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Relative contribution of P_{2U}- and P_{2Y}-purinoceptors to endothelium-dependent vasodilatation in the golden hamster isolated mesenteric arterial bed

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1 P_2-purinoceptors were characterized pharmacologically in the constantly perfused isolated mesenteric arterial vascular bed of the golden hamster. Vasoconstrictor and vasodilator responses to the nucleotides ATP, ADP, 2 methylthio ATP (2MeSATP), α,β-methylene ATP (α,β-meATP) and uridine 5′-triphosphate (UTP) and a role for ATP in sympathetic constriction were examined.

2 At basal tone nucleotides elicited dose-dependent vasoconstriction with an observed rank order of potency of α,β-meATP > > 2MeSATP > ATP = ADP > UTP (based on the doses required to elicit constrictor responses of 25 mmHg). Adenosine had no vasoconstrictor action at doses up to 5 μmol. After application of a single dose (0.5 μmol) of α,β-meATP preparations were desensitized to constriction by subsequent application of nucleotides.

3 Electrical field stimulation (4–64 Hz, 90 V, 1 ms, 30 s) elicited frequency-dependent constrictions which were abolished by guanethidine (5 μM) and by prazosin (1 μM).

4 The non-selective P_2-purinoceptor antagonist suramin (100 μM) did not significantly affect vasoconstrictor responses to ATP. The P_{2X}-selective purinoceptor antagonist pyridoxalphosphate-6-azophenyl-2′,4′-disulphonic acid (PPADS, 3 μM), virtually abolished responses to ATP. When the endothelium was removed vasoconstrictor responses to ATP and noradrenaline were augmented.

5 In preparations with tone raised with methoxamine (10–80 μM) nucleotides elicited vasodilatation with an observed potency order of ATP = UTP > ADP > > adenosine. 2MeSATP had relatively minor vasodilator effects and at the highest dose tested (50 nmol) elicited only vasoconstriction. α,β-meATP did not elicit vasodilatation but produced further constriction of the raised tone preparation. At the highest doses of ATP and ADP (0.5 μM) responses were biphasic with vasoconstriction preceding vasodilatation. After removal of the endothelium, with the exception of adenosine, vasodilator responses to purines and to UTP were abolished; vasoconstriction to ATP, ADP, UTP and 2MeSATP was evident at the highest doses.

6 Suramin (100 μM) inhibited vasodilatation to both ATP and UTP and abolished responses to 2MeSATP. PPADS (3 μM) inhibited relaxation to 2MeSATP but did not affect relaxation to ATP, UTP, adenosine and acetylcholine and ADP.

7 Reactive blue 2 (30 μM) blocked vasodilator responses to ATP, UTP, 2MeSATP and acetylcholine; it was without effect when used at 3 μM.

8 The results of this study show that ATP elicits vasoconstriction of mesenteric arteries of the golden hamster via P_{2X}-purinoceptors located on the smooth muscle, and vasodilatation via P_{2U}-receptors which are located on the endothelium. 2MeSATP has marginal vasodilator activity, suggesting that P_{2Y}-purinoceptors contribute minimally to relaxation to ATP in hamster mesenteric arteries.

Keywords: Endothelium; golden hamster; purinoceptors; mesenteric arterial bed

Introduction

Extracellular purine nucleotides and nucleosides elicit potent and diverse actions via P_1- and P_2-purinoceptors (Burnstock, 1978). Currently there are at least six defined subtypes of the P_2-purinoceptor family: P_{2X}, P_{2Y}, P_{2T}, P_{2U}, P_{2Z} and P_{2D} (Burnstock & Kennedy, 1985; Gordon, 1986; Harden *et al.*, 1995). In a recent revision of P_2-purinoceptor classification it has been proposed that these receptors can be divided into two major families, P2X and P2Y, according to whether they are intrinsic ion channels or are coupled to G-proteins respectively (Abbracchio & Burnstock, 1994). A unifying system of nomenclature for subtypes of the two main P_2-purinoceptor families will be introduced when additional information on the subtypes becomes available.

