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Evidence for a P_2-purinoceptor mediating vasoconstriction by UTP, ATP and related nucleotides in the isolated pulmonary vascular bed of the rat

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1 The vasoconstrictor effects of uridine 5'-triphosphate (UTP), uridine 5'-diphosphate (UDP), uridine 5'-monophosphate (UMP) and uridine were tested in the isolated pulmonary vascular bed of the rat. Comparison was made with the effects of adenine nucleotides, adenosine 5'-triphosphate (ATP), adenosine 5'-diphosphate (ADP), adenosine 5'-monophosphate (AMP) and with adenosine. The effect of P_{2x}-purinoceptor desensitization and blockade was compared on the vascular responses to uracil and adenine nucleotides.

2 At doses ranging from 10^{-8} to 10^{-5} mol, UTP elicited dose-dependent vasoconstriction. UDP was equiactive to UTP, while UMP and uridine did not show vasomotor activity. Similarly, ATP showed dose-related vasoconstrictor activity. ADP was less potent than ATP in eliciting vasoconstriction, while AMP was active only at the higher doses tested and adenosine was ineffective.

3 Vasoconstriction was produced by ATP analogues with the following order of potency: α,β-methylene ATP > ATPγS > β,γ-methylene ATP > 2-methylthio ATP $\geqslant$ ATP.

4 Desensitization of P_{2x}-purinoceptors by the selective agonist α,β-methylene ATP did not modify the vasoconstrictor activity of UTP and UDP and only partially reduced vasoconstrictor responses to ATP, while it abolished vascular responses to α,β-methylene ATP itself.

5 The antagonists of P_2-purinoceptors, suramin and pyridoxalphosphate-6-azophenyl-2', 4'-disulphonic acid (PPADS), did not affect vascular responses to UTP and UDP, but reduced vasoconstriction evoked by β,γ-methylene ATP and ATP by about 70 and 30%, respectively.

6 This study demonstrates that uracil nucleotides, UTP and UDP, elicit vasoconstriction in the rat pulmonary vascular bed. In addition to confirming the presence of classical P_{2x}-purinoceptors, these results also suggest the presence of a distinct purinoceptor subtype which mediates UTP- and ATP-evoked vasoconstriction in the rat pulmonary circulation.

Keywords: P_2-purinoceptors; UTP; ATP; pulmonary circulation; pulmonary vasoconstriction

Introduction

There is now good evidence in favour of extracellular effects of uridine and uracil nucleotides in several tissues, including liver and kidney and in different cell types, such as pituitary cells, macrophages and neutrophils (see Seifert & Schultz, 1989). The vasomotor activity of uridine 5'-triphosphate (UTP) and related nucleotides has been investigated in the systemic circulation. The contractile effect of UTP and uridine 5'-diphosphate (UTP) was first shown in the intra- and extracranial arteries (Urquilla, 1978; Shirasawa *et al.*, 1983) and an important role has been suggested for UTP in the pathogenesis of vasospasm following cerebral injury, as platelets and brain tissue are a rich source for uracil nucleotides (Urquilla, 1978; Shirasawa *et al.*, 1983; Seifert & Schultz, 1989). Vasomotor activity of UTP has also been described in other vascular preparations, such as rabbit ear artery (Von Kügelgen *et al.*, 1987), guinea-pig coronary artery (Vials & Burnstock, 1993), rat tail and femoral artery and dog saphenous vein (Saiag *et al.*, 1990). Both vasodilator and vasoconstrictor effects of UTP have been shown in the rat mesenteric vascular bed (Ralevic & Burnstock, 1991), which is in many respects a representative model of peripheral resistance in the systemic circulation. However, no information is available on the vasomotor activity of UTP and other uracil nucleotides in the resistance vessels of the pulmonary circulation, although the effects of adenosine and related nucleotides in the pulmonary vessels have been investigated. Both P_1- and P_2-purinoceptors specific for adenosine and adenosine 5'-triphosphate (ATP) respectively, have been shown in the pulmonary vasculature (Neely *et al.*, 1991; Lippton *et al.*, 1992). Furthermore, P_{2x}- and P_{2y}-purinoceptor subtypes which mediate vasoconstrictor and vasodilator responses to ATP, respectively, have been shown in the pulmonary circulation (Liu *et al.*, 1989; McCormack *et al.*, 1989).

