaxation to adenosine and NECA in this tissue is mediated mainly via P_1-purinoceptors. It was interesting to note that 8-SPT (100 μM) produced only a 4.7 fold antagonism of adenosine in PSS without NBTI, with a greater shift at the foot rather than the top of the concentration-effect curve. However, in the presence of NBTI where adenosine was more potent, 8-SPT (100 μM) produced an 18.2 fold antagonism of adenosine, with a parallel displacement of the concentration-effect curve. This antagonism was still less than the 97.7 fold antagonism of the more potent NECA. It is likely that relaxation to high concentrations of adenosine is mediated by another mechanism in addition to P_1-purinoceptors. This additional mechanism is unlikely to involve P_2-purinoceptors as adenosine was not antagonized by suramin (see below). Identification of the subtype of P_1-purinoceptors with which adenosine and NECA interact, whether A_1 or A_2 (Kennedy, 1990), requires further studies with selective agonists and antagonists.

It is clear that the relaxant action of ATP does not signi-

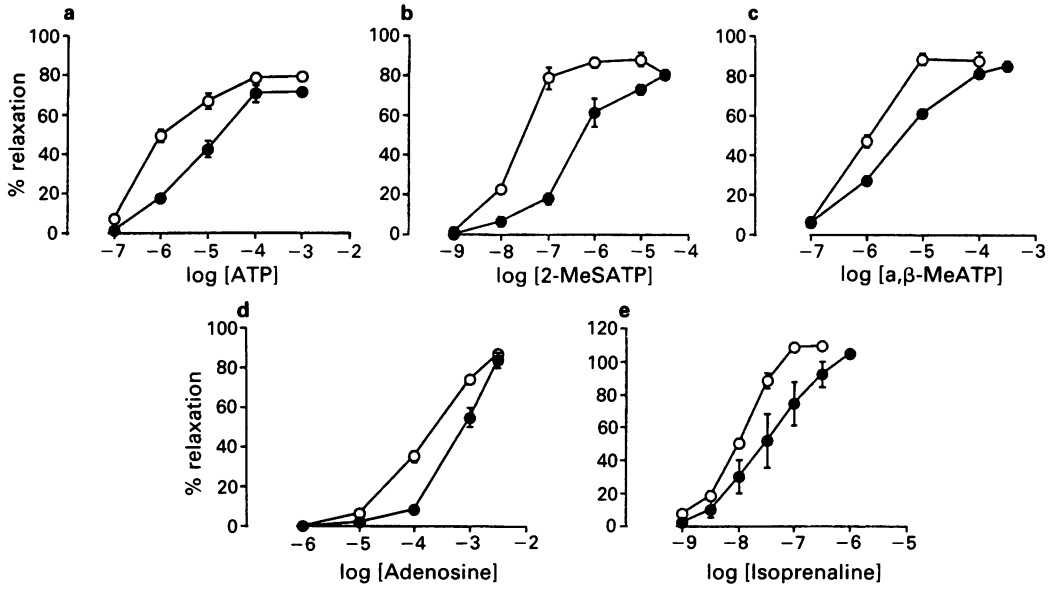

Figure 5 Non-cumulative log agonist concentration-effect curves for relaxations of the rat gastric fundus to (a) ATP, (b) 2-methylthio ATP (2-MeSATP), (c) α,β-methylene ATP (α,β-MeATP), (d) adenosine and (e) isoprenaline in the absence (open symbols) and presence (closed symbols) of reactive blue 2 (10 μM). Tone was induced with carbachol (0.1 μM) and tissues were incubated in indomethacin (10 μM) and 8-sulphophenyltheophylline (30 μM). The ordinate scale is the relaxation as a % of the tone to carbachol (0.1 μM). The abcissae are the $\log_{10}$ M concentrations of the agonist. Points represent the means and vertical lines the s.e.mean, $n = 5-6$.