Vascular P_{2X}-purinoceptors are found on the smooth muscle where they mediate vasoconstriction with a characteristic agonist potency order of α,β-methylene adenosine 5′-triphosphate (α,β-meATP) > > β,γ-methylene ATP > 2 methylthio ATP (2MeSATP) > ATP (Burnstock & Kennedy, 1985; Ralevic & Burnstock, 1991a). P_{2Y} receptors are typically located on the endothelium, although they may also be found on the smooth muscle, and elicit vasodilatation with an agonist potency order of 2MeSATP > > ATP = ADP > > α,β-meATP, uridine triphosphate (UTP). P_{2U} receptors are characterised by the equipotency of UTP and ATP, and low activity of other ATP analogues; these receptors are found on the smooth muscle and endothelium where they mediate vasoconstriction and vasodilatation respectively (O'Connor *et al.*, 1991). Although still useful in the characterization of P_2-purinoceptor subclasses it is now clear that rank orders of agonist potency may be substantially influenced by the different susceptibilities of purine analogues to degradation by ectonucleotidases (Crack *et al.*, 1994; Evans & Kennedy, 1994; Khakh *et al.*, 1995). Thus, selective antagonism is preferred.

α,β-MeATP has proved useful in the characterization of P_{2X}-purinoceptors since it causes P_{2X} receptor desensitization (Burnstock & Kennedy, 1985; Kennedy, 1990). However, in general there is a lack of potent and selective antagonists at this and other subtypes of the P_2-purinoceptor. The trypano-

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side suramin is a non-selective antagonist of P_2-purinoceptors (Dunn & Blakeley, 1988; Hoyle *et al.*, 1990; Leff *et al.*, 1990). Arylazidoaminopropionyl ATP (Hogaboom *et al.*, 1980; Fedan *et al.*, 1981) and pyridoxalphosphate-6-azophenyl-2',4'-disulphonic acid (PPADS) (Lambrecht *et al.*, 1992; Ziganshin *et al.*, 1994; Windscheif *et al.*, 1994) are relatively selective P_{2X}-purinoceptor antagonists in some tissues. The suramin analogue NF023 has recently been shown to be P_{2X}-receptor selective in the rabbit vas deferens (Ziyal *et al.*, 1994) and in rat and hamster mesenteric arteries (Ziyal *et al.*, 1995). Reactive blue 2 has been shown to antagonize selectively ATP effects at P_{2Y} receptors in some tissues, although concentration and time of exposure are critical (Hopwood & Burnstock, 1987; Burnstock & Warland, 1987; Houston *et al.*, 1987). Currently there are no selective antagonists at the P_{2U} receptor.

Species differences in the relative proportions and distributions of P_2-purinoceptor subclasses have been shown. For example, in rabbit mesenteric arteries P_{2Y} receptors are present on the smooth muscle (Mathieson & Burnstock, 1985), whereas in rat mesenteric arteries P_{2Y} receptors are located on the endothelium, where they appear to coexist with P_{2U} receptors (Ralevic & Burnstock, 1988; Windscheif *et al.*, 1994). The aim of the current study was to characterize pharmacologically P_2-purinoceptors in mesenteric arteries of the golden hamster. Responses of the isolated perfused mesenteric arterial bed were examined in the presence and absence of the endothelium to determine whether receptors are located on the smooth muscle or on endothelial cells. The contribution of ATP to vasoconstriction mediated by mesenteric sympathetic perivascular nerves was also investigated.

Methods

Isolated mesenteric arterial bed preparation

Male golden hamsters (130–150 g) were killed by asphyxiation with CO_2. Mesenteric beds were isolated and set up for perfusion essentially as for the rat mesenteric arterial bed as described previously (Ralevic *et al.*, 1995). The abdomen was opened and the superior mesenteric artery exposed and cannulated with a hypodermic needle. The superior mesenteric vein was severed, blood flushed through the preparation with approximately 0.5 ml of Krebs solution, the gut dissected away and the preparation mounted on a stainless steel grid (7 × 5 cm) in a humid chamber (custom made at University College London). The preparation was perfused at a constant flow rate of 3 ml min^{-1} using a peristaltic pump (model 7554-30, Cole-Parmer Instrument Co., Chicago, Illinois). The perfusate was Krebs solution of the following composition (mM): NaCl 133, KCl 4.7, NaH_2PO_4 1.35, $NaHCO_3$ 16.3, $MgSO_4$ 0.61, $CaCl_2$ 2.52 and glucose 7.8, gassed with 95% O_2-5% CO_2 and maintained at 37°C. Responses were measured as changes in perfusion pressure (mmHg) with a pressure transducer (model P23XL, Viggo-Spectramed, Oxnard, CA) on a side arm of the perfusion cannula, and recorded on a polygraph (model 7D, Grass Instrument Co., Quincy, Mass). Preparations were allowed to equilibrate for 30 min prior to experimentation. All doses of drugs were applied as 50 μl bolus injections into a neoprene rubber injection port proximal to the preparation. Antagonists were added to the perfusate reservoir.

