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Characterization of P2 receptors for purine and pyrimidine nucleotides in human placental cotyledons

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1 The aim of this study was to characterize P2 receptors in the arterial vascular bed of human perfused placental cotyledons. Vasoconstrictor responses to bolus injections of purine and pyrimidine nucleotides were tested at basal tone, and vasodilator responses in preparations with tone raised by perfusion with prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$; 10–50 nM).

2 At basal tone, bolus injections of the P2X-selective agonist α,β-methylene ATP (α,β-meATP; 0.5–500 nmol) elicited dose-dependent vasoconstriction. ATP (0.005–5 μmol) also elicited dose-dependent vasoconstriction, but was less potent than α,β-meATP. Vasoconstriction was also elicited by other nucleotides, but only at the highest dose tested (5 μmol): UTP > CTP = ITP ($n=6$). GTP and TTP did not cause vasoconstriction.

3 Constrictor responses to bolus injections of α,β-meATP were resistant to desensitization and were not significantly affected when carried out in the presence of 1 μM α,β-meATP added to the perfusate. However, responses to bolus injections of α,β-meATP were partially blocked by perfusion with 10 μM α,β-meATP. In contrast, responses to ATP and UTP were unaffected by 10 μM α,β-meATP. The P2X receptor antagonist pyridoxalphosphate-6-azophenyl-2′,4′-disulphonic acid (PPADS; 10 and 100 μM) had no significant effect on vasoconstriction mediated by α,β-meATP and ATP.

4 Removal of the endothelium had no significant effect on constrictor responses to α,β-meATP, ATP and UTP. Inhibition of nitric oxide (NO) synthesis with N^G-nitro-L-arginine methyl ester (L-NAME; 100 μM) had no significant effect on vasoconstriction to ATP and α,β-meATP.

5 In preparations with tone raised with $PGF_{2\alpha}$ (10–50 nM) vasodilatation was elicited by nucleotides with the following order of potency: 2MeSATP = ADP >> ATP > UTP > CTP = GTP = ITP = TTP. pD_2 values were: 2MeSATP, 10.03 ± 0.26 ($n=7$); ADP, 9.97 ± 0.40 ($n=5$); ATP, 8.89 ± 0.18 ($n=7$); UTP, 7.79 ± 0.35 ($n=7$). Maximal responses to 2MeSATP and ADP were similar and were approximately 40% greater than maximal responses to ATP and UTP.

6 Vasodilator responses to nucleotides were abolished by L-NAME (100 μM) and by removal of the endothelium.

7 In conclusion, contractile responses mediated by α,β-meATP and ATP in human placental smooth muscle are resistant to desensitization and insensitive to PPADS and, thus, show a dissimilar pharmacological profile to the classic smooth muscle $P2X_1$ receptor. There may be two subtypes of smooth muscle P2 receptor based on differential antagonism of α,β-meATP and ATP with α,β-meATP. A smooth muscle P2 receptor mediates vasoconstriction to UTP, and may indicate a further subtype. Endothelium-dependent, NO-dependent, vasodilatation to 2MeSATP and ADP may be mediated by $P2Y_1$ receptors, while endothelial $P2Y_2$ receptors are likely to mediate NO-dependent relaxation to ATP and UTP.

Keywords: Endothelium; perfusion; placenta; P2-receptors; pyrimidine receptors; PPADS

Introduction

Purine and pyrimidine nucleotides elicit diverse biological effects via distinct cell-surface P2 receptors (see Olsson & Pearson, 1990; Ralevic & Burnstock, 1991; Dubyak & El-Moatassim, 1993). These receptors have been divided into two broad groups: P2X receptors, which are intrinsic ion channels, and P2Y receptors, which are G protein-coupled receptors (Abbracchio & Burnstock, 1994; Fredholm *et al*., 1994). These families have been further subdivided according to the distinct structure of mammalian receptors. The P2X receptor family comprises subtypes $P2X_{1-6}$. From the vascular perspective $P2X_1$ (previously known as P_{2X}) is the most relevant, being found in smooth muscle. The P2Y family includes the receptors $P2Y_1$ and $P2Y_2$ (formerly known as P_{2Y} and P_{2U} respectively), the P_{2T} receptor (which has not yet been cloned), and a further five cloned subtypes ($P2Y_{3-7}$).

