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Discrimination by PPADS between endothelial P_{2Y}- and P_{2U}-purinoceptors in the rat isolated mesenteric arterial bed

[1]Vera Ralevic & Geoffrey Burnstock

Department of Anatomy and Developmental Biology, University College London, Gower Street, London WC1E 6BT

1 The main aim of this study was to characterize the antagonistic effects of pyridoxalphosphate-6-azophenyl-2',4'-disulphonic acid (PPADS) at coexisting endothelial P_{2Y}- and P_{2U}-purinoceptors. Studies were conducted in Krebs-perfused mesenteric arterial preparations isolated from the rat, with tone raised by methoxamine (5–50 μM).

2 Purine and pyrimidine compounds elicited vasodilatation with a rank order of potency of 2-methylthio ATP (2-MeSATP) = ADP > ATP = UTP > P^1, P^3-diadenosine triphosphate (Ap_3A) > P^1, P^2-diadenosine pyrophosphate (Ap_2A) > NADP > adenosine. 8-*para*-Sulphophenyltheophylline (8-PSPT; 3 μM) had no effect on vasodilator responses to 2MeSATP, ADP, ATP, UTP, Ap_3A or NADP, but blocked responses to adenosine and the maximal response to Ap_2A.

3 PPADS (3–100 μM) attenuated vasodilator responses to the P_{2Y}-selective agonists 2MeSATP and ADP, shifting the dose-response curves to the right. The pA_2 values for PPADS at 2MeSATP and ADP were 5.97 ± 0.69 and 5.98 ± 0.86 respectively. In contrast, PPADS had no effect on vasodilator responses mediated by the P_{2U}-selective agonist, UTP, or on vasodilator responses mediated by ATP.

4 PPADS (10 μM) was used to characterize responses mediated by the adenine dinucleotides; dose-response curves for vasodilator responses to Ap_3A and NADP, but not those to Ap_2A, were shifted to the right by PPADS. The estimated pA_2 values for the effect of PPADS on Ap_3A and NADP were 6.38 and 6.26 respectively.

5 Indomethacin (10 μM) had no effect on vasodilator responses to 2MeSATP, ADP, ATP or UTP.

6 In conclusion, these results show that PPADS is an antagonist at endothelial P_{2Y}- but not P_{2U}-purinoceptors in rat mesenteric arteries. These receptors cannot be discriminated by inhibition of prostaglandin synthesis; P_{2Y}-purinoceptors are, however, sensitive to ADP. Selective antagonism by use of PPADS showed that ATP acts at P_{2U}- and not P_{2Y}-purinoceptors. Ap_3A and NADP mediate vasodilatation via P_{2Y}-purinoceptors, whereas vasodilatation to Ap_2A is mediated partly via P_1- and possibly via P_{2U}-purinoceptors.

Keywords: Adenine dinucleotides; ATP; purinoceptors; pyrimidinoceptors; PPADS; rat mesenteric arterial bed; UTP

Introduction

Cell surface receptors for ATP have been divided into at least five well-defined subtypes, namely P_{2X}, P_{2Y}, P_{2U}, P_{2T} and P_{2Z}, largely on the basis of agonist potencies (Burnstock & Kennedy, 1985; Gordon, 1986). In a revision of P_2-purinoceptor nomenclature and classification these subtypes are to be embraced within two broad groups, termed P2X and P2Y according to whether they act as intrinsic ion channels or are coupled to G-proteins respectively (Abbracchio & Burnstock, 1994; Fredholm *et al.*, 1994). The originally-defined P_{2X}-purinoceptors are ligand-gated cation channels, whereas the classic P_{2Y}-, P_{2U}- and P_T-purinoceptors are G-protein-coupled. In the revised classification, subtypes of the two major families of purinoceptors have been identified as they are cloned and characterized after functional expression, and this work is still in progress. Among other subtypes P_{2Y}- (P_{2Y1}) (Webb *et al.*, 1993) and P_{2U}-purinoceptors (P_{2Y2}) (Lustig *et al.*, 1993) have been cloned. Since the correlation between all cloned and originally-defined purinoceptor subtypes is not absolute, both new and classical systems of nomenclature are currently in use.

