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Actions mediated by P_2-purinoceptorsubtypes in the isolated perfused mesenteric bed of the rat

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1 The effects of adenosine 5'-triphosphate (ATP) and its analogues on the perfusion pressure of the isolated mesenteric bed of the rat were examined in preparations at resting tone, and with tone raised by noradrenaline.

2 In the preparations at resting tone, the effect of the analogues was to produce vasoconstriction, their rank order of potency being α,β-methylene ATP > 2-methylthio ATP > ATP.

3 In raised tone preparations, dose-dependent vasodilatations were produced by ATP and 2-methylthio ATP although, at the highest doses tested, responses decreased in magnitude. The rank order of potency of the analogues in eliciting this vasodilator response was 2-methylthio ATP > ATP, while α,β-methylene ATP was without effect.

4 Following desensitization of contractile responses to α,β-methylene ATP, contractile responses to ATP and 2-methylthio ATP were abolished while their relaxant responses were potentiated.

5 Removal of the endothelium with sodium deoxycholate totally abolished the vasodilator responses and enhanced the contractile responses.

6 It is concluded that, in the rat mesentery, ATP and its analogues cause vasoconstriction via P_{2x}-purinoceptors and vasodilatation via P_{2y}-purinoceptors and that these are located on the smooth muscle and on the endothelium, respectively.

Introduction

Purinoceptors have been subclassified as P_1- and P_2-purinoceptors according to several criteria including whether they are activated preferentially by adenosine or by adenosine 5'-triphosphate (ATP), respectively (Burnstock, 1978). Burnstock & Kennedy (1985) proposed a subdivision of the P_2-purinoceptor into types P_{2x} and P_{2y} which can be principally distinguished by the rank order of potency of ATP and its structural analogues; a sensitivity of response to agonists in the order α,β-methylene ATP (α,β-meATP), β,γ-methylene ATP (β,γ-meATP) > ATP = 2-methylthio ATP (2me · S · ATP) is characteristic of the P_{2x} receptor, whilst at the P_{2y} subtype, agonist potency is in the order 2me · S · ATP > ATP > α,β-meATP, β,γ-meATP. The identification of P_{2x} and P_{2y} receptors may be substantiated by the use of specific antagonists to these receptors; at the P_{2x} receptor arylazidoaminoproprionyl ATP is a selective antagonist (Hogaboom *et al.*, 1980) and α,β-meATP is a selective desensitizing agent (Kasakov & Burnstock, 1983). The anthraquinone sulphonic acid derivative, Reactive blue 2, is an antagonist at the P_{2y}-purinoceptor (Kerr & Krantis, 1979; Manzini *et al.*, 1986; Burnstock *et al.*, 1986; Burnstock & Warland, 1987; Houston *et al.*, 1987; Reilly *et al.*, 1987).

The responses of tissues to differential activation of the P_2-purinoceptor subtypes have been studied in isolated vessels (White *et al.*, 1985; Burnstock & Warland, 1987; Houston *et al.*, 1987; Reilly *et al.*, 1987) and in isolated vascular beds including the pancrease (Bertrand *et al.*, 1987) and heart (Hopwood & Burnstock, 1987). In general it has been shown that P_{2x}-purinoceptors mediate vasoconstriction and P_{2y}-purinoceptors mediate vasodilatation. The importance of the endothelium in mediating vasodilatation was first shown for acetylcholine (ACh) in the rabbit isolated thoracic aorta (Furchgott & Zawadski, 1980) and subsequently in the rabbit isolated mesenteric vascular bed (Carvalho & Furchgott, 1981) and in the rat isolated mesenteric arterial bed (Furchgott *et al.*, 1987; Hiley

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et al., 1987). Since the first demonstrations for ACh, other vasodilators were also found to exert their relaxant action via the endothelium, and these included ATP (Furchgott, 1981; DeMey & Vanhoutte, 1981). More recent studies with ATP have shown that, in general, removal of the endothelium abolishes the P_{2y}-purinoceptor-mediated relaxation (Kennedy *et al.*, 1985; Houston *et al.*, 1987) indicating that P_{2y}-purinoceptors are located on the endothelium, although P_{2y}-purinoceptors have also been identified in the smooth muscle of some vessels (Kennedy & Burnstock, 1985; Mathieson & Burnstock, 1985). P_{2x}-purinoceptor-mediated vasoconstriction, in contrast, always occurs independently of the endothelium, the receptors being located on the smooth muscle membrane. Consistent with this conclusion, P_{2y}- but not P_{2x}-purinoceptors have been shown to be present on cultured aortic endothelial cells of the pig (Needham *et al.*, 1987).