The rat mesenteric arterial preparation was prepared as for the hamster preparation described above and perfused with Krebs solution at 5 ml min^{-1}. In this preparation vasodilator responses to ATP (0.005–5 nmol) and 2MeSATP (0.0005–5 nmol) were determined in the methoxamine (20 μM) raised-tone preparation.

Basal tone

Electrical field stimulation(EFS; 2–64 Hz, 90 V, 1 ms for 30 s) was applied to activate perivascular sympathetic nerves and produce frequency-response curves. In one set of animals EFS was followed by the addition of doses of noradrenaline (NA) and then of purines and UTP. Because of receptor desensitization a maximum of two purine compounds per preparation were tested as constrictors at basal tone, with the order being randomized between preparations, and a dose-interval of at least 5 min, which allowed a reasonably accurate assessment of the dose-response relationships. Because of pronounced receptor desensitization to α,β-meATP is was not possible to generate a complete dose-response curve in any individual preparation. Thus, a maximum of two doses of widely different potencies of α,β-meATP were tested per preparation, with a dose-interval of 15 min, which also allowed a more accurate assessment of the dose-response relationship. In another set of preparations the initial response curve to EFS was followed by equilibration of preparations for 30 min with guanethidine (5 μM) or prazosin (1 μM) and EFS repeated. Separate preparations were used to test the effects of PPADS (3 μM) or endothelium removal on responses to NA and ATP.

Effect of desensitization with α,β-meATP on vasoconstrictor responses

After the response to a high dose of α,β-meATP (0.5 μmol) had returned to baseline (approximately 2 min) single doses of the following were applied at 1.5 min intervals in the following order: α,β-meATP (0.5 μmol), 2MeSATP (0.5 μmol), ATP (5 μmol), ADP (5 μmol), UTP (5 μmol), α,β-meATP (0.5 μmol).

Raised tone

The tone of the preparations was raised by titration of methoxamine (10–80 μM) into the perfusate to achieve an increase in tone above baseline of approximately 50–100 mmHg. Vasodilator responses to the purine compounds and to UTP, acetylcholine (ACh) and sodium nitroprusside (SNP) were established. Response curves were generated by applying consecutive increasing doses of each agonist, but the order in which the response curves was generated was randomized between preparations. The effects of suramin (100 μM), reactive blue 2 (3 and 30 μM), and PPADS (3 μM) were tested in separate preparations equilibrated with antagonist for 30 min before construction of dose-response curves. In a separate set of animals the endothelial dependency of the responses was tested in a paired manner.

Endothelium removal

The endothelium was removed by controlled perfusion via the injection port of 1 ml of a solution of sodium deoxycholate (2 mg ml^{-1} in saline) through the vascular bed. The success of this treatment was evaluated by the inability of ACh, up to a maximal dose of 50 nmol, to elicit vasodilatation. Preparations were still able to relax in a dose-dependent manner to the endothelium-independent vasodilators adenosine and SNP.

Drugs used

Acetylcholine chloride, adenosine 5'-triphosphate (sodium salt), adenosine 5'-diphosphate (sodium salt), adenosine hemisulphate, α,β-meATP (lithium salt), methoxamine hydrochloride, noradrenaline bitartrate, prazosin hydrochloride, sodium deoxycholate, sodium nitroprusside, uridine 5'-triphosphate (sodium salt) and reactive blue 2 were from Sigma Chemical Co. 2-MeSATP was from Research Biochemicals Inc. Guanethidine monosulphate (Ismelin) was from Ciba-Geigy (Horsham, West Sussex). Suramin was from Bayer, Germany. Pyridoxalphosphate-6-azophenyl-2',4'-disulphonic acid (PPADS) was a generous gift from Dr G. Lambrecht, University of Frankfurt, Germany.

