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P_2-purinoceptors of two subtypes in the rabbit mesenteric artery: reactive blue 2 selectively inhibits responses mediated via the P_{2y}- but not the P_{2x}-purinoceptor

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1 α,β-Methylene ATP and ATP both produced concentration-dependent contractions of the isolated mesenteric artery of the rabbit that were not inhibited by reactive blue 2.

2 In preparations where the tone had been raised with noradrenaline, ATP and 2-methylthio ATP, but not α,β-methylene ATP, produced relaxations of the vessel. These relaxations were inhibited in the presence of reactive blue 2.

3 Reactive blue 2 did not inhibit the contractions to noradrenaline, and only slightly inhibited relaxations to adenosine and acetylcholine.

4 The rank order of potency of purine nucleotide analogues in contracting the vessel was: α,β-methylene ATP ≫ β,γ-methylene ATP = 2-methylthio ATP > ATP, and in relaxing the vessel at raised tone was: 2-methylthio ATP ≫ ATP ≫ β,γ-methylene ATP > α,β-methylene ATP.

5 It is concluded from this study that in the isolated mesenteric artery of the rabbit, purine nucleotides act via P_{2y}-purinoceptors to cause the muscle to relax and via P_{2x}-purinoceptors to cause the muscle to contract. The results also suggest that reactive blue 2 selectively inhibits responses mediated via the P_{2y}-purinoceptor, at least within a limited concentration range.

Introduction

In 1978 Burnstock proposed that there were two subclasses of the purinoceptor: adenosine acted with greater potency at the P_1-purinoceptor and was antagonized by methylxanthines, while ATP acted with greater potency at the P_2-purinoceptor (Burnstock, 1982). Later it was shown that P_2-purinoceptor-mediated responses were antagonized by arylazido-aminoproprionyl ATP ($ANAPP_3$) (Hogaboom *et al.*, 1980) and were desensitized by α,β-methylene ATP (Kasakov & Burnstock, 1983). Studies on a number of tissues have demonstrated that purine nucleotides act via P_2-purinoceptors to produce contractions in some tissues and relaxations in others (Burnstock & Brown, 1981; Burnstock & Kennedy, 1985). Recently a further division of the P_2-purinoceptor into the P_{2x} and P_{2y} subtypes has been proposed (Burnstock & Kennedy, 1985). This subdivision was largely based on the rank order of potency of purine agonists in a number of tissues. In general, the order of potency at the P_{2x}-purinoceptor was: α,β-methylene ATP, β,γ-methylene ATP > ATP = 2-methylthio ATP, while at the P_{2y}-purinoceptor it was: 2-methylthio ATP ≫ ATP > β,γ-methylene ATP, α,β-methylene ATP. Also in support of this subdivision, $ANAPP_3$, the P_2-purinoceptor antagonist, and α,β-methylene ATP, which desensitizes the P_2-purinoceptor, are in fact selective in antagonizing only the P_{2x}-purinoceptor-mediated response (see Burnstock & Kennedy, 1985). In general P_{2x}-purinoceptors mediate constriction while P_{2y}-purinoceptors mediate dilatation. Reactive blue 2, an anthraquinone sulphoric acid derivative, which can be looked upon as an ATP analogue, has been shown to antagonize inhibitory responses to ATP in the guinea-pig colon (Kerr & Krantis, 1979), internal anal sphincter (Crema *et al.*, 1983), rat duodenum and rat caecum (Manzini *et al.*, 1985; 1986), but not to antagonize specifically purinergic contractions in the rat urinary bladder (Choo, 1981).

In isolated preparations of the rabbit mesenteric

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artery, ATP acts via P_2-purinoceptors to produce both contractions and, on preparations in which tone has been raised by noradrenaline, relaxations of the vessel. In order to produce each of these responses in the rabbit isolated mesenteric artery, ATP, unlike acetylcholine, acts directly via receptors on the smooth muscle of the vessel; the endothelium is not involved (see Furchgott & Zawadzki, 1980; Vanhoutte & Rimele, 1983; Mathieson & Burnstock, 1985). After the vessel has been desensitized to α,β-methylene ATP, the contractions to ATP and α,β-methylene ATP are abolished whereas relaxations to ATP are unaffected (Mathieson & Burnstock, 1985). The aim of the present investigation was to study the rank order of potency of ATP, α,β-methylene ATP, β,γ-methylene ATP, and 2-methylthio ATP in producing contractions and relaxations of the rabbit mesenteric artery. Also the selectivity of reactive blue 2 in inhibiting the relaxant response to purine nucleotides (via the P_{2y}-purinoceptor) was investigated: relaxations to 2-methylthio ATP, ATP, acetylcholine and adenosine, and contractions to ATP, α,β-methylene ATP and noradrenaline were compared in the absence and in the presence of this drug. A preliminary report of some of this work has been presented to the British Pharmacological Society (Burnstock *et al.*, 1986).