It is widely accepted that ATP and adenine nucleotides elicit constriction of vascular and non vascular smooth muscle via activation of P_{2x}-purinoceptors, while P_{2y}-purinoceptors mediate their relaxant effects (Burnstock & Kennedy, 1985; Burnstock, 1991). The existence of other subclasses of P_2-purinoceptors, namely P_{2t}-, P_{2u}- and P_{2z}-purinoceptors and the abundant literature on the effects of adenine nucleotides has led to a recent revision of the nomenclature of P_2-purinoceptors. Based on pharmacological and biochemical evidence supported by molecular biology, Abbracchio & Burnstock (1994) suggested that two families of purinoceptors should be distinguished, P2X- and P2Y-purinoceptors. These two families represent ion-gated and G-protein-coupled purinoceptors, respectively. Subtypes of the P2Y-purinoceptor family have been proposed and the term $P2Y_2$-purinoceptor has been suggested for the P_{2u}-purinoceptor, where UTP and ATP are equipotent agonists (Abbracchio & Burnstock, 1994).

In this study we have evaluated the vasoconstrictor effects of UTP and other uridine nucleotides in the rat pulmonary vascular bed and we have compared their actions with the effects of adenine nucleotides and ATP analogues. The effects

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of antagonists of P_2-purinoceptors were also examined, including suramin (Dunn & Blakeley, 1988) and pyridoxalphosphate-6-azophenyl-2',4'-disulphonic acid (PPADS; Lambrecht *et al.*, 1992; Ziganshin *et al.*, 1994; Windscheif *et al.*, 1994).

Methods

Isolation and perfusion of pulmonary vascular bed

Male Wistar rats (250–300 g) were used for this study. Animals were killed by an overdose of sodium pentobarbitone (Sagatal, RBM Animals Health, Dagenham, Essex) and exsanguination. Lungs were removed '*en bloc*' together with the heart; the left atrium was excised and the main pulmonary artery was cannulated. The preparation was transferred into a chamber and perfused with Krebs solution of the following composition (mM): NaCl 118.0, KCl 5.0, NaH_2PO_4 1.2, $NaHCO_3$ 25.5, $MgSO_4$ 1.2, glucose 5.6, $CaCl_2$ 2.5 and 0.4% bovine serum albumin, oxygenated with 95%O_2 and 5% CO_2 to maintain pH 7.4 and kept at 37°C. Because flow through the pulmonary vascular bed was maintained constant at 8 ml min^{-1} throughout the experiment, changes in perfusion pressure (mmHg) were indicative of changes in pulmonary vascular resistance. These were measured with a pressure transducer (model P23XL, Vigo Spectramed, Oxnard, CA, U.S.A.) on a side arm of the perfusion cannula and were recorded on a polygraph (model 79D, Grass Instrument Co., Quincy, MA, U.S.A.). Preparations were allowed to equilibrate for 20 min before experimentation.

Administration of drugs

The agonists tested were applied as a bolus injection of 100 μl via an injection port proximal to the preparation. This experimental protocol was preferred to the continuous perfusion of preparations with agonists, since it reduces the duration (and consequent oedema formation) and the cost of experimentation. In order to minimize the desensitizing action of the P_{2x}-purinoceptor agonists tested, injection of two consecutive doses was at 20 min interval. No more than three dose-response curves were obtained in each preparation. In order to limit the side effects of oedema formation that occurred in prolonged experiments, when the effect of suramin and PPADS was tested, each preparation was challenged with selected doses of two agonists (UTP, UDP, ATP or ADP) and left in contact with the antagonists for 30 min; administration of the agonists was then repeated. When the effect of P_{2x}-purinoceptor desensitization was evaluated, preparations were continuously perfused in the presence of α,β-methylene ATP 10^{-6} M for 20 min and vascular responses to the agonists tested were recorded still in the presence of the desensitizing agent. The effect of indomethacin was tested by evaluating vasoconstrictor responses before and after 30 min continuous perfusion with the drug.

Materials

UTP, UDP, UMP, uridine, ATP, ADP, AMP, adenosine, ATPγS, α,β- and β,γ-methylene ATP were purchased from Sigma. 2-methylthio ATP (2-MeS ATP) was obtained from RBI (Natick, MA, U.S.A.). Suramin was a kind gift from Bayer, UK. PPADS was a generous gift of Prof. Lambrecht. All drugs were dissolved in distilled water and stored frozen.