Pharmacological characterization of purinoceptors is heavily reliant on selective antagonism. Significant progress has been made in the development of antagonists at P_{2X}- and P_{2T}-purinoceptors; however, potent and selective antagonists at P_{2Y}- and P_{2U}-purinoceptors are lacking. Suramin is a non-selective antagonist at P_2-purinoceptors (Dunn & Blakeley, 1988; Hoyle *et al.*, 1990). Pyridoxalphosphate-6-azophenyl-2',4'-disulphonic acid (PPADS) (Lambrecht *et al.*, 1992; Ziganshin *et al.*, 1993; 1994; Windscheif *et al.*, 1994), and the suramin derivative NF023 (Ziyal *et al.*, 1994; 1995) have been shown to be selective antagonists at P_{2X}-purinoceptors. PPADS has been shown to block P_{2Y}-mediated responses in turkey erythrocytes (Boyer *et al.*, 1994), but is ineffective at P_{2Y} receptors in rabbit mesenteric arteries and aorta (Ziganshin *et al.*, 1994). Reactive blue 2 has been proposed as a P_{2Y}-purinoceptor antagonist; however, it has low selectivity, acting also at P_{2X}-purinoceptors (Choo *et al.*, 1980; Bo *et al.*, 1994; Bultmann & Starke, 1994) and is effective only within a narrow concentration-range (Burnstock & Warland, 1987; Hopwood & Burnstock, 1987).

In the rat mesenteric arterial vasculature, coexisting P_{2Y}- and P_{2U}-purinoceptors are present on the endothelium where they mediate vasodilatation (Ralevic & Burnstock, 1991). Relaxation is elicited at least in part by the release of endothelium-derived relaxing factor/nitric oxide (NO) since responses are blocked by an inhibitor of NO synthase, N^G-nitro-L-arginine methyl ester (Ralevic & Burnstock, 1991). In a previous study we showed that PPADS was a selective antagonist at P_{2X}-purinoceptors in rat mesenteric arteries. However, when used at a concentration (10 μM) which virtually abolished responses at P_{2X}-purinoceptors, PPADS partially blocked endothelium-dependent vasodilator responses to 2-MeSATP, but not those to ATP and UTP, suggesting antagonism of P_{2Y}- but not P_{2U}-purinoceptors (Windscheif *et al.*, 1994). PPADS antagonism of P_{2Y}-, but not of P_{2U}-mediated responses was subsequently shown in the hamster isolated mesenteric arterial bed (Ralevic & Burnstock, 1996) and in cultured bovine aortic endothelial cells (Brown *et*

[1] Author for correspondence.

al., 1995). In bovine aortic rings indomethacin strongly blocked relaxation to 2MeSATP and ADP but had only slight effects on UTP responses, suggesting differential release of cyclo-oxygenase-derived mediators of relaxation by P_{2Y}- and P_{2U}-purinoceptors (Wilkinson *et al.*, 1994).

The aim of this study was to characterize further the effects of PPADS as an antagonist of endothelial P_2-purinoceptors in the rat isolated mesenteric arterial bed, specifically to investigate its use as a means of distinguishing between responses mediated by P_{2Y}- and P_{2U}-purinoceptors. We also tested whether indomethacin could discriminate between these receptors.

In a previous publication we studied the effects of adenine dinucleotides on mesenteric arteries and surmised that their vasodilator actions are mediated via endothelial P_{2Y}-purinoceptors (Ralevic *et al.*, 1995). Here, we use PPADS to present a more definitive characterization of the subtype of P_2-purinoceptor involved.

Methods

Isolated mesenteric arterial bed preparation

Male Wistar rats (300–350 g) were killed by asphyxiation with CO_2. Mesenteric beds were isolated and set up for perfusion 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, 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 5 ml min^{-1} by use of a peristaltic pump (model 7554-30, Cole-Parmer Instrument Co., Chicago, Illinois, U.S.A.). 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, U.S.A.) on a side arm of the perfusion cannula, and recorded on a polygraph (model 7D, Grass Instrument Co., Quincy, Mass, U.S.A.). Preparations were allowed to equilibrate for 30 min prior to experimentation.

Tone of the preparations was raised with methoxamine (5–50 μM) and dose-response curves to a maximum of three vasodilators per preparation were constructed. Doses of purine or pyrimidine compounds were applied as 50 μl bolus injections via a rubber septum proximal to the preparation. Neither indomethacin (10 μM) nor 8-PSPT (3 μM) had significant effects on the tone of the preparations, thus these agents were added to the perfusate and the preparations equilibrated for 30 min at preconstricted tone before repeating the dose-response curves. PPADS (3–100 μM) significantly augmented the methoxamine-induced tone of the preparations. Thus PPADS was added to preparations at basal tone, after washout of methoxamine. Methoxamine was titrated into the perfusate to re-constrict the preparations to approximately the same level of tone as prior to the addition of PPADS. Dose-response curves were repeated after 30 min equilibration with PPADS.

Drugs used

The following drugs were obtained from Sigma: β-nicotinamide adenine dinucleotide phosphate (sodium salt), P^1, P^2-diadenosine pyrophosphate (sodium salt), P^1, P^3-diadenosine triphosphate (ammonium salt), ADP (sodium salt), ATP (disodium salt), UTP (sodium salt), indomethacin, and methoxamine hydrochloride. 2-MethylthioATP (tetrasodium salt) and 8-*para*-sulphophenyltheophylline were from Research Biochemicals Inc. Pyridoxalphosphate-6-azophenyl-2′, 4′-disulphonic acid (PPADS) was a generous gift from Dr G Lambrecht, University of Frankfurt, Germany.