Methods

Pharmacology

Male New Zealand white rabbits (2.5–3.8 kg) were killed by a blow to the head and exsanguination. Side branches of the superior mesenteric artery were carefully dissected from the mesentery. From each branch, two ring segments 4 mm in length were excised and mounted horizontally under isometric conditions in 12 ml organ baths by inserting two tungsten wires into the lumen according to the method of Bevan & Osher (1972). The tissues were bathed in Krebs solution of the following composition (mM): NaCl 133, KCl 4.7, $NaHPO_4$ 1.35, $NaHCO_3$ 16.3, $MgSO_4$ 0.61, glucose 7.8 and $CaCl_2$ 2.52 (Bülbring, 1953). The solution was gassed with 95% O_2 and 5% CO_2 and maintained at 37°C. Preparations were allowed to equilibrate for 1–2 h under a resting tension of 0.75 g. Responses of the circular smooth muscle were recorded with a Grass FTO3C transducer and a Grass polygraph (model 79). On preparation of the vessel, the endothelium was left intact. This was verified by relaxing the noradrenaline-preconstricted vessel with acetylcholine (Mathieson & Burnstock, 1985).

In time, ATP and analogues of ATP cause desensitization of their own contractile response. So as to avoid this desensitization, ATP, α,β-methylene ATP, β,γ-methylene ATP and 2-methylthio ATP were added to the bath as single additions at intervals of 30–40 min with repeated washings between each addition. Noradrenaline did not cause desensitization of its receptor but maintained its tone and therefore was added cumulatively to the bath. Rarely, when this was not the case, subsequent relaxation-response curves were not carried out. To study relaxation, vascular tone was raised with a concentration of noradrenaline (1–10 μM) that produced approximately 50% of the maximal noradrenaline contraction. In these 'raised-tone preparations', purine nucleotides and acetylcholine did not cause desensitization of receptors and were therefore added cumulatively to the bath. At least 30 min was allowed between the start of each cumulative relaxation-response curve. Contractile and relaxant responses were repeated as appropriate to ensure that the responses were consistent with time and with repeated administration of each agonist.

Responses to drugs were compared in the absence and in the presence of three different concentrations of reactive blue 2 (21.6 μM, 46.4 μM and 0.1 mM). On its first addition at a given concentration, reactive blue 2 was left to equilibrate for 5–10 min before starting the concentration-response curve. Experiments were continued for no longer than 2 h after reactive blue 2 had been initially added to the bath, since beyond this time it tended to have a general desensitizing action on all drug responses. Hence up to 4 cumulative relaxation-response curves (or 1 non-cumulative contractile-response curve) were performed on each tissue once reactive blue 2 had been added to the bath. Reactive blue 2 was washed out of the bath at the end of each

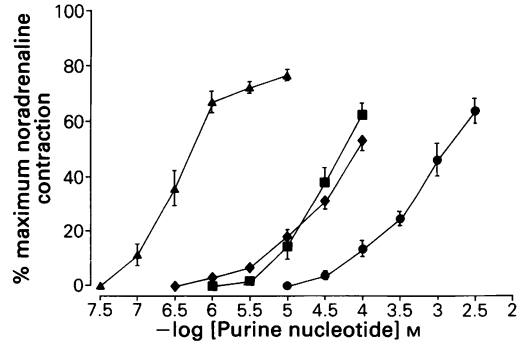

Figure 1 Isolated transverse ring preparation of the rabbit mesenteric artery at basal tone. Concentration-response curves (non-cumulative, contractions) to ATP (●) ($n = 7$), 2-methylthio ATP (■) ($n = 7$), α,β-methylene ATP (▲) ($n = 6$) and β,γ-methylene ATP (◆) ($n = 8$) are expressed as % of the maximal contraction to noradrenaline. Maximal contraction to noradrenaline was 2.7 ± 0.2 g ($n = 19$). Vertical bars denote s.e.mean.

concentration-response curve (or after each concentration of a non-cumulative concentration-response curve) and reapplied 5–10 min before the next application of agonist. Relaxant concentration-response curves to a specific agonist in the absence and presence of reactive blue 2 (21.6 μM, 46.4 μM, 0.10 mM) were compared concurrently in some cases, each concentration of reactive blue 2 being applied to a different preparation, and in others sequentially on the same preparation. Contractile concentration-response curves to noradrenaline and ATP in the absence and the presence of reactive blue 2 (0.10 mM) were compared sequentially on the same preparation. α,β-Methylene ATP, on the other hand, slightly desensitized the tissues so that low doses of α,β-methylene ATP were not repeatable 1 h after administration of the highest concentration. Therefore α,β-methylene ATP concentration-response curves in the absence and presence of reactive blue 2 (0.10 mM) were compared concurrently each on a different preparation.