Evaluation of data

Vascular responses were measured as maximal increase in perfusion pressure following bolus injection of drugs. Results are presented in the text and the figures as means ± s.e.mean, followed by number of observations in parentheses. Since a plateau was not reached in evaluating dose-related responses to some of the agonists tested, EC_{50} values could not be calculated. Therefore, the order of potency of agonists was evaluated empirically by comparing the dose-response curves obtained. Statistical significance was evaluated by Student's *t* test for paired data and results were considered significant for $P<0.05$.

Results

Vasoconstrictor responses to uridine and adenine nucleotides

The basal tone of the perfused pulmonary vascular bed was 12.5 ± 0.4 mmHg ($n=25$). Administration of increasing doses (10^{-8}–10^{-5} mol) of the uridine nucleotide, UTP, elicited dose-dependent vasoconstrictor responses and vasoconstriction achieved with the highest dose tested was 11.1 ± 2.4 ($n=7$) mmHg. UDP was active at the same doses as UTP, reaching a vasoconstriction of 9.8 ± 1.9 ($n=5$) mmHg at the higher doses tested. UMP and uridine failed to evoke vasomotor responses (Figure 1). Similarly, the adenine nucleotides ATP and ADP evoked vasoconstriction, while AMP elicited a weak vasoconstrictor response only at the highest dose tested. Adenosine lacked vasomotor activity (Figure 1). ATP was equipotent to UTP, acting at doses from 10^{-8} to 10^{-5} mol and elicited at the dose 10^{-5} mol a vasoconstriction of 11.5 ± 1.2 ($n=7$) mmHg. ADP evoked vasoconstriction at higher doses than ATP. The vasoconstrictor response to the highest dose of ADP tested was 5.9 ± 0.5 ($n=5$) mmHg (Figure 1). Treatment of the preparations with indomethacin 10^{-5} M did not affect vasoconstrictor responses to either ATP or UTP (10^{-7}–10^{-5} mol).

Vasoconstrictor responses to ATP analogues

The vasoconstrictor effect of ATP was mimicked by the selective agonists of the P_{2x}-purinoceptor subtype, α,β- and β,γ-methylene ATP. The ATP analogues were active at doses 100 and 10 fold lower than ATP, respectively (Figure 2). The ATP analogue ATPγS was also more potent than ATP, being active at doses from 10^{-9} to 10^{-6} mol, while 2-MeS ATP was equipotent to ATP in eliciting vasoconstrictor responses (Figure 2). Tachyphylaxis was observed when two consecutive doses of

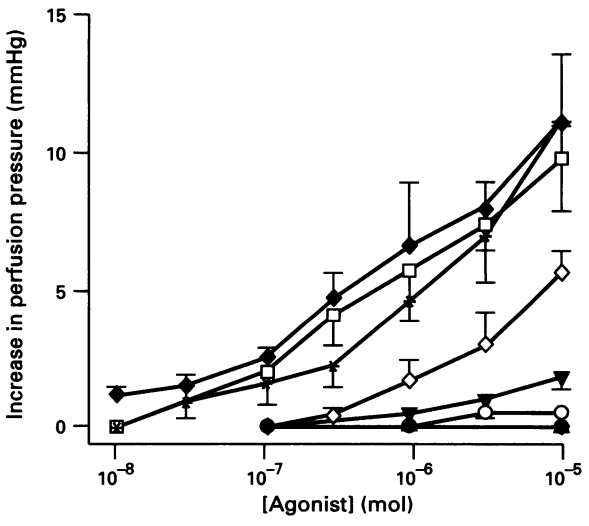

Figure 1 Dose-dependent effect of UTP (□), UDP (*), UMP (●), uracil (○), ATP (◆), ADP (◇), AMP (▼) and adenosine (▲) in the rat isolated pulmonary vascular bed. Each point is the mean of 4–7 determinations. In this and the following figures, vascular responses to the agonists were evaluated as maximal increase in the perfusion pressure after bolus injection of 100 μl drug.

any of the ATP analogues tested were injected at less than 20 min interval. Maximal vasoconstriction achieved was 12.7 ± 1.3 ($n=5$), 11.5 ± 1.5 ($n=5$) and 9.7 ± 0.9 ($n=5$) mmHg for α,β-methylene ATP, ATPγS and β,γ-methylene ATP, respectively (Figure 2).