Data analysis

Vasoconstrictor responses were evaluated as the increase in perfusion pressure in mmHg above baseline. Vasodilator responses were measured as the decrease in perfusion pressure (mmHg) and evaluated as a percentage of the methoxamine-induced tone above baseline. Results are presented as means ± s.e.mean, with the number of observations in parentheses (n). Comparison of response curves not reaching maximal values was with analysis of variance with repeated measures; differences were considered significant when $P<0.05$. pD_2 values were calculated as the negative log of the dose (in mol) that produced a half-maximal response. pD_{25} values and pD_{15} values are defined as the negative log of the dose required to produce a vasoconstrictor response of 25 mmHg and relaxant response of 15% respectively. These values were used when responses did not reach a maximum and were chosen on the basis that they were approximately half the maximally achieved response. pA_2 values were estimated from the pK_B values, calculated according to: $K_B = [B]/(DR-1)$, where B = concentration of agonist and DR (dose-ratio) = the difference between pD_2 values in the absence and presence of antagonist. Differences between means were determined by Student's t test and were considered significant when $P<0.05$.

Results

Vasoconstrictor responses at basal tone

Basal perfusion pressure of the preparations was 30.36 ± 2.22 mmHg ($n=14$). Purine compounds elicited dose-dependent vasoconstriction with the following observed order of potency: α,β-meATP > > 2MeSATP > ATP = ADP > UTP (Figure 1a), based on the doses required to elicit responses of 25 mmHg. Adenosine did not elicit vasoconstriction at doses of up to 5 μmol. With the exception of α,β-meATP responses to the purines and UTP did not reach a maximum, hence pD_{25} values were calculated. pD_{25} values were: ATP, 6.13 ± 0.10 ($n=10$); ADP, 6.00 ± 0.16 ($n=8$); UTP, 5.76 ± 0.17 ($n=4$). There was no significant difference between these pD_{25} values. Mean pD_{25} values for α,β-meATP and 2MeSATP were 8.93 ($n=4-7$ for each dose in a total of 19 preparations) and 7.09 ($n=4-6$ for each dose in a total of 10 preparations).

Electrical field stimulation of sympathetic nerves

EFS (4–64 Hz, 90V, 1 ms, 30 s) of sympathetic nerves elicited frequency-response curves which were abolished by the sympathetic neuronal blocking agent guanethidine (5 μM). Responses were also blocked by the α_1-adrenoceptor antagonist prazosin (1 μM).

Effect of desensitization with α,β-meATP on vasoconstrictor responses

The effect of desensitization of P_{2X}-purinoceptors on responses was examined in six preparations. After a high dose of α, β-meATP (0.5 μmol) there was a block of vasoconstrictor responses of the preparations to doses of the following nucleotides applied 2 min later at 1.5 min intervals in the following order: α,β-meATP (0.5 μmol); 2MeSATP (0.5 μmol); ATP (5 μmol) ADP (5 μmol) and UTP (5 μmol; Figure 1b). Responses to α,β-meATP, 2MeSATP and ADP were virtually abolished by α,β-meATP desensitization, whereas there was a residual response to ATP and UTP.

Effect of suramin and PPADS on vasoconstrictor responses

Constrictor responses to ATP (0.005–5 μmol) were unaffected by suramin (0.1 mM) (data not shown). Constrictor responses to ATP were blocked by PPADS (3 μM) (data not shown).

NA (0.005–1500 nmol) elicited dose-dependent vasoconstriction with a pD_2 value of 7.60 ± 0.05 ($n=15$). Responses to NA were unaffected by PPADS (3 μM); pD_2 7.64 ± 0.71 ($n=4$). In the presence of suramin (0.1 mM) the pD_2 value of the NA dose-response curve was significantly increased to 8.12 ± 0.07 ($n=4$).

Effect of endothelium removal on vasoconstrictor responses

Response curves to ATP were augmented by endothelium removal ($n=4$). Responses to NA were also augmented after removal of the endothelium; the pD_2 value was significantly increased from 7.60 ± 0.05 ($n=15$) to 8.57 ± 0.17 ($n=4$).

Vasodilator responses at raised tone

Methoxamine (31.6 ± 0.53 μM, $n=14$; range 10–80 μM) raised the tone of the preparations by 63.71 ± 5.13 mmHg ($n=14$). The purine compounds and UTP elicited dose-dependent vasodilatation with the empirical order of potency of: ATP = UTP > ADP > 2MeSATP > adenosine (Figure 2). pD_2 values were: ATP, 8.50 ± 0.09 ($n=17$); UTP, 8.81 ± 0.11 ($n=16$). The pD_{15} value for ADP was 8.53 ± 0.29 ($n=5$). Figure 3 is a representative trace showing the low efficacy of