Statistical methods

Dilatation was expressed as a percentage of the maximal relaxation of the raised-tone preparation, while vasoconstriction was expressed as a percentage of the maximal contraction to noradrenaline. In the figures, the response indicated for each drug concentration for a given concentration-response curve is a mean value calculated from a number of preparations, denoted as n in the figure legends, a single observation being made from each animal. Vertical bars indicate the standard error of the mean (s.e.mean). The slope for individual concentration-response curves was calculated from the regression of the percentage maximal response to log (agonist concentration) and the mean ± 95% confidence limits (CL) was determined for each group. In many cases maximal contraction or relaxation for a particular drug was not attained at the concentrations tested, therefore EC_{50} values could not be calculated. Instead, drug potencies were expressed in terms of the concentration of drug required to produce either 50% of the maximal contraction to noradrenaline or, in raised tone preparations, 40% of the maximal relaxation. Results have been analysed either by Student's paired or unpaired t tests as appropriate or, if more than two groups were compared, by analysis of variance. A probability P of <0.05 was considered significant.

Drugs

Adenosine 5'-triphosphate (ATP), lithium salt of α,β-methylene adenosine 5'-triphosphate (α,β-methylene ATP), sodium salt of β,γ-methylene adenosine 5'-triphosphate (β,γ-methylene ATP), acetylcholine chloride, hemi sulphate salt of adenosine, noradrenaline bitartrate and reactive blue 2 (60% pure) were all obtained from Sigma Chemical Company. NO^+ salt of 2-methylthio adenosine 5'-triphosphate (2-methylthio ATP) was a gift from Ciba Geigy Chemical Company (Basal), and Na salt of 2-methylthio ATP was obtained from Research Biochemicals Inc. (U.S.A.). All drugs were made up fresh each day in distilled water. Ascorbic acid (100 μM) was added to the noradrenaline solution.

Results

Rank order of potencies of purines in producing responses

Exogenous ATP (10 μM–3.0 mM), α,β-methylene ATP (0.03 μM–10 μM), β,γ-methylene ATP (1.0 μM–0.1 mM) and 2-methylthio ATP (0.3 μM–0.1 mM) each produced concentration-dependent transient contractions of the rabbit mesenteric artery which reached a peak within 5–15 s, and even in the continued presence of the drug, returned rapidly towards baseline if not washed out of the bath. β,γ-Methylene ATP, ATP and 2-methylthio ATP did not reach a maximal response over the concentration-range tested, whereas α,β-methylene ATP produced a near-maximal response. The order of potency of these purines in producing contractions of the vessel was: α,β-methylene ATP ≫ β,γ-methylene ATP = 2-methylthio ATP > ATP. α,β-Methylene ATP was 1600 times more potent than ATP in producing contraction of the vessel. There was no significant difference between the potencies of β,γ-methylene ATP and 2-methylthio ATP, but they were 30 times more potent than ATP (Figure 1). The slopes of the concentration-response curves to the 4 purine analogues were significantly different, although the variation was not great; the slopes of the concentration-response curves to α,β-methylene ATP (52.9 ± 12.8 CL) and to β,γ-methylene ATP (44.3 ± 11.8 CL) were greater, and the slope of the concentration-response curve to 2-methylthio ATP (28.6 ± 10.8 CL) was not greatly different from that of ATP (31.3 ± 5.4 CL).

In preparations of the rabbit mesenteric artery in which tone has been raised with noradrenaline, exogenous ATP (0.03–30 μM), β,γ-methylene ATP (0.1–30 μM) and 2-methylthio ATP (1.0 nM–3 μM) produced sustained, concentration-dependent relaxations. Often there was an initial transitory contraction of the vessel before this sustained relaxation (Figure 3). Both the NO^+ salt (which was the salt used regularly throughout the experiments) and the Na salt of 2-methylthio ATP were equipotent in their relaxant responses. (This indicates that the NO^+ component of the agonist is not itself acting as an uncoupler of oxidation phosphorylation to relax the smooth mus-