Effect of desensitization of P_{2x}-purinoceptors by α,β-methylene ATP

Perfusion of the pulmonary vascular bed with a desensitizing concentration of α,β-methylene ATP (10^{-6} M) for 20 min almost abolished vasoconstrictor responses to a bolus injection of α,β-methylene ATP itself. In contrast, ATP-evoked vasoconstriction was always obtained in the presence of α,β-methylene ATP (Figures 3 and 4). After desensitization, control vasoconstrictor responses of comparable magnitude to α,β-methylene ATP (10^{-7} mol) and ATP (10^{-5} mol) were reduced by about 80 and 30%, respectively (Figure 4). Vasoconstrictor responses to UTP were not reduced by desensitization of P_{2X}-purinoceptors. Increased responsiveness to lower doses of UTP was observed in the presence of α,β-methylene ATP although statistical significance was not reached (Figure 4). Desensitization of P_{2x}-purinoceptors did not affect vasoconstrictor responses to UDP and ADP (10^{-7}–10^{-5} mol).

Antagonism by suramin and PPADS

In the presence of suramin 10^{-4} M, vasoconstrictor responses to UTP were unaffected (Figure 5). At the same concentration, suramin had a weak inhibitory effect on the ATP-evoked vasoconstriction. Vasoconstrictor responses to 10^{-6} and 10^{-5} mol ATP were significantly reduced by about 40 and 26%, respectively, while vascular responses to β,γ-methylene ATP 10^{-6} mol were significantly reduced by about 68% (Figure 5). Similarly, a fully active concentration of the P_{2x}-purinoceptor antagonist, PPADS (3×10^{-5} M) did not modify vascular responses to UTP and UDP (Figure 6). ATP-evoked vasoconstriction was significantly reduced in the presence of PPADS. However, the extent of antagonism appeared to be less that that versus the P_{2x} agonist, β,γ-methylene ATP. Comparable vasoconstrictor responses to ATP (10^{-6} mol) and β,γ-methylene ATP (10^{-7} mol) were reduced by about 35 and 70% respectively (Figure 6).

Discussion

The results of this study support the original observation that the uridine nucleotides, UTP and UDP, elicit vasoconstriction of the rat pulmonary vascular bed. UDP potency was comparable to that of UTP while UMP and uridine were devoid of vasoconstrictor actions. These results are in line with previous observations obtained in systemic blood vessels such as renal artery, rabbit ear and basilar artery where UDP was either equipotent or less potent than UTP, while UMP and uridine were only weak vasoconstrictors (Macdonald *et al.*, 1984; Von Kügelgen *et al.*, 1987; Von Kügelgen & Starke, 1990).

In the pulmonary vascular bed, vascular responses to UTP were insensitive to the unselective P_2-purinoceptor antagonist, suramin. This finding is not surprising as suramin has been shown to have little if any antagonism of UTP-mediated effects in several tissues (Wilkinson *et al.*, 1994; Dainty *et al.*, 1994). UTP- and UDP- evoked vasoconstriction of the pulmonary vascular bed was also resistant to desensitization and blockade of P_{2x}-purinoceptors by α,β-methylene ATP and PPADS, thus indicating that uridine nucleotides activate a receptor system distinct from the classical P_{2x}-purinoceptor subtype. Resistance of UTP-evoked vasoconstriction to desensitization and blockade of P_{2x}-purinoceptors observed in this study is consistent with previous observations which led to the proposal that separate receptors mediate the vasoconstrictor effects of UTP and ATP in several blood vessels of the systemic circulation (Von Kügelgen *et al.*, 1987; Von Kügelgen & Starke, 1990; Saiag *et al.*, 1990; Ralevic & Burnstock, 1991). However, evidence obtained in this study suggests that in the pulmonary circulation, UTP and ATP may share a common P_2-purinoceptor distinct from the classical P_{2x} subtype.

As previously reported (Liu *et al.*, 1989; McCormack *et al.*, 1989) the results of the present study confirm the presence of P_{2x}-purinoceptors in the rat pulmonary vasculature since the ATP analogues tested elicited vasoconstriction with an order of potency which defines the pharmacological profile of the classical P_{2x}-purinoceptor subtype as originally described by Burnstock & Kennedy (1985). Moreover, when vascular responses to α,β-methylene ATP, β,γ-methylene ATP, ATPγS