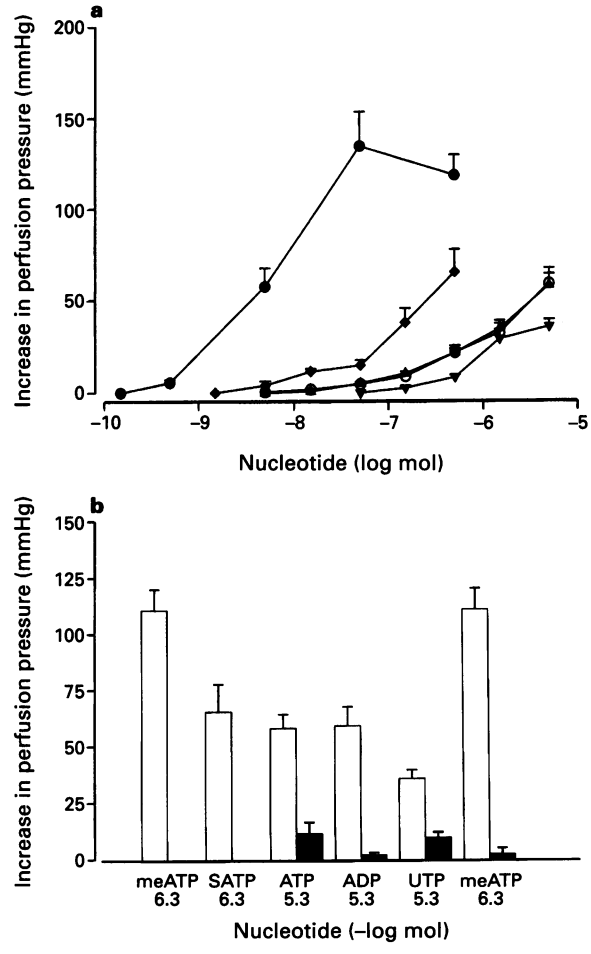

Figure 1 (a) Constrictor responses of the hamster isolated mesenteric arterial bed to: α,β-meATP (●, $n=4-7$); ATP (▲, $n=10$), 2MeSATP (◆, $n=4-6$); UTP (▼, $n=4$); ADP (○, $n=8$). (b) Effect of application of a dose of α,β-meATP (0.5 μmol) on constrictor responses (solid columns) to subsequent consecutive application of doses of nucleotides approximately 2 min later at 1.5 min intervals in the following order: α,β-meATP (meATP; 0.5 μmol), 2MeSATP (SATP; 0.5 μmol), ATP (5 μmol), ADP (5 μmol), UTP (5 μmol) and α,β-meATP (meATP; 0.5 μmol). Responses to SATP were abolished. For comparison control responses are represented by the open columns.

vasodilator action of 2MeSATP in the golden hamster mesenteric arterial bed, compared to its pronounced vasodilator effect in the rat isolated perfused mesenteric arterial bed. At the highest dose of 2MeSATP (50 nmol) there was no vasodilatation and only a constrictor response, 27.25±3.31 mmHg ($n=16$), was revealed. At the highest dose of ATP (500 nmol) there was a biphasic response in which vasoconstriction, 17.33±1.79 mmHg ($n=15$), preceded vasodilatation. UTP, ADP and adenosine did not elicit constriction at raised tone.

Effect of suramin, PPADS and reactive blue 2 on vasodilator responses

In the presence of antagonists the increases in perfusion pressure above baseline and the concentrations of methoxamine used to elicit these increases fell within the range achieved under control conditions and were as follows: suramin (0.1 mM), 73.0±10.95 mmHg using 9.5±0.05 μM methox-

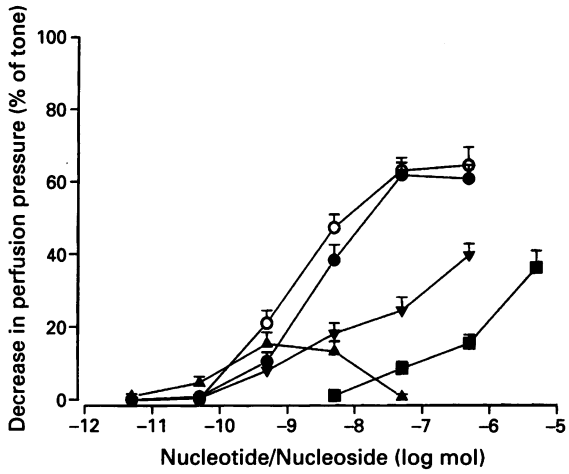

Figure 2 Vasodilator responses of the hamster isolated mesenteric arterial bed with tone raised with methoxamine (10–80 μM) to: ATP (0.005–500 nmol; ●, $n=18$); UTP (0.005–500 nmol; ○, $n=16$); 2MeSATP (0.005–50 nmol; ▲, $n=16$); ADP (0.005–500 nmol; ▼, $n=5$); adenosine (5–5000 nmol; ■, $n=14$). At the highest dose of 2MeSATP vasoconstriction and no vasodilatation was typically observed.