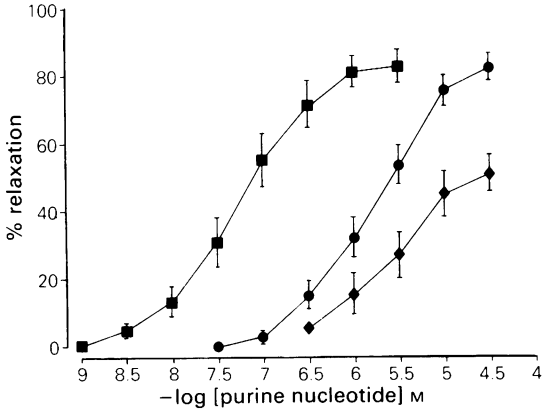

Figure 2 Isolated transverse ring preparation of rabbit mesenteric artery with tone raised and maintained by noradrenaline (3–10 μM). Concentration-response curves (cumulative, relaxations) to ATP (●) ($n = 15$), 2-methylthio ATP (■) ($n = 16$) and β,γ-methylene ATP (◆) ($n = 10$) are expressed as % of the maximal relaxation. Vertical bars denote s.e.mean. Note that α,β-methylene ATP caused contraction of this preparation.

cle.) 2-Methylthio ATP and ATP each reached a maximal relaxation of approximately 80% of the total possible relaxation. β,γ-Methylene ATP, at the concentrations tested, did not quite reach its maximal relaxation, and only relaxed the preparation to just over 50% (Figure 2). α,β-Methylene ATP (0.3–10 μM), on the other hand, did not produce relaxation of the raised-tone preparation but caused a further contraction of the vessel (results not shown). The order of potency of purines in producing relaxation of the isolated rabbit mesenteric artery was: 2-methylthio ATP ≫ ATP > β,γ-methylene ATP > α,β-methylene ATP (Figure 2). 2-Methylthio ATP was 30 times more potent than ATP. Although β,γ-methylene ATP appears to be less potent than ATP in producing relaxations of the vessel, this difference is not significant. The mean slope of the concentration-response curve to ATP (44.4 ± 9.3 CL) was not significantly different from the slopes of the concentration-response curves to 2-methylthio ATP (40.6 ± 8.8 CL) or β,γ-methylene ATP (33.0 ± 5.5 CL).

Action of reactive blue 2 on drug responses

With increasing concentrations of reactive blue 2 in the bath (21.6 μM, 46.4 μM, 0.10 mM) there was a progressive reduction in the maximal relaxation produced to ATP and to 2-methylthio ATP, and a progressive reduction in the slope of the relaxant concentration-response curves to these two purines. In the presence of reactive blue 2 (0.10 mM), the relaxant response to ATP and 2-methylthio ATP was abolished (Figures 3, 4a,b, Table 1). On the other hand, the maximal relaxations produced to adenosine and to acetylcholine, in the presence of reactive blue 2, were