Pharmacological characterization of purine receptors relies on agonist selectivities and potency orders as there is a lack of selective antagonists at purine and uridine nucleotide-specific receptors. The stable analogue of adenosine 5′-triphosphate (ATP), α,β-methylene ATP (α,β-meATP), is a potent agonist and desensitizing agent at the smooth muscle $P2X_1$ receptor (Burnstock & Kennedy, 1985). Rapid desensitization is characteristic of $P2X_1$ and $P2X_3$ receptors, whereas all other subtypes of P2X receptor are resistant to desensitization (Evans & Surprenant, 1996). Pyridoxalphosphate-6-azophenyl-2′,4′-disulphonic acid (PPADS) is a selective antagonist at smooth muscle $P2X_1$-receptors in rabbit and rat blood vessels (Windscheif *et al*., 1994; Ziganshin *et al*., 1994), but can also block $P2Y_1$ responses (Ralevic & Burnstock, 1996). 2-MethylthioATP (2MeSATP) is a potent agonist at vascular $P2Y_1$ receptors and is selective for these versus $P2Y_2$ receptors. However, it is also active at $P2X_1$ receptors. The $P2Y_2$ receptor is characterized by the equipotency of ATP and uridine 5′-triphosphate (UTP). In addition to its effects on $P2Y_2$ receptors UTP also acts on uridine nucleotide-specific receptors or pyrimidinoceptors, which are not activated by purines (von Kugelgen *et al*., 1987; Saiag *et al*., 1990; 1992; Ralevic & Burnstock, 1991),

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and which may be equivalent to $P2Y_4$ and/or $P2Y_6$ receptors (Chang *et al*., 1995; Communi *et al*., 1996a,b).

This study characterizes the P2 receptors mediating responses to purine and pyrimidine nucleotides in the arterial bed of human placental cotyledons. Read and colleagues (1993) described P2X and P2Y receptors in human placental cotyledons, but did not examine the effects of pyrimidines. $P2Y_2$ receptors have been described in human trophoblastic cells where they were shown to mediate an increase in intracellular Ca^{2+} (Petit & Bélisle, 1995). A G protein-coupled P2 receptor, $P2Y_1$, has been cloned from human placenta, although its agonist activity profile was not determined (Leon *et al*., 1995). $P2Y_4$, cloned from human genomic DNA and showing strong expression in placenta, was activated by UTP = UDP > ATP = ADP (Communi *et al*., 1996a,b). A preliminary account of our results has been presented at the Purines 1996 Meeting (Ralevic *et al*., 1996).

Methods

Tissue source

Placentae were obtained from 16 healthy women (20–43 years) undergoing elective Caesarean section with epidural anaesthesia. None of the women had received any drug treatment known to have chronic vascular effects. Two or three placental cotyledons were isolated from each placenta. For each cotyledon the third or fourth chorionic plate branch of the umbilical artery was cannulated, the corresponding vein for outflow was severed and the cotyledon was flushed with 2 ml of heparine treated saline (10 iu ml^{-1}). Cotyledons were cut away from the surrounding tissue and removed for perfusion.

Perfusion of placental cotyledons

Preparations were placed in a humid chamber (custom made at University College London) and perfused at a constant flow rate of 3 ml min^{-1} with a peristaltic pump (model 7554-30, Cole-Parmer Instrument Co., Chicago, Illinois). The perfusate was Krebs solution (pH 7.4) 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, with bovine serum albumin (20 g l^{-1}), 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).

Experimental protocol

Preparations were allowed to equilibrate for 20 min before experimentation. Preparations not achieving a stable perfusion pressure of less than 50 mmHg were discarded. At basal tone responses of the cotyledons were tested to doses of guanosine 5′-triphosphate (GTP), cytidine 5′-triphosphate (CTP), inosine 5′-triphosphate (ITP), UTP, ATP and α,β-meATP. Response curves were reproducible and were repeated in the same preparations 30 min after one of the following treatments: (a) endothelium removal (see below), (b) addition of PPADS (10 and 100 μM) to the perfusate, (c) addition of α,β-meATP (10 and 100 μM) to the perfusate. In separate preparations tone was raised with prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$; 10–50 nM) by approximately 100 mmHg above baseline, and dose-response curves to purine and pyrimidine nucleotides were constructed. Nucleotides were reapplied after endothelium removal or 30 min after addition of N^G-nitro-L-arginine methyl ester (L-NAME, 100 μM) to the perfusate. Doses of purine or pyrimidine compounds were applied as 50 μl bolus injections via a rubber septum proximal to the preparation.