Data analyses

Vasodilator responses were measured as changes in perfusion pressure (mmHg) and evaluated as a percentage of the methoxamine-induced increase in tone above baseline. Results are presented as mean ± s.e.mean. Where dose-response curves did not reach a maximum these were compared by analysis of variance with repeated measures. pA_2 values were determined by Schild-Analysis (Arunlakshana & Schild, 1959). Estimated pA_2 values were estimated from the pK_B, which was evaluated from the formula $K_B = [B]/(DR-1)$, 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

Vasodilator responses to purines and pyrimidines

Purines and pyrimidines elicited dose-dependent vasodilatation with a rank order of potency of 2MeSATP = ADP > ATP = UTP > Ap_3A > Ap_2A > NADP > adenosine (Figure 1). pD_2 values were: 2MeSATP, 10.30 ± 0.12 (n = 8); ADP, 10.06 ± 0.12 (n = 8); ATP, 9.10 ± 0.11 (n = 7); UTP, 9.22 ± 0.07 (n = 8); Ap_3A 8.79 ± 0.13 (n = 8); Ap_2A, 7.13 ± 0.05 (n = 7).

Hill slopes were: 2MeSATP, 0.74 ± 0.08 (n = 8); ADP, 0.66 ± 0.05 (n = 8); ATP, 0.68 ± 0.09 (n = 7); UTP, 0.65 ± 0.05 (n = 8); Ap_3A, 0.61 ± 0.07 (n = 8); Ap_2A, 0.86 ± 0.04 (n = 7). Response curves to NADP and adenosine did not reach a maximum, thus pD_2 values and Hill slopes could not be calculated.

Effect of 8-para-sulphophenyltheophylline on vasodilator responses

8-PSPT had no significant effect on the tone of the preparations. 8-PSPT (3 μM) had no significant effect on dose-dependent vasodilatation to 2MeSATP, ADP, ATP, UTP, Ap_3A or NADP, but blocked dose-dependent responses to adenosine and the maximal response to Ap_2A (achieved at 0.5 μmol) (n = 4–8; results not shown).

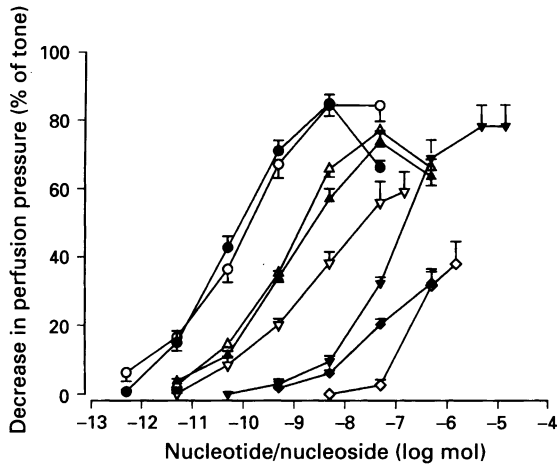

Figure 1 Vasodilator dose-response curves to nucleotides and nucleosides in the rat isolated mesenteric arterial bed: (●) 2MeSATP (n = 8); (○) ADP (n = 8); (▲) ATP (n = 7); (△) UTP (n = 8); (▽) Ap_3A (n = 8); (▼) Ap_2A (n = 7); (◆), NADP (n = 8); (◇) adenosine (n = 4). s.e.means are shown.

Effect of PPADS on vasodilator responses to purine and pyrimidine mononucleotides

PPADS potentiated constriction caused by perfusion with methoxamine, added to raise the tone of the preparations. Thus, PPADS was added at basal tone after washout of methoxamine and, after equilibration for 30 min, methoxamine was re-titrated into the perfusate to a lower final concentration to achieve a similar increase in perfusion pressure as prior to the addition of PPADS. In the absence of PPADS the increase in perfusion pressure above baseline was 59.57±2.44 mmHg with 25.9±0.28 μM methoxamine ($n=32$). In the presence of PPADS (3, 10 and 100 μM) increases in perfusion pressure and the concentrations of methoxamine used to produce these increases were: 3 μM PPADS, 53.67±4.16 mmHg with 9.5±0.19 μM methoxamine ($n=8$); 10 μM PPADS, 65.51±3.69 mmHg with 10.9±0.05 μM methoxamine ($n=20$); 100 μM PPADS, 51.38±6.66 mmHg with 5.0 μM methoxamine ($n=4$).