Table 1 Effect of reactive blue 2 (10 μM), or its solvent (distilled water) in time-matched controls, on parameters of relaxation and spasm concentration-effect curves to several purine analogues

		Test		*Time-matched controls*	
Compound		*Initial*	*Reactive blue 2*	*Initial*	*Solvent*
Relaxation					
ATP	pD_2	6.19 ± 0.07	5.04 ± 0.13*	6.02 ± 0.05	5.99 ± 0.14
	α (%)	78.8 ± 2.0	71.0 ± 1.1*	82.3 ± 1.5	84.0 ± 1.4
2-MeSATP	pD_2	7.59 ± 0.03	6.45 ± 0.10*	7.55 ± 0.18	7.39 ± 0.13
	α (%)	80.8 ± 0.4	80.0 ± 2.0	83.4 ± 3.0	88.8 ± 2.8
α,β-MeATP	pD_2	6.07 ± 0.08	5.48 ± 0.05*	6.01 ± 0.05	5.85 ± 0.12
	α (%)	87.6 ± 4.4	85.2 ± 2.5	93.2 ± 1.8	92.2 ± 2.0
Adenosine	pD_2	3.79 ± 0.10	3.23 ± 0.09*	3.62 ± 0.13	3.77 ± 0.19
	α (%)	86.8 ± 2.0	84.0 ± 3.7	93.6 ± 4.0	96.6 ± 3.80
Isoprenaline	pD_2	7.97 ± 0.03	7.39 ± 0.26*	8.13 ± 0.04	8.03 ± 0.08
	α (%)	109.3 ± 1.9	104.2 ± 2.6	108.0 ± 1.9	106.4 ± 1.2
Spasm					
ATP	pD_2	5.33 ± 0.11	NM	5.43 ± 0.05	5.25 ± 0.30
	α (%)	44.8 ± 4.9	8.0 ± 5.1*	32.8 ± 3.9	41.0 ± 4.9*
2-MeSATP	pD_2	7.42 ± 0.15	6.49 ± 0.42*	7.62 ± 0.18	7.49 ± 0.24
	α (%)	52.0 ± 2.6	16.2 ± 3.0*	34.0 ± 5.5	31.4 ± 4.0

Data are means ± s.e.mean; $n = 5-6$. *indicates significantly different from data in initial concentration-effect curve ($P < 0.05$). α(%) = maximum response as a % of spasm to carbachol (0.1 μM). NM indicates not measurable. The PSS contained indomethacin (10 μM) and 8-sulphophenyltheophylline (30 μM) throughout. 2-MeSATP = 2-methylthio ATP and α,β-MeATP = α,β-methylene ATP.

ficantly involve P_1-purinoceptors either indirectly, via the metabolism to adenosine, or directly as the potency of ATP was unaffected by NBTI or 8-SPT.

Interaction between the two responses to ATP

ATP produced an initial phasic relaxation followed by a maintained spasm. The degree of tone modified the magnitude of the two responses, as reported previously by Lefebvre & Burnstock (1990), but we have found that the potencies for these two effects were unaffected by the extent of tone. Lefebrve & Burnstock (1990) suggested that the spasm was rebound, that is secondary to the relaxation. However, we consider that the two phases are separate but functionally interact. The spasm cannot be significantly rebound in nature as indomethacin increased the potency of ATP as a relaxant but reduced spasmogenic responses to ATP. Additionally, desensitization with α,β-MeATP markedly reduced the relaxant response to ATP but induced a considerable augmentation of the spasmogenic response to ATP. Lefebvre & Burnstock (1990) suggested that the spasmogenic response to ATP involved stimulation of prostaglandin biosynthesis. We concur that indomethacin markedly reduced the spasmogenic response to ATP. However, there was a large spasmogenic response to ATP, after desensitization to α,β-MeATP, in the presence of indomethacin (10 μM, Figure 2b), a concentration sufficient to abolish prostaglandin biosynthesis (Vane, 1971). It is proposed that ATP interacts with different purinoceptors