The purpose of this study was to identify P_2-purinoceptor subtypes P_{2x} and P_{2y} in the isolated perfused mesenteric bed of the rat by determination of the rank order of potency of ATP and its analogues α,β-meATP and 2me·S·ATP in causing changes of vascular tone. The P_2-purinoceptors were further characterized using the selective P_{2x}-desensitizing agent α,β-meATP. Responses of the mesenteric bed to ATP and its agonists were investigated before and after removal of the endothelium with sodium deoxycholate to determine the location in the vasculature of the P_{2x}- and P_{2y}-receptors.

Methods

General procedures

Male Wistar rats (300–400 g) were used in the study. The animals were anaesthetized (sodium pentobarbitone, 150 mg kg^{-1}, i.p.) and injected with heparin (1000 units i.p.). The superior mesenteric artery was isolated and cannulated and the gut dissected away (McGregor, 1965). The isolated mesentery was perfused at a constant rate of 3.2 ml min^{-1} with Krebs solution containing (mM): NaCl 133, KCl 4.7, NaH_2PO_4 1.35, $NaHCO_3$ 16.3, $MgSO_4$ 0.61, glucose 7.8 and $CaCl_2$ 2.52 (Bülbring, 1953). Bovine serum albumin (Sigma) was added (5 g l^{-1}) to raise the colloid osmotic pressure and prevent tissue oedema (Byfield *et al.*, 1986).

The perfusate was gassed with 95% O_2 and 5% CO_2 and maintained at 37°C. The same solution was also superfused over the outside of the preparation at 1 ml min^{-1} (Longhurst *et al.*, 1986). Vasoconstrictor and vasodilator responses were measured as changes in perfusion pressure using a Gould P23 pressure transducer on a side arm of the main perfusion cannula, and the changes were recorded on a Grass model 79D polygraph.

After a 30 min equilibration period, drugs were administered as 50 μl bolus injections through neoprene rubber tubing proximal to the tissue. Vasoconstrictor responses to bolus injections of α,β-meATP were always established following responses to the other purines or on separate animals to avoid receptor desensitization by this potent analogue. For vasodilator responses the tone of the preparation was raised by constant perfusion with 30 μM noradrenaline (NA) added to the perfusate. For prolonged exposure α,β-meATP (1 μM or 10 μM) was also administered via the perfusate reservoir. In control experiments conducted at basal tone, two different doses of NA (50 μl boluses of 30 and 100 μM) were applied before and after continuous perfusion with α,β-meATP to confirm that the action of α,β-meATP was selective for blockade of purinoceptors.

Removal of endothelium

To remove the endothelium the preparation was perfused with 2 ml of sodium deoxycholate solution (Sigma) in saline over 30 s via an alternative perfusion line into the main cannula (Welling & Grantham, 1972; Byfield *et al.*, 1986). For preconstricted mesenteric bed preparations the perfusate was changed to one that was free of NA and the preparation washed through until the tone had returned to its original value. Addition of sodium deoxycholate was accompanied by a transient increase in perfusion pressure. The preparation was washed through for 15 min until the pressure had returned to that of the preparation at basal tone. Vasoconstrictor responses were then re-examined in the preparation at basal tone, while for vasodilator responses the mesenteric bed was reconstricted with NA (30 μM) added to the perfusate. When the endothelium was present, a dose-dependent vasodilatation to ACh (50 μl boluses of 0.1, 1 and 10 μM) was found to occur. Complete removal of the endothelium was established in each preparation by the lack of a vasodilator response to ACh. Control experiments using sodium nitroprusside (50 μl boluses of 10 and 100 μM) were performed to confirm the viability of the preparation following removal of the endothelium.

Drugs

Adenosine 5'-triphosphate (ATP, sodium salt), α,β-methylene ATP (lithium salt), acetylcholine chloride and noradrenaline bitartrate were obtained from Sigma. 2-methylthio ATP (sodium salt) was obtained from Research Biochemicals Inc. (U.S.A.). Nor-

adrenaline was made up daily for use as a 10 mM stock solution in 0.1 mM ascorbic acid to prevent oxidation.