PPADS (3–100 μM) produced concentration-dependent antagonism of vasodilator responses to 2MeSATP and ADP, shifting the dose-response curves to the right (Figures 2, 3).

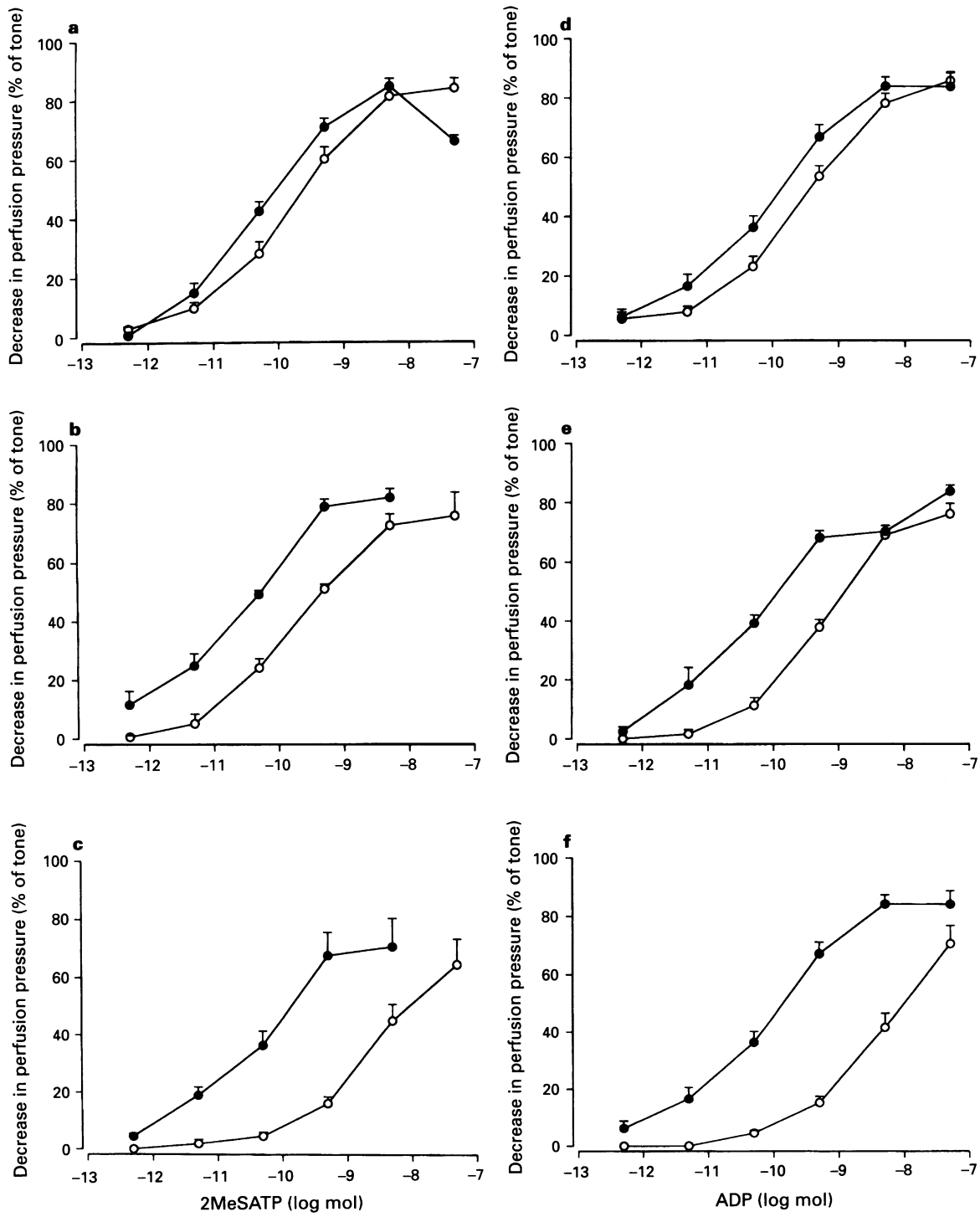

Figure 2 Effect of pyridoxalphosphate-6-azophenyl-2',4'-disulphonic acid (PPADS; 3–100 μM) on vasodilator responses to 2MeSATP (a–c) and ADP (d–f) in the rat isolated mesenteric arterial bed. (●) Responses in the absence of drugs; (○) responses in the presence of PPADS. (a, d) PPADS (3 μM) on responses to 2MeSATP and ADP. (b, e) PPADS (10 μM) on responses to 2MeSATP and ADP. (c, f) PPADS (100 μM) on responses to 2MeSATP and ADP. Data shown are results of paired experiments, except for (f) which are unpaired. s.e.means are shown.