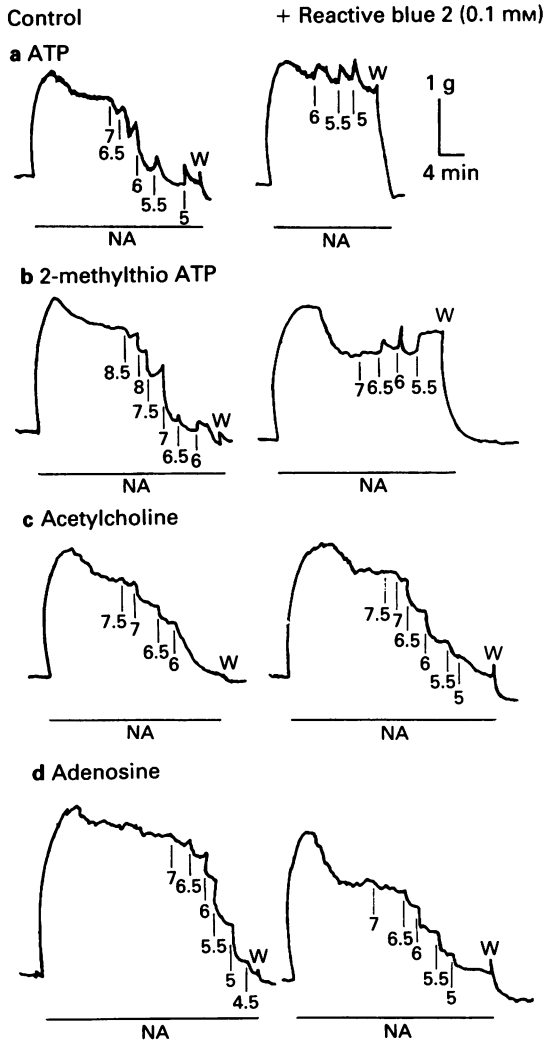

Figure 3 Isolated transverse ring preparation of rabbit mesenteric artery with tone raised and maintained by noradrenaline (NA, 3–10 μM, indicated by the horizontal line). Concentration-response curves (cumulative, relaxation) to (a) ATP, (b) 2-methylthio ATP, (c) acetylcholine and (d) adenosine in the absence (first trace) and in the presence (second trace) of reactive blue 2 (0.10 mM). Drug concentrations indicated on the traces are expressed as − log (drug concentration) M. Horizontal bar indicates 4 min, and vertical bar 1 g.

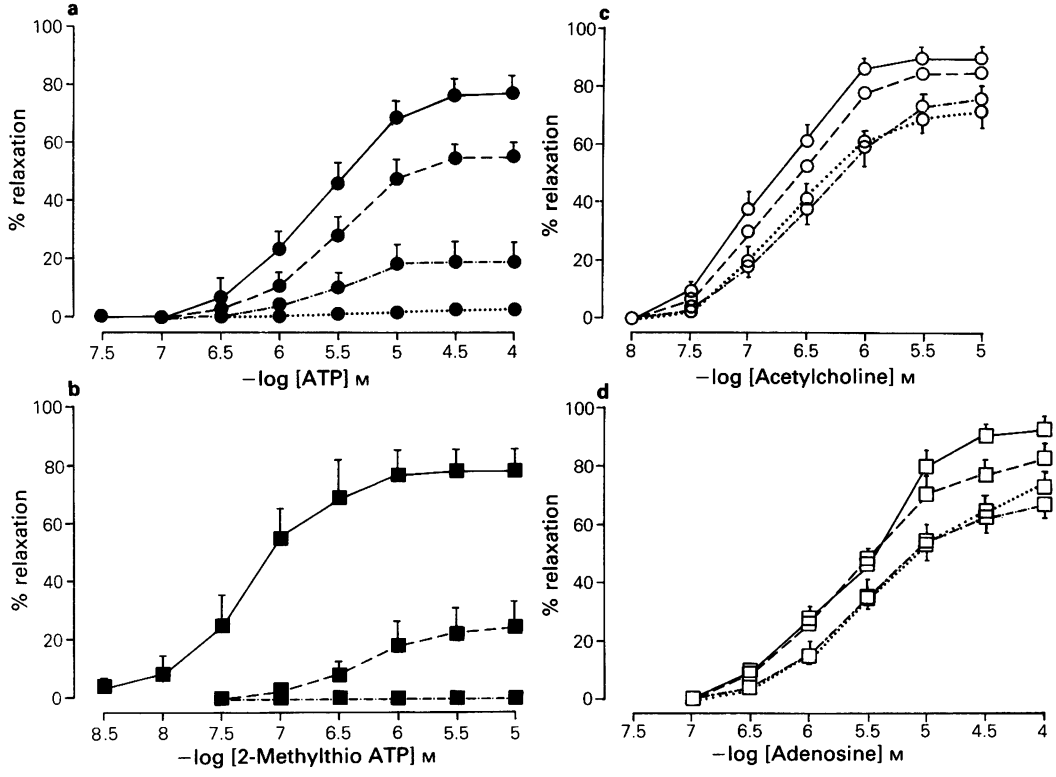

Figure 4 Isolated transverse ring preparation of rabbit mesenteric artery with tone raised and maintained by noradrenaline (3–10 μM). Concentration-response curves (cumulative, relaxation) to (a) ATP (●) ($n = 8$), (b) 2-methylthio ATP (■) ($n = 7$), (c) acetylcholine (○) ($n = 10$) and (d) adenosine (□) ($n = 8$) in the absence of reactive blue 2 and in the presence of 21.6 μM(---), 46.4 μM (–·–·–) and 0.10 mM (.....) reactive blue 2. Relaxations are expressed as % of the maximal relaxation. Vertical bars denote s.e.mean.