Statistical analysis

Vasoconstrictor and vasodilator responses were recorded as changes in perfusion pressure (mmHg). For the dose-response curves, data were plotted as the mean (±s.e.mean) of the responses at each dose. Statistical significance was evaluated by Student's t test ($P < 0.05$ was taken to be significant).

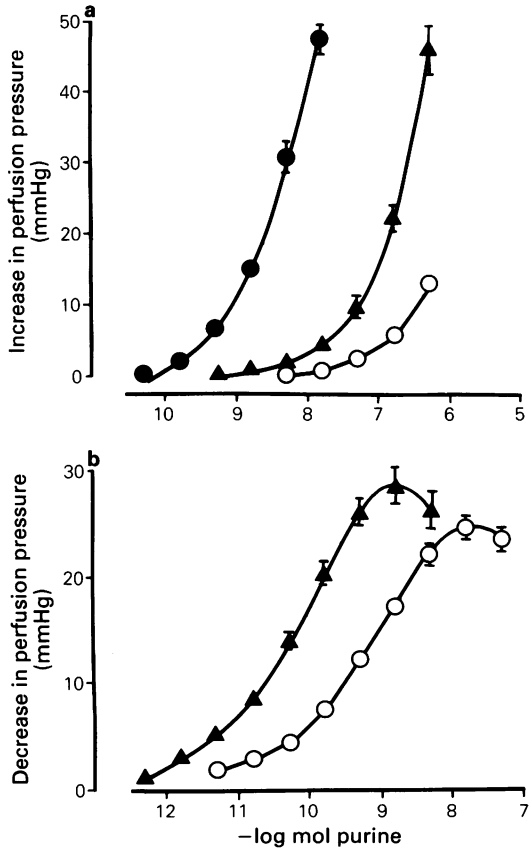

Figure 1 (a) Dose-response curves for the contractile responses to α,β-methylene ATP (α,β-meATP; ●) ($n = 19$), 2-methylthio ATP (2me·S·ATP; ▲) ($n = 14$) and ATP (○) ($n = 19$) of the isolated perfused mesenteric bed of the rat at basal tone. The bolus volume of individual doses was 50 μl. Data shown are means with vertical lines indicating s.e.mean. Where no error bar is shown it falls within the area covered by the symbol. (b) Dose-response curves for the relaxant responses to 2me·S·ATP (▲) ($n = 18$) and ATP (○) ($n = 18$) of the rat isolated mesenteric bed with tone raised by continuous perfusion with 30 μM noradrenaline. α,β-MeATP did not elicit vasodilatation.

Results

The effect of ATP and its analogues on the mesenteric bed at basal perfusion pressure

The basal perfusion pressure of the isolated mesenteric bed, perfused at a constant flow rate of 3.2 ml min^{-1}, was 20.73 ± 1.46 mmHg ($n = 26$). Bolus injections (50 μl) of ATP and its analogues 2me·S·ATP and α,β-meATP produced dose-dependent, reproducible vasoconstrictor responses. The order of potency of the purines in producing contractions was: α,β-meATP > 2me·S·ATP > ATP (Figure 1a, see also Figure 2a). At applied concentrations greater than 30 μM the desensitizing effect of

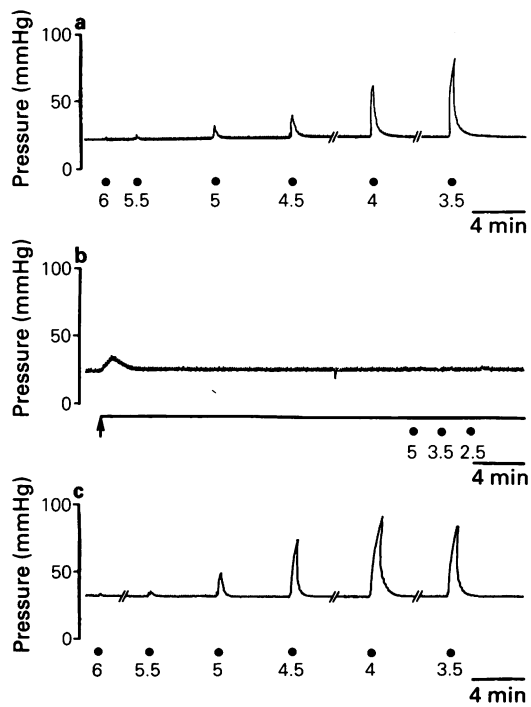

Figure 2 Isolated mesenteric bed preparation of the rat at basal tone. Dose-dependent vasoconstrictor responses to α,β-methylene ATP (α,β-meATP; indicated by ●, with concentrations of doses expressed as −log M) (a), were totally abolished by the addition of α,β-meATP (10 μM) to the perfusate (b) (indicated by horizontal line; point of application indicated by arrow). Removal of the endothelium with sodium deoxycholate (c) produced a potentiation of the vasoconstrictor response to equivalent concentrations of α,β-meATP.