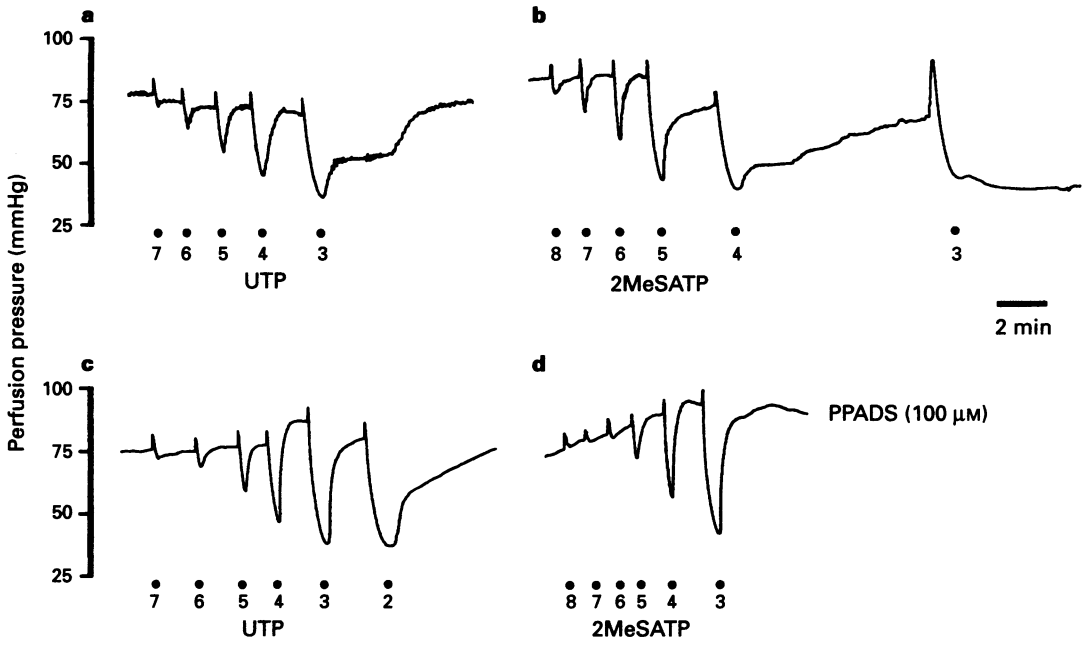

Figure 3 Representative traces from a single rat mesenteric arterial preparation showing vasodilator responses to UTP and 2MeSATP in the absence (a, b) and presence (c, d) of 100 μM PPADS. Nucleotides were applied as doses of 50 μl bolus injections. Tone of the preparation was raised by 10 μM methoxamine in the absence of PPADS and 5 μM methoxamine in the presence of PPADS. PPADS had no effect on dose-dependent vasodilatation to UTP, but antagonized vasodilatation to 2MeSATP. Doses are indicated as concentration of drug (M) in the 50 μl bolus. For UTP this corresponds to a dose-range of -11.3 to -6.3 log mol (or 0.005–500 nmol). For 2MeSATP this corresponds to a dose range of -12.3 to -7.3 log mol (or 0.0005–50 nmol).

pA_2 values for PPADS at 2MeSATP and ADP were 5.97 ± 0.69 ($n=14$) and 5.98 ± 0.86 ($n=14$) respectively. At each concentration of PPADS, inhibition of responses to 2MeSATP and ADP was surmountable and the curves were shifted in a parallel manner suggesting that the antagonism was competitive. However, the Schild slopes for PPADS versus 2MeSATP and ADP were 0.76 in each case.

In contrast PPADS (10 and 100 μM) had no effect on vasodilator responses to UTP (Figures 3, 4a) and at 10 μM PPADS had no effect on vasodilator responses to ATP (Figure 4b). PPADS (10 μM) also had no effect on dose-dependent vasodilator responses to adenosine (Figure 5d).

Effect of PPADS on vasodilator responses to purine dinucleotides

PPADS (10 μM) antagonized the dose-dependent responses to Ap_3A and NADP with estimated pA_2 values of 6.38 and 6.26 respectively (Figure 5a, c). In contrast, PPADS (10 μM) had no significant effect on responses to Ap_2A (Figure 5b).

Effect of indomethacin on vasodilator responses

Indomethacin had no significant effect on the tone of the preparations. Indomethacin (10 μM) had no significant effect on dose-response curves for vasodilatation to 2MeSATP, ADP, ATP or UTP ($n=4$; results not shown).