Table 1 Agonist potencies on raised-tone preparations of rabbit isolated mesenteric artery in the absence and in the presence of reactive blue 2 (RB2)

(i) Slope

Agonist	*Control* ± 95%CL	+ 21.6 μM RB2 ± 95%CL	+ 46.4 μM RB2 ± 95%CL	+ 0.1 mM RB2 ± 95%CL	n†	P‡
ATP	41.4 ± 9.2	37.4 ± 16.0	14.8 ± 9.0	1.3 ± 3.3	7	*
2 Met-ATP	40.6 ± 8.2	16.3 ± 14.0	0	0	7	*
Ado	41.4 ± 8.8	39.1 ± 5.9	36.3 ± 7.8	34.5 ± 8.8	8	NS
ACh	45.4 ± 4.6	42.0 ± 7.8	36.1 ± 6.0	36.1 ± 5.0	10	*

(ii) % maximal relaxation

Agonist	*Control* ± s.e.	+ 21.6 μM RB2 ± s.e.	+ 46.4 μM RB2 ± s.e.	+ 0.1 mM RB2 ± s.e.	n†	P‡
ATP	79.5 ± 5.8	54.1 ± 4.7	19.0 ± 5.1	2.3 ± 1.5	7	*
2 Met-ATP	78.4 ± 7.2	24.7 ± 8.8	0	0	7	*
Ado	91.0 ± 3.4	83.0 ± 5.0	63.4 ± 4.6	64.8 ± 5.9	8	*
ACh	89.7 ± 3.4	84.4 ± 5.2	76.6 ± 4.4	72.1 ± 5.2	10	*

All values are given as mean ±95% CL/s.e.

† n signifies the number of preparations (one from each animal).

‡ Significant difference P (* = $P < 0.05$, NS = no significant difference) between responses in the absence and in the presence of RB2 (analysis of variance).

Abbreviations: 2-methylthio ATP (2 Met-ATP); adenosine (Ado) and acetylcholine (ACh).

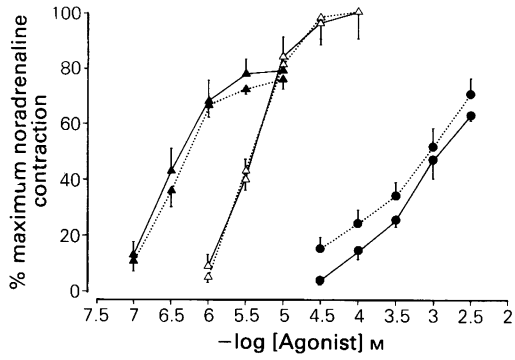

Figure 5 Isolated transverse ring preparation of rabbit mesenteric artery at basal tone. Concentration-response curves (contraction) to ATP (●) ($n = 6$), α,β-methylene ATP (▲) ($n = 6$) and noradrenaline (△) ($n = 5$) in the absence of reactive blue 2 (solid line) and presence of reactive blue 2 (0.10 mM) (dotted line). Responses are expressed as % of the maximal noradrenaline contraction. Maximal contraction to noradrenaline was 2.7 ± 0.2 g ($n = 19$). Vertical bars denote s.e.mean.

significantly reduced, but this reduction was only a small proportion of the maximal relaxation (Figures 3, 4c,d, Table 1). The slope of the acetylcholine concentration-response curve, but not the slope of the adenosine concentration-response curve, was significantly reduced (although not to a great extent) in the presence of reactive blue 2. Concentration-dependent contractions of the vessel to noradrenaline, ATP and α,β-methylene ATP were not significantly altered in the presence of 0.10 mM reactive blue 2: the slope of the concentration-response curve to these drugs, the potency, and (when attained) the maximal contraction were not significantly altered. However, although there is no significant difference in the potency of ATP in producing contractions in the absence and presence of reactive blue 2, a small leftward shift in the ATP concentration-response curve was observed, but there was no noticeable difference in the rate of development of the transitory contraction for each concentration of ATP (Figure 5). The action of reactive blue 2 was irreversible. It stained the tissue blue and after several hours caused a general reduction of tissue sensitivity to drugs.

Discussion

From this study it can be concluded that the isolated mesenteric artery of the rabbit relaxes to purines via the P_{2y}-purinoceptor and contracts to purines via the P_{2x}-purinoceptor. The agonist potency order for purine nucleotides at the P_{2x}-purinoceptor is: α,β-methylene ATP ≫ β,γ-methylene ATP = 2-methylthio ATP > ATP, whereas the order at the P_{2y}-purinoceptor is: 2-methylthio ATP ≫ ATP ≫ β,γ-methylene ATP > α,β-methylene ATP. Reactive blue 2 selectively inhibits the P_{2y}-purinoceptor-mediated response.