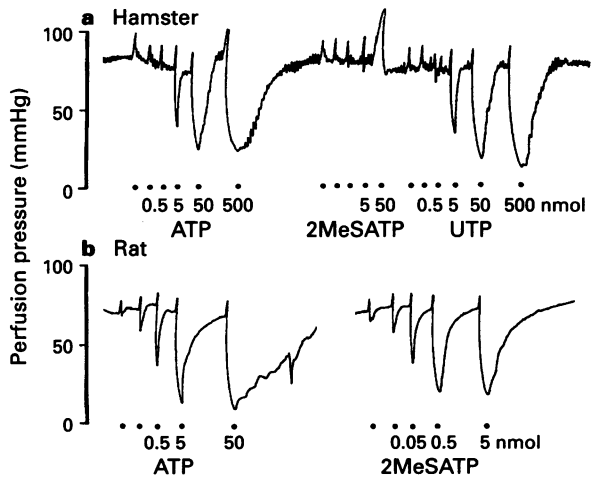

Figure 3 (a) Representative trace showing dose-dependent vasodilator responses to ATP (0.005–500 nmol) and UTP (0.005–500 nmol) in the hamster isolated mesenteric arterial bed with tone raised with methoxamine (15 μM). 2MeSATP (0.005–50 nmol) had no vasodilator effect in this preparation, and at the highest dose elicited vasoconstriction. In other preparations 2MeSATP gave weak vasodilator responses. (b) Shown for comparison is a representative trace from the rat isolated perfused mesenteric arterial bed with tone raised with methoxamine (20 μM), where 2MeSATP (0.0005–5 nmol) is more potent than ATP (0.005–5 nmol) as a vasodilator.