Discussion

In this study we characterize the antagonistic effects of PPADS at endothelial P_2-purinoceptors in rat mesenteric arteries. We show that PPADS produces concentration-dependent inhibition of responses mediated by P_{2Y}- but not P_{2U}-purinoceptors. This confirms and extends our previous findings in rat (Windscheif *et al.*, 1994) and hamster mesenteric arterial beds (Ralevic & Burnstock, 1995). In agreement with this are the results of Brown *et al.* (1996) who reported that PPADS (30 μM) inhibits P_{2Y}-, but not P_{2U}-mediated accumulation of

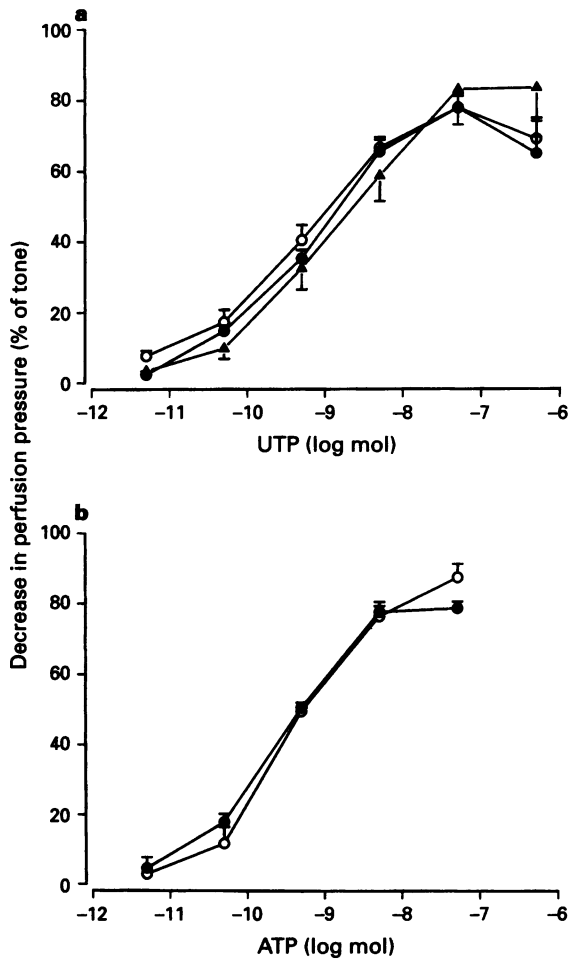

Figure 4 Effect of pyridoxalphosphate-6-azophenyl-2',4'-disulphonic acid (PPADS) (○, 10 μM; ▲, 100 μM) on vasodilator responses of the rat isolated mesenteric arterial bed to: (a) UTP (●), (b) ATP (●); s.e.means are shown.