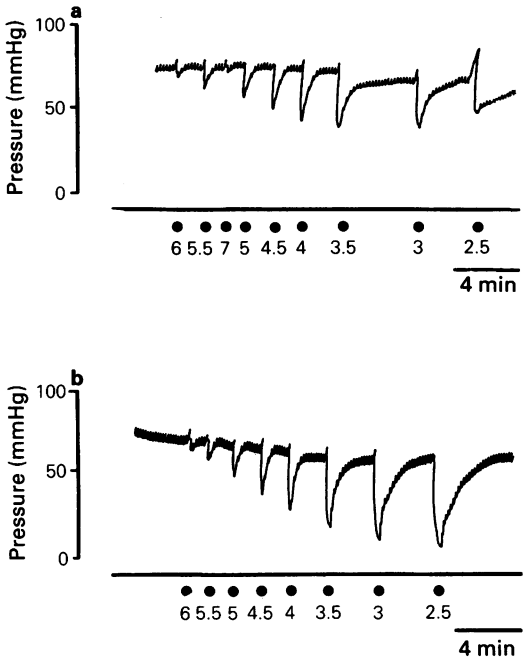

Figure 3 Isolated mesenteric bed preparation of the rat with tone raised by 30 μM noradrenaline added to the perfusate (indicated by horizontal line). The effect of continuous perfusion of 10 μM α,β-methylene ATP (α,β-meATP) on vasodilator responses to 50 μl doses of ATP (indicated by ●, with concentrations of doses expressed as $-\log$ M). Note that in the control responses (a) doses of ATP greater than an applied concentration of 30 μM produce a decreased magnitude of vasodilatation and that the responses were biphasic with the relaxation being preceded by a contraction. In the presence of α,β-meATP (b) the relaxations tended towards a maximum and no vasoconstrictor response was observed.

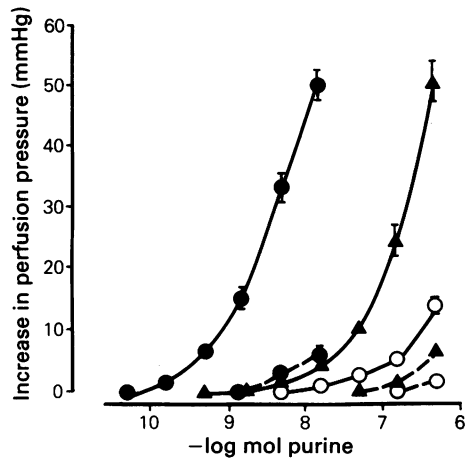

Figure 4 The desensitizing effect of α,β-methylene ATP (α,β-meATP; continuous perfusion at 1 μM) on the vasoconstrictor responses to α,β-meATP (●) ($n = 5$), 2-methylthio ATP (2me·S·ATP; ▲) ($n = 5$) and ATP (○) ($n = 5$) (broken lines) in the rat perfused mesenteric arterial vasculature. Solid lines denote control responses to α,β-meATP ($n = 12$), 2me·S·ATP ($n = 9$) and ATP ($n = 13$). Results shown as mean with vertical lines indicating s.e.mean. Error bars falling within the area covered by a symbol are not shown.

α,β-meATP became apparent, as constrictor responses of the mesenteric bed to subsequent higher doses of this purine were of a decreased magnitude. Receptor sensitivity did not recover even after 20 min of drug-free perfusion. The responses to α,β-meATP were not blocked by the α_1-adrenoceptor antagonist, prazosin, whereas those to NA were abolished (results not shown).