Endothelium removal

The endothelium was removed by perfusion of the cotyledons with 2 ml of a solution of sodium deoxycholate (2 mg ml^{-1} in saline) via the injection port, as described for the rat perfused mesenteric arterial bed (Ralevic & Burnstock, 1988).

Drugs used

The following drugs were obtained from Sigma: ATP (disodium salt), UTP (sodium salt), CTP, GTP, ITP, thymidine 5′-triphosphate (TTP), α,β-methylene ATP (lithium salt), adenosine 5′-diphosphate (ADP; sodium salt), sodium nitroprusside (SNP). 2-MethylthioATP (tetrasodium salt) was from Research Biochemicals Inc. PPADS was a generous gift from Dr G. Lambrecht (University of Frankfurt, Germany) and from Tocris Cookson (Bristol, UK.).

Data analyses

Vasodilator responses were measured as changes in perfusion pressure (mmHg) and evaluated as a percentage of the $PGF_{2\alpha}$-induced increase in tone above baseline. Results are presented as mean ± s.e.mean. pD_2 is defined as the negative log of the dose of agonist required to produce 50% of the maximal response. Where dose-response curves did not reach a maximum these were compared by analysis of variance with repeated measures, with *post hoc* analysis by Student's *t* test. Differences between means were determined by Student's *t* test and were considered significant when $P < 0.05$.

Results

Baseline characteristics

Basal perfusion pressure of the placental cotyledons was 32.4 ± 3.1 mmHg ($n = 36$).

Effects of purine and pyrimidine nucleotides at basal tone

At basal tone, α,β-meATP (0.5–500 nmol) and ATP (0.005–5 μmol) elicited dose-dependent vasoconstriction of the placental cotyledons, with α,β-meATP being the most potent constrictor ($n = 14$) (Figure 1). Responses to α,β-meATP and ATP were slow to reach a maximum (approximately 30–60 s) and were long-lasting, particularly at high doses, taking up to 10 min to return to baseline. The following nucleotides elicited vasoconstriction only at the highest dose tested (5 μmol): UTP > CTP = ITP ($n = 6$–8). GTP and TTP did not cause vasoconstriction ($n = 4$). Dose-response curves to the nucleotides were reproducible within the same preparation.

Effect of α,β-meATP on vasoconstrictor responses to nucleotides

Addition of 1 μM α,β-meATP produced an increase in basal perfusion pressure of 8.0 ± 4.1 mmHg ($n = 4$) which was maintained in the presence of α,β-meATP. Responses to doses of α,β-meATP were not significantly affected by α,β-meATP (1 μM) added to the perfusate (Figure 2a). α,β-meATP at 10 μM produced an increase in basal perfusion pressure of 11.6 ± 4.5 mmHg ($n = 5$) which was maintained, and did not return to baseline. At 10 μM α,β-meATP responses to the lowest doses of α,β-meATP were abolished (Figure 2a). In contrast, responses to ATP (Figure 2b) and UTP were unaffected by α,β-meATP (1 and 10 μM).

Effect of PPADS on vasoconstrictor responses to nucleotides

PPADS (100 μM) did not have any significant effect on the basal tone of the preparations. Vasoconstrictor responses to

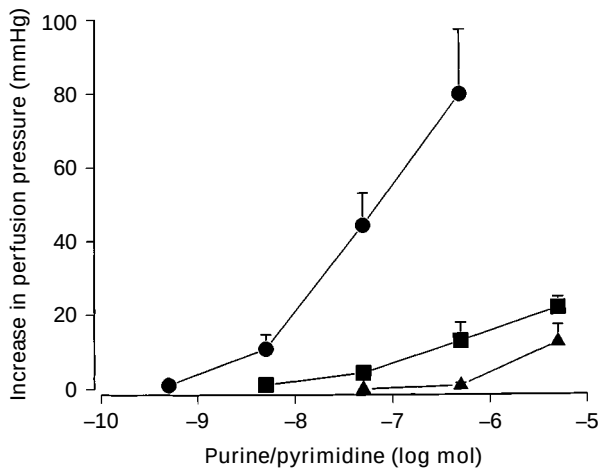

Figure 1 Dose-response curves to α,β-meATP (●, =14), ATP (■, =14) and UTP (▲, =12) in the arterial bed of human placenta. Vertical lines show s.e.mean.