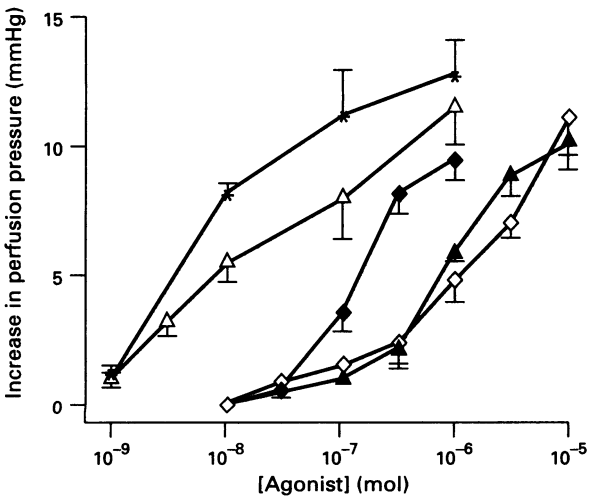

Figure 2 Dose-dependent effect of α,β-methylene ATP (*), β,γ-methylene ATP (◆), ATPγS (△) and 2-MeS ATP (▲) in the rat isolated pulmonary vascular bed. The dose-response curve to ATP (◇) presented in Figure 1 has been reproduced here for comparison with the ATP analogues. Each point is the mean of 4–7 determinations.

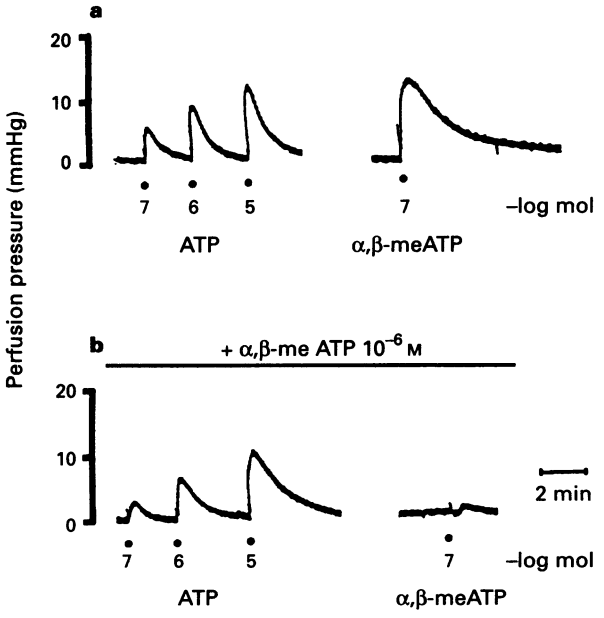

Figure 3 Tracing of a typical experiment showing vascular responses to increasing doses of ATP before (a) and after (b) treatment with the desensitizing agent α,β-methylene ATP (α,β-meATP). In the presence of α,β-methylene ATP, vasoconstrictor response to a bolus injection of the agonist itself was almost abolished, while constrictor responses to ATP were still present.