The effect of ATP and its analogues on the preconstricted mesenteric bed of the rat

Only those preparations in which continuous perfusion with 30 μM NA raised the tone by at least 30 mmHg were used (average increase in tone was 47.59 ± 1.36 mmHg, $n = 27$). ATP and 2me·S·ATP elicited dose-dependent relaxations of the preconstricted rat mesenteric bed (Figure 1b, see also Figure 3a). α,β-MeATP at applied concentrations of less than 1 μM did not produce relaxation and at higher doses caused further contraction. The order of potency for the relaxant response was 2me·S·ATP > ATP, while α,β-meATP was without effect. At the EC_{50} level, 2me·S·ATP was about 10 times more potent than ATP (EC_{50} 2me·S·ATP 1.22 ± 0.18 μM, $n = 18$; EC_{50} ATP 11.8 ± 0.18 μM, $n = 18$). At higher doses of ATP (applied concentrations > 0.3 mM) and 2me·S·ATP (applied concentrations > 30 μM), the magnitude of the relaxations decreased. In some preparations a biphasic response was observed at these concentrations with the relaxation being preceded by an initial contraction (Figure 3a).

The effect of desensitization of the P_{2x}-purinoceptor with α,β-methylene ATP

Following desensitization of P_{2x}-purinoceptors with α,β-meATP (1 μM), vasoconstrictor responses of the perfused mesenteric bed to ATP, 2me.S.ATP and α,β-meATP were significantly reduced ($n = 5$), as was reflected by a large shift to the right of the dose-response curves (Figure 4). At the higher concentration of 10 μM α,β-meATP in the perfusate, there was a total block of the responses to purines applied at concentrations of up to 1 mM (Figure 2b). Vasocon-

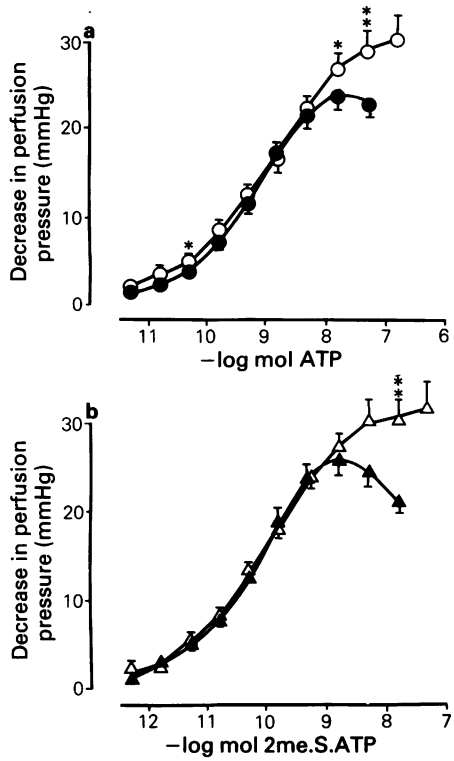

Figure 5 The effect of α,β-methylene ATP (α,β-meATP; continuous perfusion at 10 μM) on the vasodilator responses to (a) ATP (○) ($n = 5$–8) and (b) 2-methylthio ATP (2me·S·ATP; △) ($n = 5$–8) in the rat perfused mesenteric bed. Filled symbols denote control responses in the absence of α,β-meATP ($n = 5$–8). Data shown are means with vertical lines indicating s.e.mean. Error bars falling within the area covered by a symbol are not shown. A statistically significant difference is shown by * $P < 0.05$ and ** $P < 0.01$.