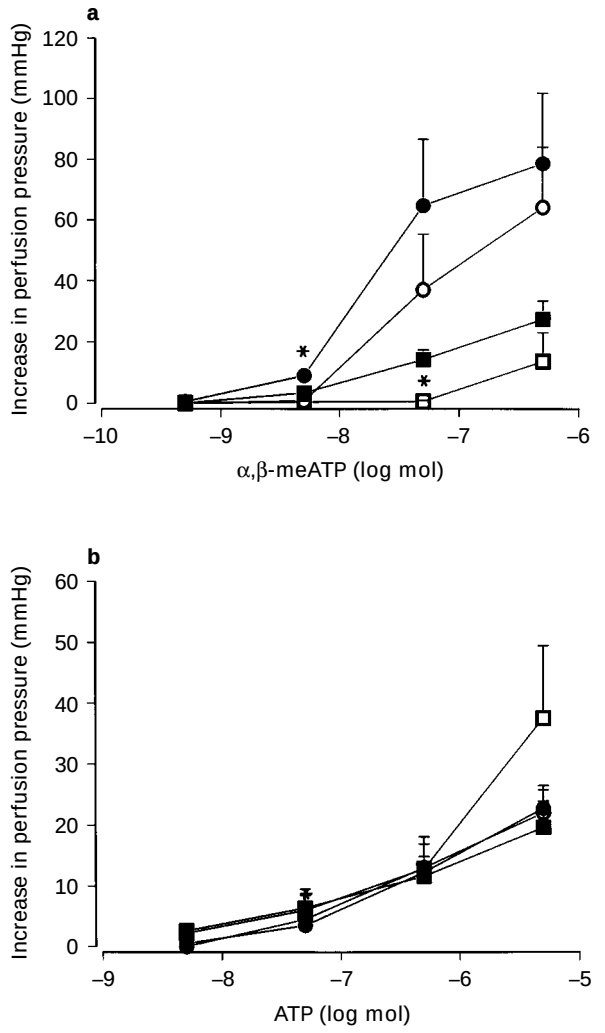

Figure 2 Effect of α,β-meATP (1 and 10 μM) on responses to (a) α,β-meATP, (b) ATP. (a) Dose-response curves to α,β-meATP in the absence (●) and presence (○) of 1 μM α,β-meATP ($n=4$), and in the absence (■) and presence (□) of 10 μM α,β-meATP ($n=5$). *Denotes significant difference between responses in controls and in the presence of 10 μM α,β-meATP ($P<0.05$). (b) Dose-response curves to ATP in the absence (●) and presence (○) of 1 μM α,β-meATP ($n=4$), and in the absence (■) and presence (□) of 10 μM α,β-meATP ($n=5$). Reproducibility of responses allowed response curves in the absence and presence of α,β-meATP to be generated consecutively in the same preparations. Vertical lines show s.e.mean.

UTP, ATP and α,β-meATP were not significantly affected by PPADS (10 and 100 μM) ($n=4-6$) (data not illustrated).

Effect of endothelium removal and L-NAME on vasoconstrictor responses to nucleotides

The NO synthase inhibitor L-NAME (100 μM) increased basal tone by 9.0 ± 3.7 mmHg in four out of five preparations. Responses to α,β-meATP, ATP and UTP in the presence of L-NAME were not significantly different from controls (Figure 3a). Removal of the endothelium produced a significant increase in basal tone of the preparations of 14.4 ± 4.6 mmHg ($n=5$). Removal of the endothelium did not significantly affect responses to α,β-meATP and ATP, although there appeared to be a trend towards augmentation (Figure 3b).