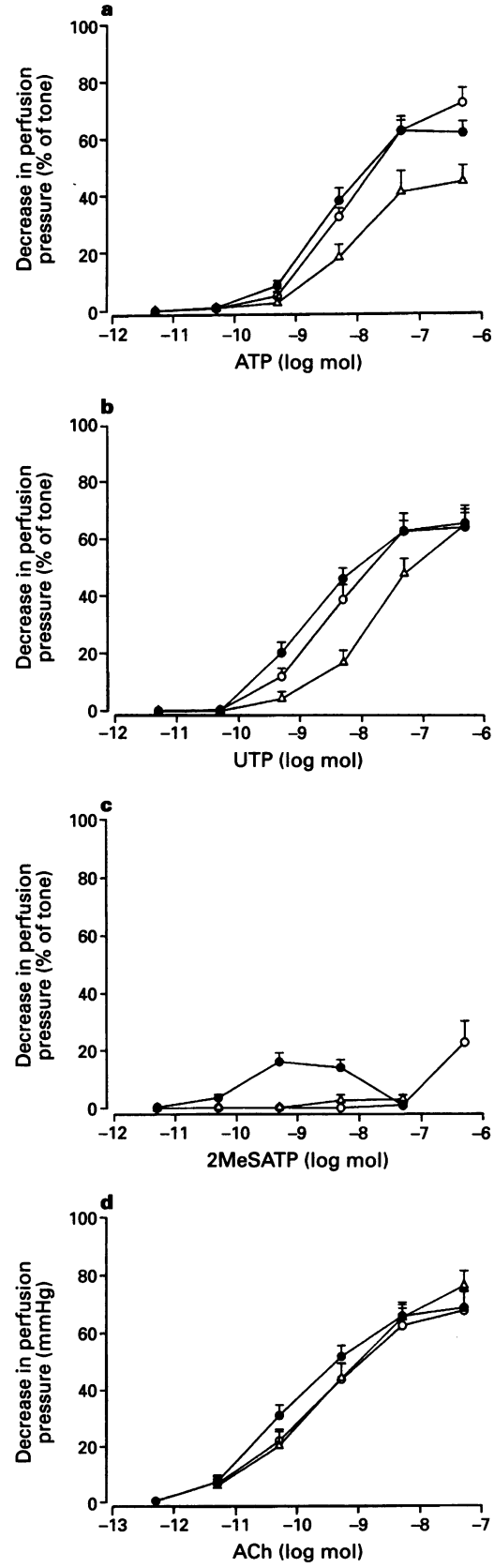

Figure 4 Vasodilator responses of the methoxamine raised-tone hamster isolated mesenteric arterial bed to: (a) ATP, (b) UTP, (c) 2MeSATP, (d) acetylcholine (ACh), in control preparations (●, $n=15–17$), in the presence of suramin (100 μM) (△, $n=6–7$), and in the presence of pyridoxalphosphate-6-azophenyl-2',4'-disulphonic acid (PPADS, 3 μM) (○, $n=7$).

amine ($n=7$); PPADS (3 μM), 64.29±7.51 mmHg using 19.6±0.44 μM methoxamine ($n=7$); reactive blue 2 (3 μM), 63.63±4.65 mmHg using 7.8±0.10 μM methoxamine ($n=8$); reactive blue 2 (30 μM), 45.0±1.92 mmHg using 9.0±0.07 μM methoxamine ($n=4$).

Suramin (0.1 mM) antagonised responses to ATP and UTP (Figure 4a,b). The pD_2 value for ATP was significantly reduced from 8.50±0.09 ($n=17$) in the absence to 7.84±0.16 ($n=6$) in the presence of suramin. The maximal vasodilator response was also significantly smaller in the presence of suramin. the pD_2 value for UTP was reduced from 8.81±0.11 ($n=16$) to 7.72±0.09 ($n=7$) by suramin, but there was no difference in the maximal relaxation. Estimated pA_2 values for the effects of suramin on ATP and UTP were not significantly different at 4.60 and 4.89 respectively. Responses to 2MeSATP were abolished (Figure 4c). The pD_2 value for ACh in the presence of suramin was significantly decreased from 10.07±0.13 ($n=17$) to 9.64±0.16 ($n=7$) (Figure 4d).

Vasodilator dose-response curves to ATP, UTP, 2MeSATP and ACh in the presence of PPADS (3 μM) are shown in Figure 4. The pD_2 value for ATP was significantly decreased from 8.50±0.09 ($n=17$) to 8.19±0.11 ($n=7$). pD_2 values for UTP, 8.48±0.12 ($n=7$) and ACh, 9.71±0.16 ($n=7$) were unchanged. Responses to 2MeSATP were virtually abolished by PPADS ($n=5$). Responses to ADP were also attenuated by PPADS, with a significant decrease in the pD_{15} value to 7.54±0.28 ($n=7$). Responses to adenosine were unaffected by PPADS (data not illustrated).

Data using reactive blue 2 (3 and 30 μM) were inconclusive because of its non-selectivity of action (data not shown).

Effect of endothelium removal on vasodilator responses

Removal of the endothelium abolished vasodilator responses to ACh (up to 50 nmol), UTP and to all of the purines except for adenosine. The response to adenosine at 5 μmol was unaffected being 35.87±4.68 mmHg ($n=14$) in the presence and 38.91±11.80 mmHg ($n=4$) in the absence of the endothelium, indicating unimpaired smooth muscle vasodilator function. The responses to SNP were similar in the absence of the endothelium (pD_2 9.32±0.21; $n=3$) and in endothelium-intact preparations (pD_2 9.11±0.07; $n=14$). In the absence of the endothelium constrictions to ATP (0.5 μmol), UTP (0.5 μmol), 2MeSATP (0.05 μmol) were revealed.

Discussion

The results of the studies at basal tone indicate that mesenteric arteries of the golden hamster have P_{2X}-purinoceptors which mediate vasoconstriction with an observed order of potency of α,β-meATP > > 2MeSATP > ATP = ADP > UTP. Although the relative stability of α,β-meATP and instability of ATP and other analogues of ATP to degradation may contribute to this rank order of potency (Crack *et al.*, 1994; Evans & Kennedy, 1994; Khakh *et al.*, 1995), this nevertheless is useful in the characterization of P_2-purinoceptors. The P_{2X}-purinoceptors were on the vascular smooth muscle since endothelium removal did not abolish responses to ATP, as also shown in rat mesenteric arteries (Ralevic & Burnstock, 1988). Responses to NA were also augmented, suggesting that the endothelium presents a barrier to diffusion of vasoactive substances to the smooth muscle and/or that it causes inhibitory modulation of constrictor responses by the release of endothelial factors.