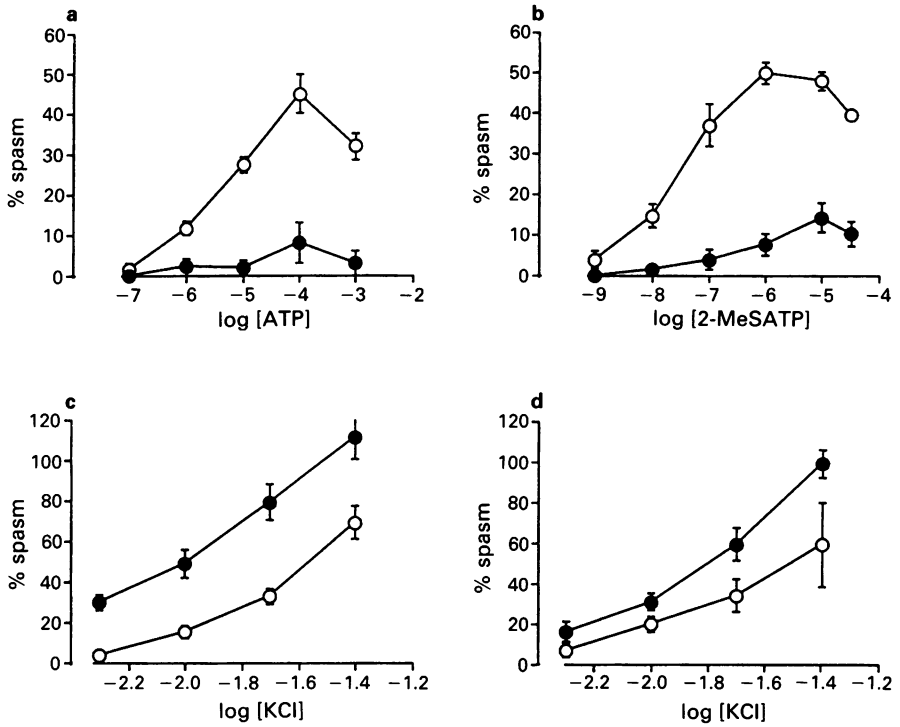

Figure 6 Non-cumulative log agonist concentration-effect curves for the rat gastric fundus for spasm to (a) ATP, (b) 2-methylthio ATP (2-MeSATP), (c) KCl in the absence (open symbols) and presence (closed symbols) of reactive blue 2 (10 μM) and (d) to KCl in the absence (○) and presence (●) of the solvent for reactive blue 2 in time-matched controls. Tone was induced with carbachol (0.1 μM) and tissues were incubated in indomethacin (10 μM) and 8-sulphophenyltheophylline (30 μM). The ordinate scale is the spasm as a % of the tone to carbachol (0.1 μM). The abcissae are the $\log_{10}$ M concentrations of the agonist. Points represent the means and vertical lines the s.e.mean, $n = 5$–6.

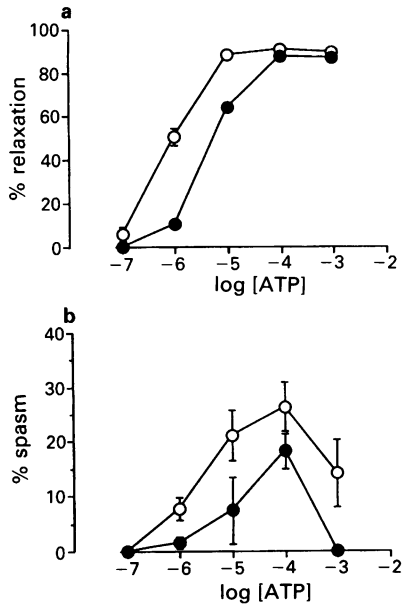

Figure 7 Non-cumulative log agonist concentration-effect curves in rat gastric fundus for (a) relaxation and (b) spasm to ATP in the absence (○) and presence (●) of suramin (100 μM). Tone was induced with carbachol (0.1 μM) and tissues were incubated in indomethacin (10 μM). The ordinate scale is the relaxation as a % of the tone to carbachol (0.1 μM). The abcissae are the $\log_{10}$ M concentrations of ATP. Points represent the means and vertical lines the s.e.mean, $n = 5$.

mediating relaxation and spasm and that the magnitude of each effect is functionally affected by the size of the other response. In addition, prostaglandin biosynthesis plays only a minor role in mediating the spasm.

Relaxant responses to ATP, 2-methylthio ATP and α,β-methylene ATP

Usually P_{2Y}-purinoceptors mediate relaxation of smooth muscle (Burnstock & Kennedy, 1985). The rank order of agonist potency in rat gastric fundus for relaxation was 2-MeSATP ≫ α,β-MeATP > ATP which does not accord with the commonly accepted rank order for the P_{2Y}-purinoceptor which is 2-MeSATP ≫ ATP > α,β-MeATP (Burnstock & Kennedy, 1985). The rank order found in the present study, where tissues were exposed to 8-SPT and indomethacin, is the same as that reported by Lefebvre & Burnstock (1990), where these modifying agents were absent. In the present study the three agonists exhibited similar slopes and maxima for their concentration-effect curves in contrast to the previous study where the relaxants had different slopes and maxima.