Effects of purine and pyrimidine nucleotides at raised tone

Addition of $PGF_{2\alpha}$ ($32\pm$nM) raised the tone of the preparations by 87.5 ± 11.1 mmHg ($n=10$) above baseline. Dose-dependent vasodilatation was elicited by nucleotides with the following order of potency: 2MeSATP = ADP >> ATP > UTP > CTP = GTP = ITP = TTP ($n=5$) (Figure 4). Maximal

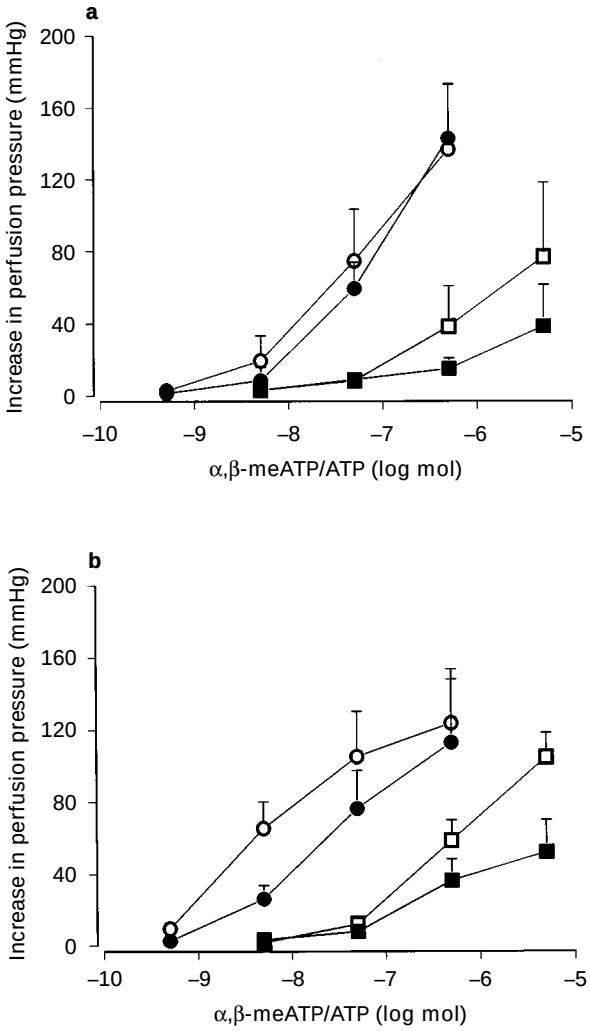

Figure 3 (a) Responses to α,β-meATP (circles, $n=5$) and ATP (squares, $n=5$) in the absence (solid symbols) and presence (open symbols) of N^G-nitro-L-arginine methyl ester (L-NAME; 100 μM). There was no significant difference between the mean responses. (b) Responses to α,β-meATP (circles, $n=5$) and ATP (squares, $n=5$) in the presence (solid symbols) and absence (open symbols) of endothelium. There was no significant difference between the mean responses. Vertical lines show s.e.mean.

relaxations were not significantly different between 2MeSATP, 55.75±5.71 ($n=7$) and ADP, 46.76±9.00 ($n=5$), or between ATP, 31.74±6.56 ($n=7$) and UTP 30.38±4.06 ($n=7$). pD_2 values for 2MeSATP and ADP were not significantly different, being 10.03±0.26 ($n=7$) and 9.97±0.40 ($n=5$), respectively. The pD_2 value for ATP, 8.89±0.18 ($n=7$), was significantly greater than that for UTP, 8.05±0.28 ($n=7$) ($P<0.05$).

Effect of endothelium removal and L-NAME on vasodilator responses to nucleotides

Vasodilator responses to purine and pyrimidine nucleotides were abolished following removal of the endothelium with sodium deoxycholate ($n=3$; Figure 5). Vasodilator responses to nucleotides were also abolished by L-NAME (100 μM; $n=4$) (Figure 6). After each of these treatments the ability of the smooth muscle to relax was unimpaired, as evidenced by responses to the direct smooth muscle relaxing agent SNP (0.005–50 nmol), which were augmented (Figures 5 and 6).