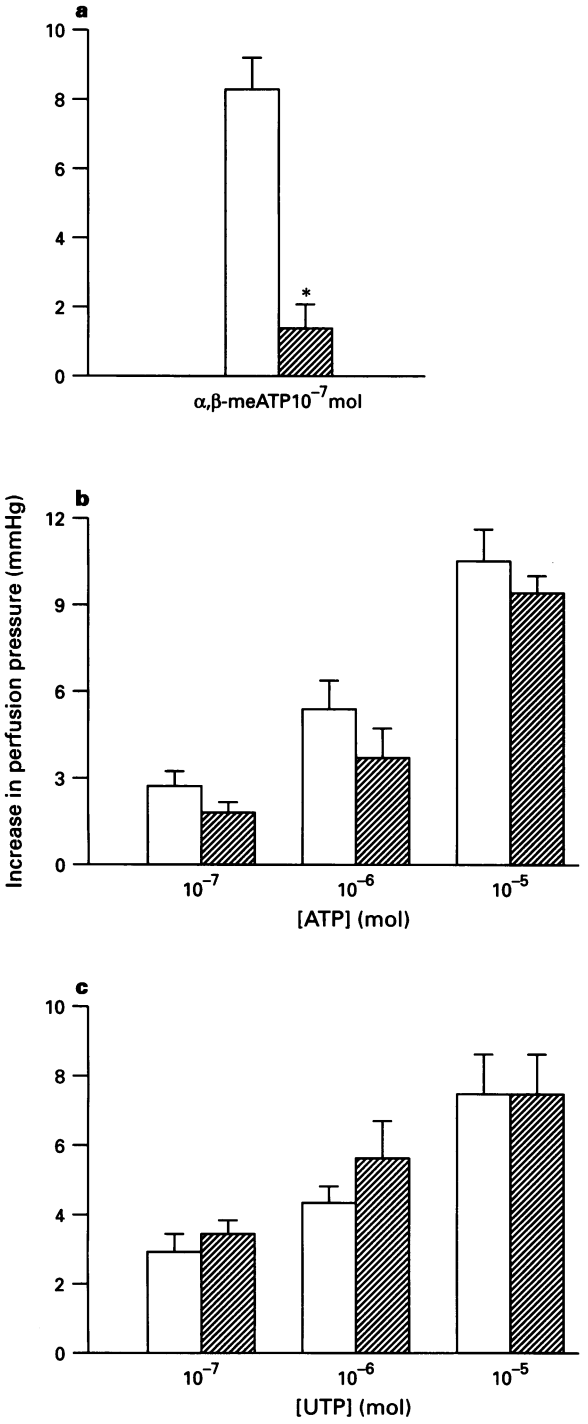

Figure 4 Vasoconstrictor responses to (a) α,β-methylene ATP (α,β-meATP), (b) ATP and (c) UTP before (open columns) and after (hatched columns) desensitization of P_{2x}-purinoceptors by continuous perfusion of the preparations with α,β-methylene ATP 10^{-6} M. Each column is the mean of 4 determinations. Vascular responses to α,β-methylene ATP were significantly reduced. No significant differences in vascular responses to ATP and UTP were observed before and after desensitization. * for at least $P<0.05$.

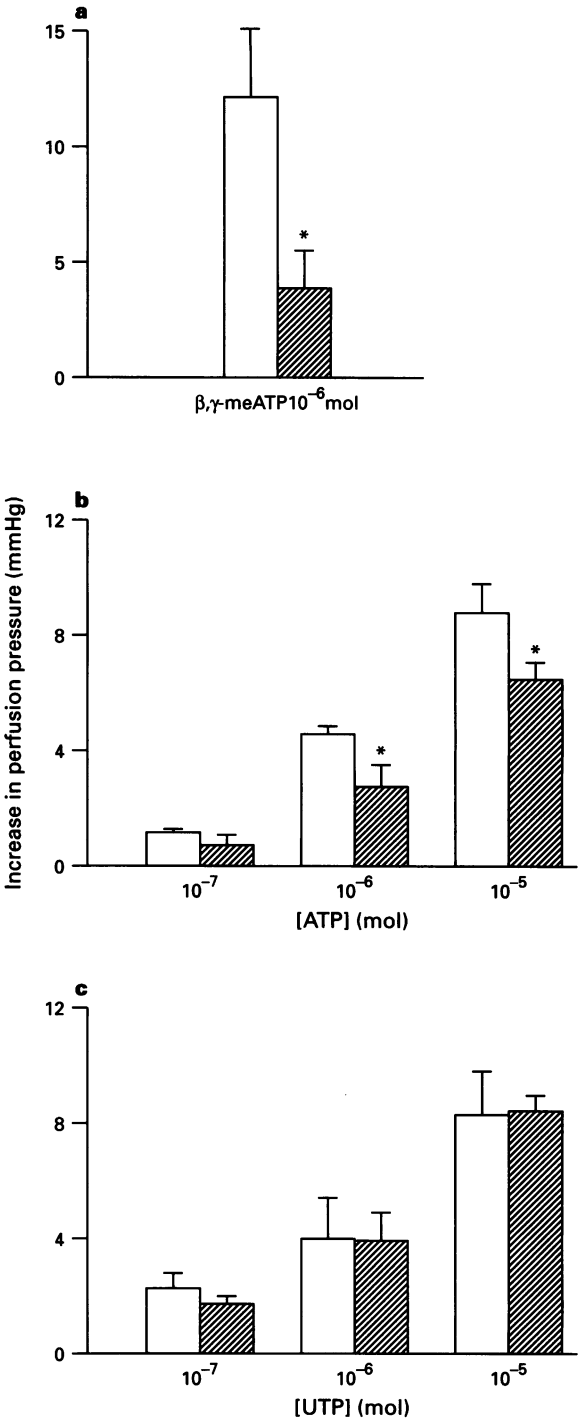

Figure 5 Vasoconstrictor responses to (a) β,γ-methylene ATP (β,γ-meATP), (b) ATP and (c) UTP in the absence (open columns) and in the presence (hatched columns) of suramin 10^{-4} M. Each column is the mean of 4–6 determinations. In the presence of suramin a significant reduction was observed in the vascular responses to β,γ-methylene ATP and to ATP 10^{-6} and 10^{-5} mol. * for at least $P<0.05$.