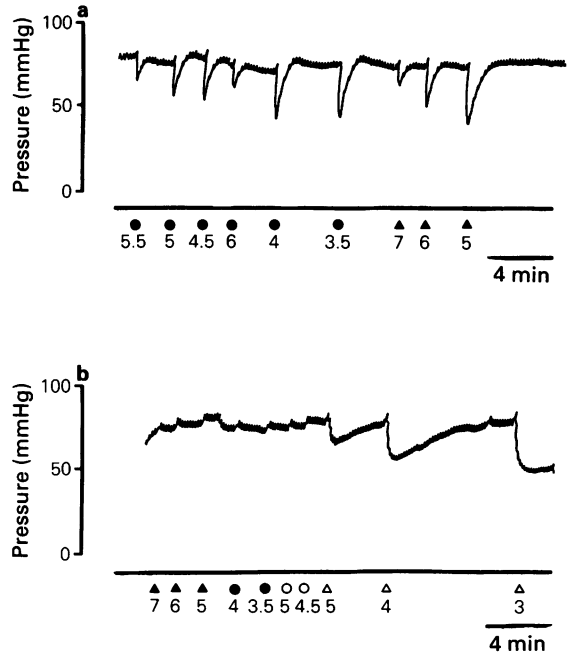

Figure 6 Rat isolated mesenteric bed preparation with tone raised by 30 μM noradrenaline added to the perfusate (indicated by horizontal line). The effect of ATP (50 μl doses, indicated by ●) on the preconstricted mesenteric bed before (a) and after (b) removal of the endothelium with sodium deoxycholate. Concentrations of all doses are expressed as −log M. Note that vasodilator responses to acetylcholine (ACh; 50 μl boluses of 0.1, 1 and 10 μM) (indicated by ▲) were totally abolished confirming that the endothelium had been removed. The vasodilator responses to 2-methylthio ATP (indicated by ○) were also abolished by removal of the endothelium. The preparation dilated in a dose-dependent manner to sodium nitroprusside (50 μl boluses of 0.01, 0.1, and 1 mM, indicated by △) independently of the endothelium.

strictor responses to NA (50 μl boluses of 30 μM and 0.1 mM) gave increases in perfusion pressure of 14.0 ± 3.85 mmHg and 36.6 ± 8.43 mmHg, respectively ($n = 5$), that were not inhibited by α,β-meATP, but were potentiated; the increases in perfusion pressure were now 25.6 ± 6.9 mmHg and 67.0 ± 10.9 mmHg ($n = 5$) for the two concentrations of NA.

Addition of α,β-meATP to the perfusate of the preconstricted mesenteric bed produced a transient increase in tone of the preparation. This was allowed to return to its initial constricted level of tone before repeating the doses of ATP and 2me·S·ATP. There was no significant difference in the level of tone of the preparation before and after the addition of α,β-meATP (10 μM) to the perfusate (mean perfusion pressure was 46.31 ± 2.6 mmHg and 50.25 ± 3.26 mmHg, respectively, $n = 4$). In the presence of α,β-meATP (1 and 10 μM), biphasic responses to ATP and 2me·S·ATP were no longer observed, the initial contractile response having been abolished (Figure 3). There was no significant effect on the vasodilator responses to ATP (Figure 5a) and 2me·S·ATP (Figure 5b) at lower concentrations of these purines (<0.3 mM for ATP and <0.1 mM for 2me·S·ATP). At higher concentrations, however, the relaxations were significantly greater (Figure 5, $P < 0.05$) and reached a maximal response for each preparation.

Effect of endothelium removal on responses of the rat mesenteric bed in conditions of low and raised tone

Removal of the endothelium with sodium deoxycholate did not significantly affect the increase in tone of the preparation produced by the addition of 30 μM NA. However, vasodilator responses to ATP and 2me·S·ATP were completely abolished (Figures 6, 7a); at the higher concentrations of purines, contractions were elicited above the increased tone produced by NA. Vasodilatations to ACh (50 μl boluses of 0.1, 1 and 10 μM) were also abolished after removal of endothelium, but the preparation still relaxed in a dose-dependent manner in response to sodium nitroprusside (10 and 100 μM; Figure 6b).

In contrast, the vasoconstrictor responses of the rat mesenteric bed to α,β-meATP, 2me·S·ATP and ATP were not destroyed by removal of the endothelium, but were significantly increased (Figure 7b, $P < 0.05$, see also Figure 2c). That the endothelium had been removed was confirmed by raising the tone of the preparation by perfusion with 30 μM NA and establishing that vasodilator responses to ACh had been abolished.

Discussion

The results of this study demonstrate the presence of P_2-purinoceptor subtypes in the isolated perfused mesenteric bed of the rat. These occur at two separate locations within the vasculature: P_{2x}-receptors are found on the vascular smooth muscle surface and mediate vasoconstriction and P_{2y}-receptors are found on the endothelial surface where they mediate endothelium-dependent vasodilatation.

The rank order of ATP and its analogues in eliciting vasoconstrictor and vasodilator responses of the rat mesenteric bed was used to identify the P_{2x}- and P_{2y}-purinoceptor subtypes. In the perfused mesenteric bed at basal tone, dose-dependent contractile responses to ATP and its analogues were observed. The agonist potency order for eliciting the vasoconstrictor response was α,β-meATP > 2me·S·ATP > ATP which conforms to the pattern originally observed for the P_{2x}-purinoceptor (Burnstock & Kennedy, 1985). The P_{2y}-purinoceptor was similarly identified by the order of agonist potency in producing vasodilatation of the preconstricted mesenteric bed: 2me·S·ATP was more potent than ATP while α,β-meATP had no vasodilator effect. In this preparation we have found that adenosine is not a good relaxing agent even at doses up to 5×10^{-7} mol (greater than the maximum concentration of ATP used) (results not shown). There-