It has previously been reported that in rabbit isolated mesenteric artery, ATP and α,β-methylene ATP act via the same receptor to produce concentration-dependent transitory contractions, and that α,β-methylene ATP is 5000 times more potent than ATP in this action (Mathieson & Burnstock, 1985). In raised-tone preparations the vessel relaxes to ATP directly via the muscle, rather than via the endothelium as is the case in a number of other blood vessels (Vanhoutte & Rimele, 1983; Mathieson & Burnstock, 1985). This relaxation is not antagonized by the P_1-purinoceptor antagonist, 8-phenyltheophylline, which suggests that ATP is not broken down to adenosine but rather acts directly via the P_2-purinoceptor. After desensitization of the P_2-purinoceptor with α,β-methylene ATP, contractions to ATP are abolished whereas relaxations to ATP are not affected (Mathieson & Burnstock, 1985). These results suggest that purine nucleotides are not acting via a homogeneous P_2-purinoceptor to produce contractions and relaxations of the rabbit isolated mesenteric artery.

The heterogeneity of the P_2-purinoceptor of the rabbit isolated mesenteric artery was further supported in the present study of the order of purine nucleotide potencies in producing contractions and relaxations of the vessel. The rank potency order of purine nucleotides in producing contractions of the rabbit isolated mesenteric artery was: α,β-methylene ATP ≫ β,γ-methylene ATP = 2-methylthio ATP > ATP, whereas the rank potency order of these purine nucleotides in producing relaxation of the vessel was: 2-methylthio ATP ≫ ATP ≫ β,γ-methylene ATP > α,β-methylene ATP. Similar studies of purine nucleotide potencies have been carried out in a number of other tissues and a comparative agonist potency order has been demonstrated (see Burnstock & Kennedy, 1985). In the guinea-pig vas deferens (Fedan *et al.*, 1982; Burnstock *et al.*, 1983; 1985), the rat and guinea-pig urinary bladder (Brown *et al.*, 1979; Burnstock *et al.*, 1983), the perfused pancreas vascular bed (Chapal & Loubatieres-Mariani, 1983), the rat aorta and femoral artery (Kennedy *et al.*, 1985; White *et al.*, 1985) and the rabbit ear artery (Kennedy & Burnstock, 1985a), P_2-purinoceptors mediate contraction. A rank potency order of α,β-methylene, β,γ-methylene ATP > ATP = 2-methylthio ATP is indicated in these tissues. In the guinea-pig taenia coli (Satchell & Maguire, 1975; Maguire & Satchell, 1979; Burnstock *et al.*, 1983), the rabbit portal vein (Kennedy & Burnstock, 1985b), the pig and rat aorta (Martin *et al.*, 1985a; White *et al.*, 1985) and the rat

femoral artery (Kennedy *et al.*, 1985), P_2-purinoceptors mediate relaxations. A rank order of potency in these tissues is 2-methylthio ATP ➤ ATP > α,β-methylene ATP, β,γ-methylene ATP. Largely from these potency studies of the structural analogues of ATP, the P_2-purinoceptor was further divided into P_{2x} and P_{2y} subclasses. At the P_{2y}-purinoceptor, 2-methylthio ATP is more potent than ATP, and α,β-methylene ATP is less potent than ATP in producing a response, whereas at the P_{2x}-purinoceptor, phosphate-modified analogues of ATP are more potent than ATP in producing a response (Burnstock & Kennedy, 1985).

The potency order of purine nucleotides in producing contractions and relaxations of the rabbit isolated mesenteric artery is not precisely the same as in other tissues. Nevertheless, in accordance with the subdivision of the P_2-purinoceptor suggested by Burnstock & Kennedy (1985), it is clear that in this artery, purine nucleotides contract the vessel via the P_{2x}-purinoceptor and relax it via the P_{2y}-purinoceptor. Comparative studies of agonist potency should ideally be carried out in the absence of metabolism and uptake of the agonist. At present the influence of metabolism and uptake on the action of P_2-purinoceptor agonists is unclear since selective potent inhibitors of metabolic enzymes involved in the degradation of ATP and its analogues have yet to be developed. Both α,β-methylene ATP and β,γ-methylene ATP have been shown to be broken down more slowly than ATP (Pearson *et al.*, 1981; Moody & Burnstock, 1982; Cusack *et al.*, 1983; Pearson & Cusack, 1985). Thus the use of rank potency order of purine nucleotide agonists to characterize receptors suffers the limitation that differences in tissue sensitivity to the agonist may be due to differences in their metabolism rather than differences in potency at the receptor.