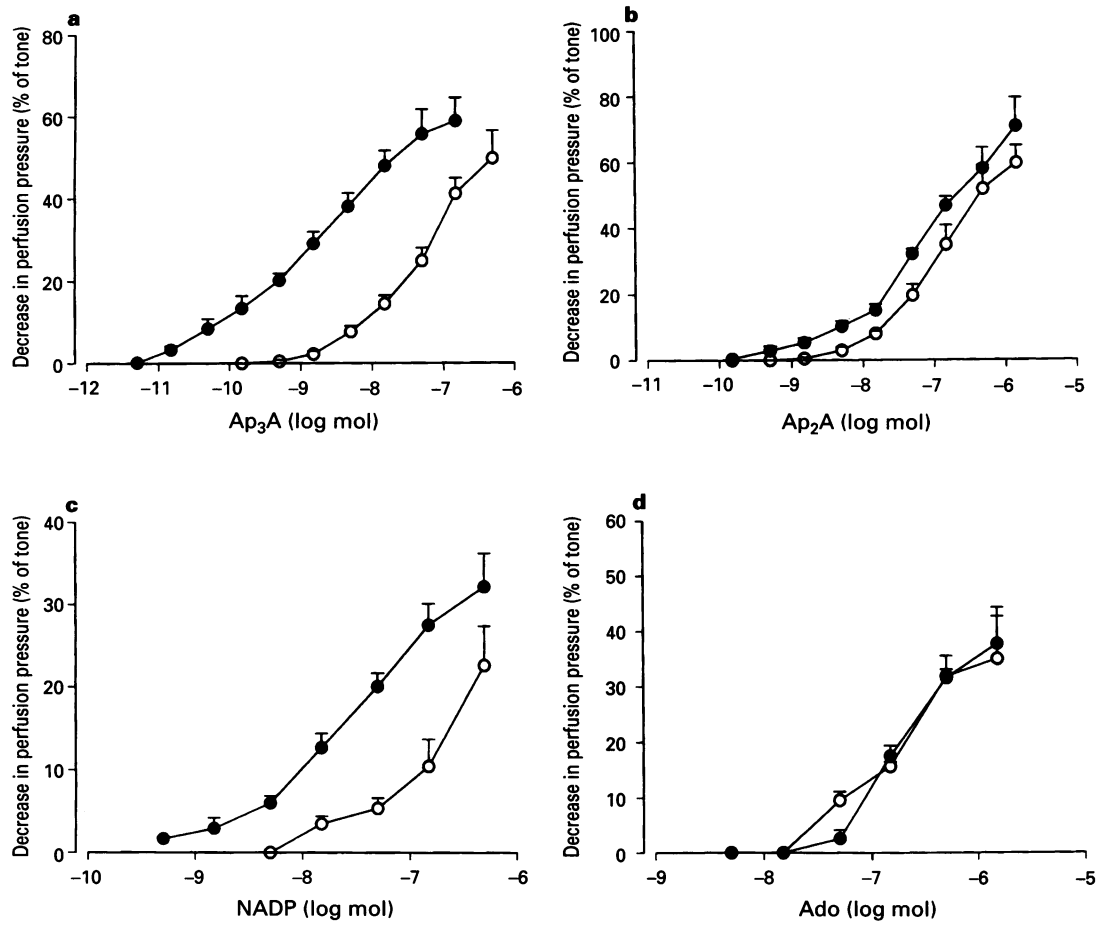

Figure 5 Effect of PPADS (10 μM) on vasodilator responses of the rat isolated mesenteric arterial bed to: (a) P^1, P^3-diadenosine triphosphate (Ap_3A); (b) P^1, P^2-diadenosine pyrophosphate (Ap_2A), (c) nicotinamide adenine dinucleotide (NADP), (d) adenosine (Ado). s.e.means are shown. (●) Responses in the absence of drugs; (○) responses in the presence of PPADS.

total inositol-[^{3}H]-polyphosphates in bovine aortic endothelial cells in culture. In the present study the clear separation of dose-response curves into equipotency of 2MeSATP and ADP and equipotency of ATP and UTP supports the existence of two distinct types of endothelial P_2-purinoceptors.

In an earlier study we proposed that PPADS is a selective antagonist at P_{2X}-purinoceptors with little effect at 2_{2Y}-purinoceptors in rat mesenteric arteries (Windscheif *et al.*, 1994). The present study is not at odds with this report since a concentration of PPADS (3 μM) which virtually abolished constrictor responses to α,β-meATP and 2MeSATP at P_{2X}-purinoceptors (Windscheif *et al.*, 1994) produced only a slight inhibition of P_{2Y}-purinoceptor-mediated vasodilatation. Thus, PPADS is selective for P_{2X}-purinoceptors. Responses mediated by 2MeSATP and ATP at smooth muscle P_{2X}-purinoceptors do not have to be considered in the present study since they significantly oppose the endothelial P_2-mediated vasodilatation only at the highest dose used (50 nmol) (Ralevic & Burnstock, 1988). Hence, in systems where the P_{2X}-purinoceptor is not present or does not participate in the functional response (such as in the present study), or in endothelial cells in culture, PPADS can be used to discriminate between P_{2Y}- and P_{2U}-purinoceptors. Interestingly, PPADS antagonism of P_{2X}-purinoceptors in rat and rabbit vessels is non-competitive (Windscheif *et al.*, 1994; Ziganshin *et al.*, 1994), whereas at P_{2Y}-purinoceptors antagonism appears to be competitive.

For many years it was assumed that responses mediated by ATP in tissues having P_{2Y}-purinoceptors, as shown by use of the classical P_{2Y}-purinoceptor agonist, 2MeSATP, were via actions at P_{2Y}-purinoceptors. We were under this impression with respect to the vasodilator actions of ATP at endothelial P_2-purinoceptors in rat mesenteric arteries, which we suggested were of the P_{2Y} subtype (Ralevic & Burnstock, 1988). In 1991, O'Connor and colleagues drew attention to the possibility that the actions of ATP may be mediated by a nucleotide receptor characterized by the equipotency of ATP and UTP (O'Connor *et al.*, 1991). In rat mesenteric arteries dose-dependent endothelium-dependent vasodilatation to ATP=UTP was shown, suggesting the presence of a nucleotide or P_{2U}-purinoceptor (Ralevic & Burnstock, 1991). However, in the absence of selective antagonists, characterization of this receptor and in particular definitive evidence to show that this was distinct from the P_{2Y}-purinoceptor was lacking. The results of the present study confirm the existence of distinct P_{2Y}- and P_{2U}-purinoceptors in rat mesenteric arteries. In addition, lack of antagonism by PPADS of responses to ATP suggests that ATP acts as a vasodilator at P_{2U}-purinoceptors and is not an agonist at classic P_{2Y}-purinoceptors in rat mesenteric arteries. Similarly, ATP appears to act at P_{2U}- and not at P_{2Y}-purinoceptors in mesenteric arteries of the golden hamster (Ralevic & Burnstock, 1995). The superimposable dose-response curves for ATP and UTP, lying to the right of the superimposable dose-response curves for 2MeSATP and ADP, is consistent with an action of ATP via P_{2U}- and not P_{2Y}-purinoceptors. This intriguing result challenges the concept of actions of ATP at P_{2Y}- versus P_{2U}-purinoceptors in other vascular systems.