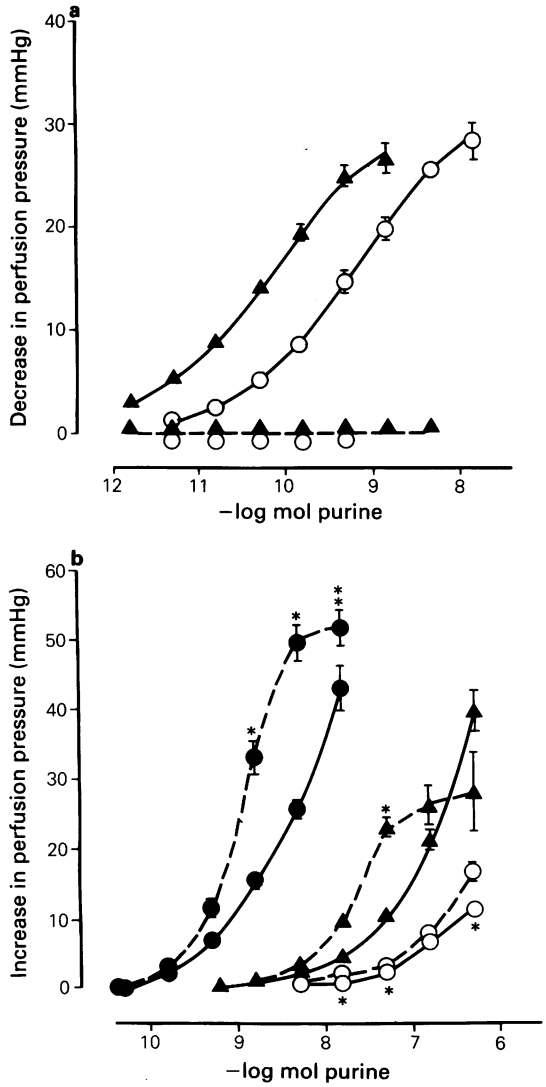

Figure 7 (a) The lack of effect of 2-methylthio ATP (2me·S·ATP; ▲) ($n = 6$) and ATP (○) ($n = 6$) on the preconstricted mesenteric bed of the rat after removal of the endothelium with sodium deoxycholate (broken lines). Solid lines show the control vasodilator responses to 2me·S·ATP ($n = 17$–20) and to ATP ($n = 11$–17) when the endothelium was preserved. Vertical lines show s.e.mean. Error bars falling within the area covered by a symbol are not shown. (b) Dose-response curves to vasoconstrictor responses of the perfused mesenteric bed of the rat to α,β-methylene ATP (α,β-meATP; ●) ($n = 7$), 2me·S·ATP (▲) ($n = 4$) and ATP (○) ($n = 6$) with intact endothelium (solid lines) and after the endothelium had been removed with sodium deoxycholate (broken lines). A statistically significant difference is denoted by * $P < 0.05$ and ** $P < 0.01$.

fore, it seems unlikely that adenosine formed from the breakdown of ATP contributes to the relaxations elicited by ATP. Rather, any conversion of ATP in this way would decrease the effective concentration of ATP, resulting in smaller responses. At higher doses of 2me·S·ATP and ATP ($>1.5 \times 10^{-9}$ mol for 2me·S·ATP and $>1.5 \times 10^{-8}$ mol for ATP) the magnitude of the vasodilator response decreased. In some preparations a biphasic response was observed in which an initial contraction preceded the vasodilatation. At these concentrations an opposing vasoconstriction caused by ATP and 2me·S·ATP acting on P_{2x}-receptors appears to come into effect, reducing the magnitude of the vasodilatation and in some cases causing contraction. Such a biphasic response to ATP and 2me·S·ATP has also been observed in the raised tone coronary vascular bed (Hopwood & Burnstock, 1987).

Further evidence for subclassification of P_2-purinoceptors in the rat mesenteric bed came from experiments in which desensitization to α,β-meATP was used to antagonize P_{2x}-purinoceptors. α,β-meATP is specific for the P_{2x}-purinoceptor (Kasakov & Burnstock, 1983) and has been used as a desensitizing agent in numerous tissues including the rat portal vein (Reilly & Burnstock, 1987), the rabbit mesenteric artery (Kügelgen & Starke, 1985), the dog mesenteric artery (Muramatsu, 1986) and the guinea-pig and mouse vas deferens (Stjärne & Åstrand, 1985).