and 2-MeS ATP were tested, tachyphylaxis occurred, as described following activation of classical P_{2x}-purinoceptors (Burnstock & Kennedy, 1985; Burnstock, 1991; Abbracchio & Burnstock, 1994). Continuous perfusion with α,β-methylene ATP and consequent desensitization of P_{2x}-purinoceptors almost abolished vascular responses to α,β-methylene ATP itself. Furthermore, PPADS, which has been shown to antagonize responses mediated by P_{2x}-purinoceptors in several vascular preparations (Ziganshin *et al.*, 1994; Windscheif *et al.*, 1994), drastically reduced vascular responses to β,γ-methylene ATP, thus further supporting the presence of P_{2x}-purinoceptors in the rat pulmonary vascular bed. However, the vasoconstrictor effect of ATP was partially resistant to desensitization and blockade of P_{2x}-purinoceptors by α,β-methylene ATP and PPADS. A reduction of the vasoconstrictor effects of ATP was observed during continuous perfusion of the vascular tree with the desensitizing agent α,β-methylene ATP, but vascular responses to all doses of ATP tested were still detectable. Furthermore, PPADS when used at a concentration that drastically reduced P_{2x}-mediated responses to ATP in other

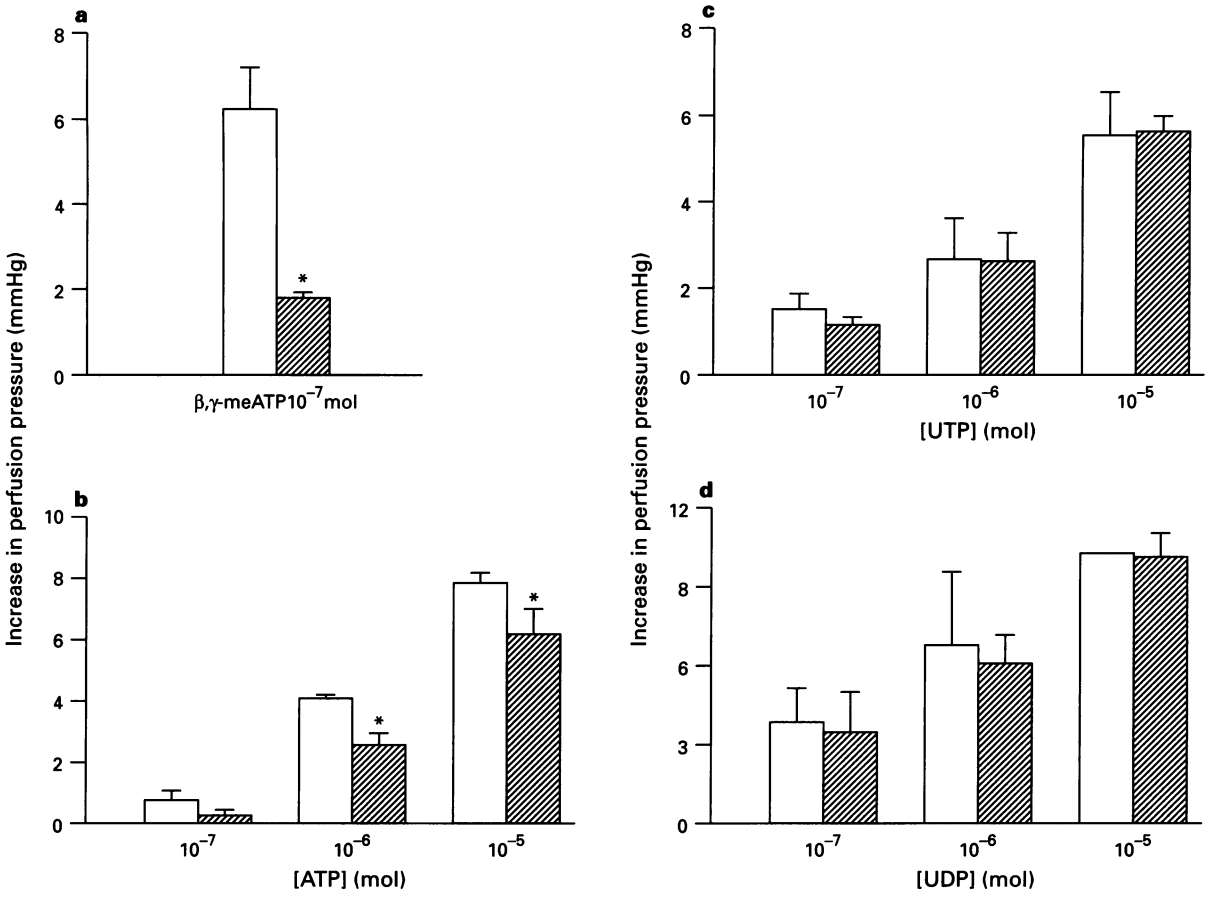

Figure 6 Vasoconstrictor responses to (a) β,γ-methylene ATP (β,γ-meATP), (b) ATP, (c) UTP and (d) UDP in the absence (open columns) and in the presence (hatched columns) of PPADS, 3×10^{-5} M. Each column is the mean of 4–5 determinations. A significant reduction was observed in the vascular responses to β,γ-methylene ATP and to ATP 10^{-6} and 10^{-5} mol. * for at least $P<0.05$.

vascular preparations (Ziganshin *et al.*, 1994; Windscheif *et al.*, 1994) as well as in this experimental model (see effect of PPADS on β,γ-methylene ATP-evoked vasoconstriction), reduced by only 30% ATP-mediated vasoconstriction. Similarly, suramin, used at a concentration that is fully active in antagonizing functional responses mediated by activation of P_{2x}-purinoceptors (Dunn & Blakeley, 1988; Leff *et al.*, 1990), only partially reduced vasoconstriction elicited by ATP. The effectiveness of suramin on P_{2x}-purinoceptors was proved in the present study by its drastic antagonism of β,γ-methylene ATP-evoked vasoconstriction. The inhibitory effect of suramin on the activity of ecto-ATPase (Crack *et al.*, 1994; Ziganshin *et al.