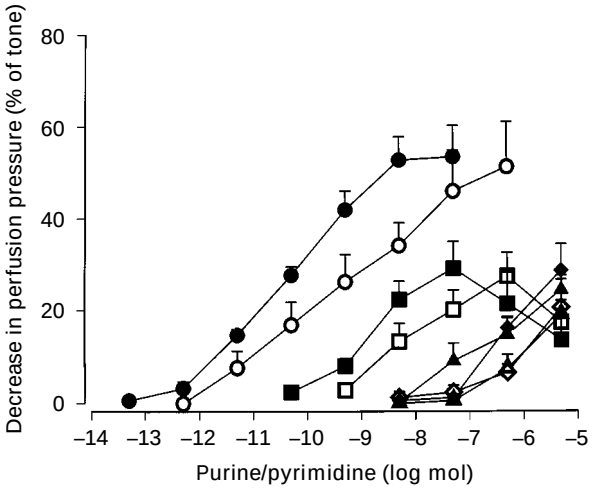

Figure 4 Vasodilator dose-response curves to purine and pyrimidine nucleotides in human placental cotyledons with tone raised by $PGF_{2\alpha}$ (10–50 nM). (●) 2MeSATP ($n=7$); (○) ADP ($n=5$); (■) ATP ($n=7$); (□) UTP ($n=7$); (◆) GTP ($n=5$); (▲) ITP ($=5$); (◇) CTP ($n=6$); (△) TTP ($n=5$). Vertical lines show s.e.mean.

Discussion

Several factors currently contribute to obscure the conclusions which can be drawn from pharmacological approaches to P_2 purine receptor characterization. These include: a lack of subtype-selective P2 receptor agonists and antagonists, the fact that most commercially available nucleotides are not pure and the fact that most nucleotides are extensively modified by interconversion or degradation into products with widely different pharmacological activities (including lack of activity). Further complications are introduced when bolus injections are used because of spatio-temporal gradients of nucleotides. These considerations must be taken into account when drawing conclusions based on rank orders of agonist potencies, which still play an important role in the characterization of P2 receptors. The division of P2X receptors into desensitizing and non-desensitizing phenotypes, a property which is independent of the above-mentioned problems, presents an invaluable handle on P2X receptor characterization.

The present study confirms and extends previous findings regarding characterization of P2 receptors in the human placental vasculature. Dose-dependent vasoconstriction to α,β-meATP indicates that P2X receptors are present in the arterial vasculature of the human placental circulation, consistent with the findings of Read and coworkers (1993). A major novel finding is that responses to both α,β-meATP and ATP were resistant to desensitization, as indicated by the reproducibility of the responses and the fact that contractions produced during continuous perfusion with α,β-meATP were sustained. This was unexpected since rapid desensitization is a characteristic of vascular smooth muscle $P2X_1$ receptors (Burnstock & Kennedy, 1985). In addition, responses to bolus injection of α,β-meATP were not blocked by continuous perfusion with 1 μM α,β-meATP. Lack of desensitization of responses to α,β-meATP has also been shown in human placental chorionic surface arteries (Dobronyi *et al*., 1997).

Block of responses to bolus injections of α,β-meATP, but not to ATP, by perfusion with a relatively high concentration (10 μM) of α,β-meATP suggests that there may be at least two different subtypes of smooth muscle P2 receptor in human placenta. The partial block of responses to bolus injections of α,β-meATP during continuous perfusion with 10 μM α,β-meATP may be due to competition for P2X receptors rather than desensitization. The greater potency of α,β-meATP compared to ATP may be due at least in part to its greater resistance to degradation by ectonucleotidases (Crack *et al*., 1994).