Accordingly, contractile responses to ATP, 2me·S·ATP and α,β-meATP, but not those to NA, were significantly reduced by this desensitizing agent at 1 μM, providing further evidence for the presence of P_{2x}-purinoceptors. P_{2y}-mediated vasodilator responses at the lower doses of ATP and 2me·S·ATP were unaffected by α,β-meATP at 1 and 10 μM. At the higher concentrations the biphasic responses were no longer observed due to abolition of the contractile component and the relaxant responses were potentiated, reaching a maximum for each preparation. These effects are consistent with α,β-meATP acting as an antagonist at the P_{2x}-purinoceptor, thereby removing an opposing constrictor effect that was causing damping of the vasodilatations. The P_{2y}-purinoceptor antagonist, Reactive blue 2, could not be used in this study since, at concentrations needed to be effective, it caused a drop in tone of the raised tone mesenteric bed preparation.

In this study, removal of the endothelium with the detergent sodium deoxycholate abolished P_{2y}-mediated relaxant responses of the mesenteric bed to ATP and to 2me·S·ATP, but not responses to sodium nitroprusside which acts directly on the smooth muscle to cause relaxation by an endothelium-independent mechanism (Rapoport & Murad, 1983). Removal of the endothelium was verified by the fact that dose-dependent relaxation of the preconstricted mesenteric bed to ACh was completely abolished (Hiley *et al.*, 1987; Furchgott *et al.*, 1987). These results indicate that P_{2y}-purinoceptors are located on the endothelium and that relaxation via these receptors probably occurs by an endothelium-dependent mechanism. In contrast, P_{2x}-purinoceptor-mediated contractions were not endothelium-dependent, demonstrating that the P_{2x}-purinoceptors are confined to the smooth muscle cells. P_{2x} prejunctional receptors have not previously been demonstrated in any model system, making it unlikely that ATP evokes a constriction via interaction with the nerve terminal and release of NA. A direct postsynaptic action of ATP is also suggested since the responses to α,β-meATP were not blocked by the α_1-adrenoceptor antagonist, prazosin, whereas those to NA were abolished (results not shown).

Removal of the endothelium appeared to potentiate the P_{2x}-mediated vasoconstrictor response as was reflected in the leftward shift in the dose-response curves to α,β-meATP, 2me·S·ATP and ATP. In the case of 2me·S·ATP and ATP this potentiation is probably due to the removal of an opposing relaxant response via P_{2y}-purinoceptors located on the endothelium. ATP and 2me·S·ATP acting solely on P_{2x}-purinoceptors would be able to produce contractions of increased magnitude. The reason for the potentiation of α,β-meATP contractile responses is not so obvious as this agent is selective for P_{2x}-purinoceptors. The potentiation of α,β-meATP responses in the absence of endothelium has also been observed in the rat isolated femoral artery (Kennedy *et al.*, 1985). Similarly, endothelium removal has been found to attenuate significantly the adenosine-induced relaxation of the pig aorta (Gordon & Martin, 1983) and rat aorta and femoral artery (Konishi & Su, 1983), despite the fact that this purine is believed to cause relaxation in an endothelium-independent manner in these and most other vessels by acting directly on P_1-purinoceptors located on smooth muscle cells (Burnstock & Kennedy, 1986). It may be that the local concentration of agonist at the smooth muscle receptor is increased by reducing diffusion across a layer of endothelial cells (Gerlach *et al.*, 1985). Alternatively the endothelium may have a role in modulating smooth muscle receptor responses; Chiba & Tsukada (1984) showed that, in the dog mesenteric artery, vasoconstriction induced by calcium chloride was markedly potentiated in the absence of the endothelium and they proposed that the endothelium might regulate calcium entry from the extracellular space.

In conclusion, two subtypes of the

P_2-purinoceptor have been identified in the rat isolated mesenteric bed. P_{2y}-purinoceptors have been shown to mediate vasodilatation and are located on the endothelium, and P_{2x}-purinoceptors mediate vasoconstriction and are found on smooth muscle cells. The implication of having these subtypes is that ATP may exert different responses on a vessel depending on the concentration of the purine, on the tone of the vessel and on endothelial integrity and it is thus likely to have an important role in maintaining vascular homeostasis (Burnstock, 1987).

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