PPADS may have potential for development as an antagonist at P_{2Y}-purinoceptors. The inhibition by PPADS of 2MeSATP- and ADP-mediated responses and the lack of inhibition of ATP- and UTP-mediated responses clearly shows

that it is able to discriminate between endothelial P_{2Y}- and P_{2U}-purinoceptors. How this occurs is not yet known. P_{2Y}- and P_{2U}-purinoceptors 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).

PPADS potentiated the constrictor effects of methoxamine, as observed previously in this preparation (Windscheif *et al.*, 1994). The reason for this is not clear but may be due to membrane depolarization, as observed in the guinea-pig vas deferens (McLaren *et al.*, 1994).

In contrast to the antagonism of P_{2Y}-purinoceptors in rat and golden hamster mesenteric arteries (present study; Ralevic & Burnstock, 1996) PPADS had no inhibitory effect on smooth muscle P_{2Y}-purinoceptors in rabbit mesenteric arteries and aorta (Ziganshin *et al.*, 1994), suggesting heterogeneity of smooth muscle and endothelial P_{2Y}-purinoceptors. Heterogeneity of P_{2Y}-purinoceptors is also indicated by PPADS antagonism of P_{2Y}-purinoceptors in turkey erythrocytes but not in C6 rat glioma cells (Boyer *et al.*, 1994). In turkey erythrocytes PPADS antagonized P_{2Y}-purinoceptor stimulated phospholipase C activity with a pK_B of 5.9 (Boyer *et al.*, 1994), which is comparable to the pA_2 values reported in the present study. To put this in perspective, the P_{2X}-inhibitory activity of PPADS in rabbit vas deferens and rat mesentery yielded apparent pK_B values of 6.34 and 6.38 respectively (Lambrecht *et al.*, 1996).

In bovine aortic rings, indomethacin strongly blocked relaxation to 2MeSATP and ADP but had only slight effects on responses to UTP, suggesting differential release of cyclooxygenase-derived mediators of relaxation in the responses to these receptors (Wilkinson *et al.*, 1994). In the present study indomethacin had no inhibitory effects on vasodilatation mediated by either P_{2Y}- or P_{2U}-purinoceptors.

PPADS appears to be more selective for P_{2Y}- versus P_{2U}-purinoceptors than the non-selective P_2-antagonist suramin, which is able to discriminate between P_{2Y}- and P_{2U}-purinoceptors in some but not all systems. In bovine aortic endothelial cells, suramin blocked responses to 2MeSATP but not to ATP and UTP (Wilkinson *et al.*, 1994). In rat mesenteric arteries suramin antagonized responses to 2MeSATP and not those to UTP (Ziyal *et al.*, 1996). However, in mesenteric arteries of the golden hamster, suramin blocked vasodilator responses mediated by both P_{2U}- and P_{2Y}-purinoceptors (Ralevic & Burnstock, 1996). Antagonism by suramin of responses at P_{2U}-purinoceptors has also been reported in mouse myotubes (Henning *et al.*, 1993), in rat pituitary gonadotropes (Chen *et al.*, 1994) and mouse cortical thick ascending limb segments (Paulais *et al.*, 1995).

We utilized the discriminatory effects of PPADS at P_{2Y}- and P_{2U}-purinoceptors to characterize further the purinoceptor subtype(s) mediating vasodilator responses of rat mesenteric arteries to adenine dinucleotides. In a previous study we speculated that responses to Ap_3A, Ap_2A and NADP are mediated at endothelial P_{2Y}-purinoceptors (Ralevic *et al.*, 1995). In the present study we confirm that actions of Ap_3A and NAPD are mediated at P_{2Y}-purinoceptors. In contrast, the vasodilator actions of Ap_2A are not mediated at P_{2Y}-purinoceptors or at P_1-purinoceptors, since responses are not antagonized by PPADS. The action of this compound at P_{2U}-purinoceptors remains a possibility.

In conclusion, PPADS is an antagonist at endothelial P_{2Y}- and not at P_{2U}-purinoceptors and can be used to discriminate between these subtypes in systems where P_{2X}-purinoceptors are not present or do not contribute to the functional response. ATP mediates vasodilatation via P_{2U}- and not P_{2Y}-purinoceptors in rat mesenteric arteries. Ap_3A and NADP, but not Ap_2A mediate responses via P_{2Y}-purinoceptors.

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