In many blood vessels the archetypal P_{2Y}-purinoceptor agonist 2MeSATP is a more potent vasodilator than ATP (Burnstock & Kennedy, 1985; Martin *et al.*, 1985). Our data suggest that 2MeSATP had a good potency for P_{2Y} receptors but was much less efficacious as a vasodilator than ATP or UTP. A similar situation has been noted in the rat aorta (O'Connor *et al.*, 1991). The P_{2Y}-purinoceptor agonist ADP was also more efficacious than 2MeSATP, suggesting that 2MeSATP may be acting as a partial agonist at P_{2Y}-purinoceptors. The equipotency of ATP and UTP as vasodilators is indicative of actions at P_{2U}-purinoceptors (O'Connor *et al.*, 1991). Suramin blocked responses to ATP and UTP with similar estimated pA_2 values, consistent with their actions at a single receptor subtype. However, maximal responses to ATP, but not those to UTP were significantly reduced by suramin, suggesting a possible action of ATP at both P_{2U}- and P_{2Y}-purinoceptors. Coexisting endothelial P_{2Y}- and P_{2U}-purinoceptors have been described in other vessels, including rat mesenteric arteries (Ralevic & Burnstock, 1991b; Windscheif *et al.*, 1994). In hamster and in rat mesenteric arteries P_{2Y} and P_{2U} receptors are present on the endothelium since vasodilator responses to 2MeSATP, ATP and UTP are abolished by endothelium removal.

Suramin did not appear to be able to discriminate between the P_{2U} and P_{2Y} subtypes in hamster mesenteric arteries, in contrast to those in bovine aortic endothelial cells where suramin blocked responses to 2MeSATP but not to ATP and UTP (Wilkinson *et al.*, 1994). It was also not possible to discriminate between P_{2U} and P_{2Y} receptors using reactive blue 2 (3 or 30 μM) since this agent had non-selective inhibitory effects on vasodilator responses. However, inhibition by PPADS of responses to 2MeSATP and ADP, but not those to ATP and UTP is consistent with an action of these compounds at P_{2Y} and P_{2U} receptors, respectively. PPADS has previously been shown to attenuate P_{2Y}-, but not P_{2U}-mediated vasodilator responses in rat mesenteric arteries (Windscheif *et al.*, 1994), to inhibit P_{2Y}-purinoceptor stimulated phospholipase C activity in turkey erythrocytes (Boyer *et al.*, 1994) and to inhibit P_{2Y}- but not P_{2U}-mediated accumulation of total [^{3}H]-inositol (poly)phosphates in bovine aortic endothelial cells (Brown *et al.*, 1995). Further, our results with PPADS suggest that the poor efficacy of 2MeSATP was not due to an opposing constrictor action at P_{2X}-purinoceptors since vasodilatation was not augmented. P_{2Y} and P_{2U} receptors have been suggested to couple differently to G-proteins on the basis of pertussis toxin attenuation of P_{2U}- but not P_{2Y}-mediated inositol phosphate accumulation in bovine aortic endothelial cells (Motte *et al.*, 1993) In bovine aortic rings indomethacin strongly blocked relaxation to 2MeSATP and ADP but had only slight effects on UTP responses, suggesting differential release of cyclooxygenase-derived mediators of relaxation (Wilkinson *et al.*, 1994). The relatively small contribution of 2MeSATP to relaxation in hamster mesenteric arteries suggests that this may be a valuable functional system in which to study P_{2U}-purinoceptor signal transduction mechanisms.

The weak vasodilator actions of adenosine in hamster mesenteric arteries, mediated via receptors on the smooth muscle, is similar to the situation observed in the rat mesenteric arterial bed (Ralevic, 1995; Rubino *et al.*, 1995).

In conclusion, hamster mesenteric arteries have P_{2X}-purinoceptors which are present on the smooth muscle and mediate vasoconstriction and P_{2U}-purinoceptors on the endothelium which mediate vasodilatation. The marginal activity of 2MeSATP suggests that P_{2Y}-purinoceptors have a minimal role in ATP-mediated vasodilatation in these vessels. The hamster mesenteric arterial preparation may be useful in the study of P_{2U}-purinoceptor signalling pathways.

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