The results with the antagonists help to confirm the role of P_2-purinoceptors mediating relaxation to these three agonists but not the sub-type of receptor. Reactive blue 2 is purported to be a slightly selective antagonist at P_{2Y}-purinoceptors (Burnstock & Warland, 1987; Hopwood & Burnstock, 1987; Taylor *et al.*, 1989). However, in the present study reactive blue 2, at a relatively low concentration of 10 μM (Burnstock & Warland, 1987; Lefebvre & Burnstock, 1990), produced marked antagonism of ATP and 2-MeSATP as relaxants as well as an approximately 4 fold antagonism of other relaxants (α,β-MeATP, adenosine and isoprenaline). The latter two compounds do not act via P_2-purinoceptors. Reactive blue 2 may be, at least partially, non-selective preventing relaxation at a post-receptor site.

Recent reports have suggested that suramin is a selective antagonist at P_2-purinoceptors relative to other receptors, but it does not distinguish between P_{2X}- and P_{2Y}-purinoceptors (Dunn & Blakeley, 1988; Den Hertog *et al.*, 1989; Hoyle *et al.*, 1990; Von Kugelgen *et al.*, 1990). Lefebvre & Burnstock

Table 2 Effect of suramin (100 μM), or its solvent (distilled water) in time-matched controls, on parameters of relaxation and spasm concentration-effect curves to purine analogues

		Test		*Time-matched controls*	
Purine		*Initial*	*Suramin*	*Initial*	*Solvent*
Relaxation					
ATP	pD_2	6.11 ± 0.08	5.26 ± 0.04*	6.02 ± 0.05	5.99 ± 0.14
	α(%)	91.8 ± 1.9	89.4 ± 1.2	82.3 ± 1.5	84.0 ± 1.4
Adenosine	pD_2	4.73 ± 0.05	4.68 ± 0.06	4.79 ± 0.19	4.72 ± 0.17
	α(%)	89.0 ± 1.3	88.8 ± 1.4	90.2 ± 2.2	95.6 ± 3.2
Spasm					
ATP	pD_2	5.59 ± 0.11	4.96 ± 0.15*	5.43 ± 0.05	5.25 ± 0.30
	α(%)	26.4 ± 4.5	18.2 ± 3.3*	32.8 ± 3.9	41.0 ± 4.9*

Data are means ± s.e.mean; $n = 6$. *indicates significantly different from data in initial concentration-effect curve ($P < 0.05$). α(%) = maximum response as a % of spasm to carbachol (0.1 μM). The PSS contained indomethacin (10 μM) throughout.

(1990) failed to see any antagonism of ATP, as a relaxant, in rat gastric fundus by suramin (100 μM). It is likely that their period of incubation (10 or 20 min) was too short. Recently Leff *et al.* (1990) have shown that suramin is a slowly equilibrating but competitive antagonist at P_{2X}-purinoceptors in rabbit isolated ear artery. They estimated the times necessary for different concentrations of suramin to achieve at least 95% occupancy at the P_{2X}-purinoceptors in rabbit ear artery and found this to be 86 min for suramin (100 μM). Thus, in the present study the rat gastric fundus was incubated with suramin (100 μM) for 90 min. Suramin was found to produce a parallel shift in the concentration-effect curve to ATP, as a relaxant, with no antagonism of adenosine, demonstrating its selectivity as an antagonist.