Although agonist potencies, acting via a given receptor, do vary, there should not be any difference in the maximal response and the slope of the concentration-response relationships between different agonists acting on the same receptor so long as they have full agonist action and are stable. In the current study of the contractile responses in the rabbit isolated mesenteric artery, the maximal contraction to purine nucleotides could not be compared since they were not attained at the concentrations tested. The slopes of the concentration-response curves to the purine analogues were significantly different. The slopes of the concentration-response curves to α,β-methylene ATP and to β,γ-methylene ATP were greater than that of ATP, while the slope of the concentration-response curve to 2-methylthio ATP was not greatly different from that of ATP. A differential rate of metabolism of purine nucleotides may explain these differing slopes. Another explanation may be that ATP and 2-methylthio ATP also act potently on the P_{2y}-purinoceptor to produce relaxation of the vessel, and as a result this relaxant response may somewhat override the contractile response. In raised-tone preparations of the rabbit isolated mesenteric artery, ATP and 2-methylthio ATP each produced a maximal relaxation of approximately 80% of the total possible relaxation and the slopes of their concentration-response curves were not significantly different. This supports the concept that both nucleotides are acting via the same purinoceptor to produce relaxation. The concentration-response curve to β,γ-methylene ATP, on the other hand, although its slope is not significantly different from that of ATP, is tending towards a somewhat smaller maximal relaxation than ATP. Again this may be due to its opposing contractile action on the P_{2x}-purinoceptor.

In order to provide more conclusive evidence for the division of the P_2-purinoceptor into P_{2x} and P_{2y} subtypes, an antagonist for each subdivision is required. $ANAPP_3$ is generally described as a P_2-purinoceptor antagonist, and α,β-methylene ATP is described as being able to desensitize this purinoceptor (Hogaboom *et al.*, 1980; Kasakov & Burnstock, 1983). In fact, both $ANAPP_3$ and α,β-methylene ATP only act at the P_{2x}-purinoceptor and have little or no effect at the P_{2y}-purinoceptor (see Burnstock & Kennedy, 1985; Mathieson & Burnstock, 1985). In rabbit isolated mesenteric artery, after desensitization of the P_2-purinoceptor with α,β-methylene ATP, the ATP contractile response (via the P_{2x}-purinoceptor) is nearly abolished, whereas the ATP relaxant response (via the P_{2y}-purinoceptor) is not significantly affected (Mathieson & Burnstock, 1985). The present study demonstrates that in the rabbit isolated mesenteric artery, reactive blue 2, over a small concentration-range, selectively inhibits responses mediated via the P_{2y}-purinoceptor. It inhibits these purine nucleotide relaxant responses in a non-competitive manner: it reduces the slope of the concentration-response curve and also the maximal relaxation. Its action is not surmountable. On the other hand, it does not inhibit contractions to noradrenaline (via the α-adrenoceptor) nor does it inhibit contractions to α,β-methylene ATP or ATP (via the P_{2x}-purinoceptor). However in the presence of reactive blue 2, there is a small, but not significant, enhancement of the ATP contractile response. Such an enhancement may be due to reactive blue 2's inhibition of the P_{2y}-purinoceptor-mediated response, and hence the contractile response to ATP is no longer opposed by its own relaxant response via the P_{2y}-purinoceptor. α,β-Methylene ATP does not act via the P_{2y}-purinoceptor, therefore its contractile response was not enhanced in the presence of reactive blue 2. Reactive blue 2 does have some non-specific action; it inhibits the relaxant response to acetylcholine and adenosine to a small extent. Reactive blue 2 is irreversible in its action, and after several hours has a general depressant action on drug responses.

Reactive blue 2, within a concentration-range of 0.1–10 μM, appears to act as a competitive antagonist in the taenia coli to inhibit the P_{2y}-purinoceptor-mediated responses, producing parallel shifts of the concentration-response curves and a slope of the Schild plot of 0.93, not significantly different from unity (Burnstock *et al.*, 1986). Therefore it seems likely that reactive blue 2 also acts as a P_{2y}-purinoceptor antagonist in the rabbit isolated mesenteric artery, although in this case its action is non-competitive. This may be because reactive blue 2 also acts at a post-receptor intracellular site in the tissue, perhaps on enzymes concerned in purine metabolism (Weber *et al.*, 1979). A post-receptor mode of action of reactive blue 2 would be analogous to that of methylene blue or haemoglobin (Gruetter *et al.*, 1981; Martin *et al.*, 1985b).

The reactive blue 2 used in this experiment was only 60% pure. Purification of the drug or a small alteration in its molecular structure may enhance its specificity towards the P_{2y}-purinoceptor-mediated response. Its selective action on the P_{2y}-purinoceptor-mediated responses needs to be verified on a wide range of preparations. A preliminary study of the action of reactive blue 2 on the guinea-pig taenia coli and the perfused rat heart have been reported (Burnstock *et al.*, 1986).

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