*, 1995) might lead to misinterpretation of the results obtained when suramin was used as an antagonist of P_2-purinoceptors. However, in the present study, data obtained with suramin were qualitatively and quantitatively in line with the observations obtained with the P_2-purinoceptor antagonist PPADS, thus ruling out the possibility that weak or lack of antagonism of suramin on ATP and UTP respectively resulted from its inhibitory actions on the ectonucleotidases. P_1-purinoceptors of the A_1 subtype which mediate vasoconstrictor responses to adenosine have been demonstrated in the feline pulmonary circulation (Neely *et al.*, 1991; Lippton *et al.*, 1992). However, the lack of vasoconstrictor responses to exogenous adenosine observed in our preparation argues against the possibility that the effect of ATP and ADP is mediated by their breakdown product, adenosine, via activation of P_1-purinoceptors.

Overall these observations suggest that an additional purinoceptor distinct from the classical P_{2x} subtype mediates ATP-evoked vasoconstriction of the rat pulmonary vascular bed. It is tempting to suggest that ATP shares with UTP a suramin-resistant vasoconstrictor P_2-purinoceptor subtype. Failure of suramin to inhibit ATP-evoked contraction has been shown in the guinea-pig vas deferens, suggesting the presence of a suramin-insensitive ATP-activated contractile purinoceptor in this tissue. However, in the guinea-pig vas deferens UTP was only a weak contractile agonist, making it unlikely that UTP and ATP share a receptor site in this preparation (Bailey & Hourani, 1994). In the pulmonary vascular bed, ATP evoked vasoconstriction in a similar way to UTP in that both agonists were active in the same range of doses and produced similar vasoconstrictor effects. Furthermore, as for UTP-evoked vasoconstriction, ATP-evoked responses were partially resistant to desensitization of P_{2x}-purinoceptors and to antagonism by suramin and PPADS. Based on these observations we suggest the presence of a vasoconstrictor P_2-purinoceptor, distinct from the classical P_{2x}-subtype, where UTP and ATP are both agonists.

According to the most recent classification of P_2-purinoceptors (Abbracchio & Burnstock, 1994), the functional evidence from the present study would suggest including this novel purinoceptor subtype in the P2X-purinoceptor family as a subtype that would be the counterpart of the $P2Y_2$-purinoceptor (also called P_{2u}-purinoceptor) which mediates vasodilatation to ATP and UTP (O'Connor *et al.*, 1991; Barnard *et al.*, 1994; Abbracchio & Burnstock, 1994). However, further investigation is needed to explore the second messenger systems coupled to the ATP/UTP vasoconstrictor purinoceptor in the pulmonary circulation before including this novel subtype in the taxonomy of P_2-purinoceptors.

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