Desensitization was readily produced to α,β-MeATP with marked cross-desensitization to ATP but only limited cross-desensitization to 2-MeSATP. The cross-desensitization was selective as none was seen to adenosine. Also the high potency of 2-MeSATP as a relaxant must be considered. One explanation of the data is that the relaxation to α,β-MeATP and to ATP is mediated via a common mechanism (perhaps P_{2X}-purinoceptors) while that to 2-MeSATP is mediated partly via a mechanism which exhibits desensitization with α,β-MeATP, possibly the P_{2X}-purinoceptors, and also partly via an additional mechanism, which does not exhibit desensitization with α,β-MeATP. 2-MeSATP showed a larger maximal response in the absence compared with the presence of α,β-MeATP, perhaps due to the addition of the two actions proposed. Dainty *et al.* (1990) have reported in the rat thoracic aorta that 2-MeSATP is a relaxant and acts as a partial agonist via a mechanism not common to ATP as 2-MeSATP failed to antagonize ATP. A second explanation is that the 3 agonists interact at a common novel purinoceptor which exhibits a rank order of agonist potency of 2-MeSATP ≫ α,β-MeATP > ATP, shows desensitization with α,β-MeATP and at which suramin is an antagonist. The differential cross-desensitization (ATP ≫ 2-MeSATP) could be due to possible differences in intrinsic efficacies of the two agonists at this novel receptor. Responses to an agonist of lower intrinsic efficacy are more subject to a decrease in tissue response capability in the presence of a desensitizing agent and, therefore, not all agonists will be equally affected by receptor desensitization (Kenakin, 1987). If ATP has a lower intrinsic efficacy than 2-MeSATP, at this novel receptor, then the responses to ATP would be preferentially depressed over those to 2-MeSATP. A third explanation is that 2-MeSATP interacts with a P_{2X}-purinoceptor while ATP interacts with this receptor plus another receptor. O'Connor *et al.* (1991) have suggested the presence of a novel 'nucleotide' receptor in certain smooth muscles and other tissues at which ATP and uridine 5'-triphosphate are agonists and with which 2-MeSATP does not interact. In these tissues 2-MeSATP produces a smaller maximum response than ATP. However, in rat fundus the maximum reponses to ATP and 2-MeSATP were essentially the same. Also, there was greater cross-desensitization between α,β-MeATP and ATP than between α,β-MeATP and 2-MeSATP. The first of the three explanations is currently more likely as there is more evidence in support and fewer assumptions have to be made.

Spasm to 2-methylthio ATP and ATP

2-MeSATP and ATP produced spasm of the rat gastric fundus, with 2-MeSATP being 55 fold as potent as ATP, in the presence of 8-SPT and indomethacin. If these two compounds are acting at a common receptor, the relative potency accords with a P_{2Y}-purinoceptor (Burnstock & Kennedy, 1985; Berrie *et al.*, 1989; Kennedy, 1990). α,β-MeATP failed to produce spasm and desensitization with α,β-MeATP enhanced the magnitude of the spasm to ATP and 2-MeSATP. These latter results are in accord with the idea that spasm was mediated via P_{2Y}-purinoceptors. Bailey & Hourani (1990) have provided the first evidence for a P_{2Y}-purinoceptor mediating spasm, in the rat isolated colon.

Reactive blue 2 (10 μM) abolished spasms to ATP and 2-MeSATP with no effect on spasms to KCl or carbachol. Therefore, reactive blue 2 appears to be a selective antagonist against spasm to purinoceptor agonists but not to their relaxation in rat gastric fundus. Burnstock & Warland (1987) suggested that reactive blue 2 is a selective antagonist at P_{2Y}-purinoceptors in rabbit ear artery where purine analogues produced relaxation. By analogy, present data with reactive blue 2 provides further evidence that ATP and 2-MeSATP cause spasm via P_{2Y}-purinoceptors. Suramin selectively antagonized ATP as a spasmogen without effect on responses to KCl or carbachol. The results with the two antagonists support the role of P_2-purinoceptors in the spasmogenic actions of ATP and 2-MeSATP but only aid the classification of the sub-type of receptor involved to a small extent.

In conclusion, rat gastric fundus exhibits relaxation and spasm to a number of purine analogues. It is proposed that adenosine produces relaxation partially via a P_1-purinoceptor and exhibits nucleoside-specific uptake. ATP- and α,β-MeATP-induced relaxation most probably occurs via a P_{2X}-purinoceptor, which is the first report of such a receptor mediating relaxation. 2-MeSATP-induced relaxation may occur via both a P_{2X}-purinoceptor and an additional mechanism. ATP and 2-MeSATP also cause spasm, probably via a P_{2Y}-purinoceptor. Further studies of the complex mixture of purinoceptors in this tissue will require the development of more selective antagonists.

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