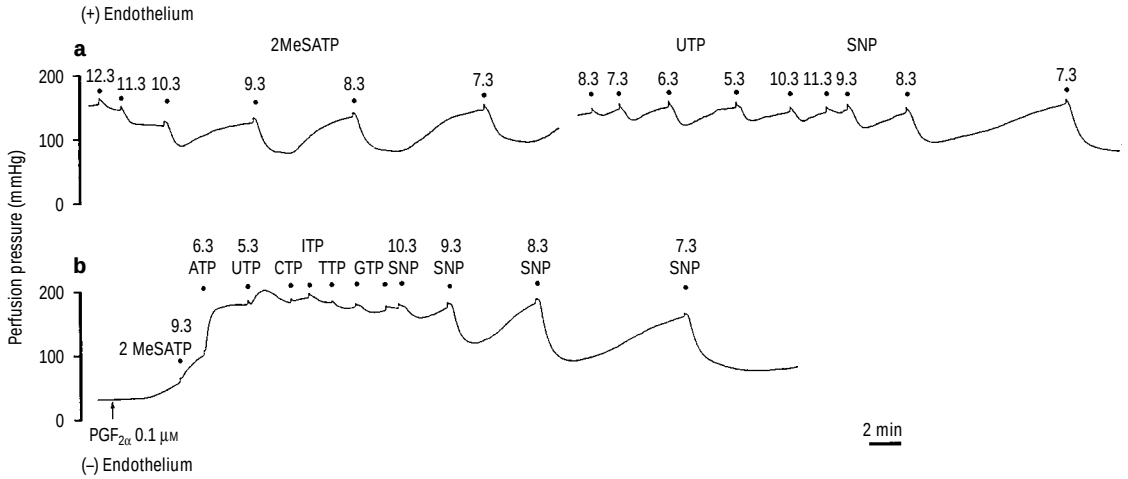

Figure 5 Representative trace showing vasodilator responses (decrease in perfusion pressure, mmHg) of a single human placental cotyledon to purine and pyrimidine nucleotides and to sodium nitroprusside (SNP; 10.3–7.3) before (a) and after (b) removal of the endothelium. Doses are given as −log mol. In (b), UTP, CTP, ITP and TTP were all applied as doses of 5.3 (−log mol; equivalent to 5 μmol). GTP was applied at 6.3 and 5.3 (−log mol). Tone of the preparations was raised with 20 nM $PGF_{2\alpha}$ in the presence of endothelium (not shown; basal perfusion pressure 27 mmHg) and 100 nM $PGF_{2\alpha}$ in the absence of endothelium (basal perfusion pressure 34 mmHg).

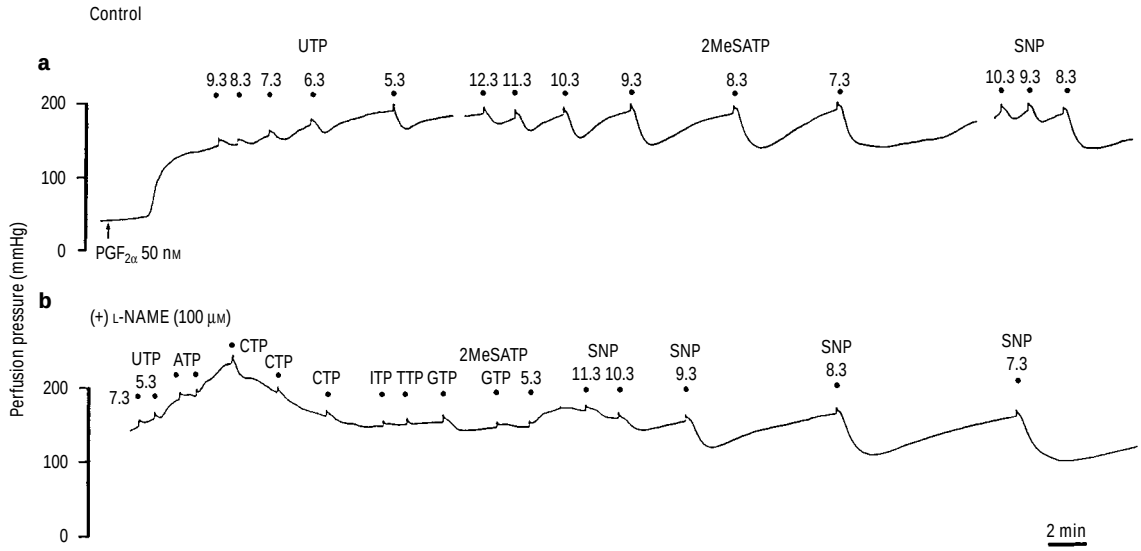

Figure 6 Representative trace showing vasodilator responses (decrease in perfusion pressure, mmHg) of a single human placental cotyledon to purine and pyrimidine nucleotides and to sodium nitroprusside (SNP) in the absence (a) and presence (b) of N^G-nitro-L-arginine methyl ester (L-NAME; 100 μM). Doses are given as $-$log mol. In (b), UTP, ITP and TTP were all applied as doses of 5.3 ($-$log mol; equivalent to 5 μmol). ATP was applied at doses of 6.3 and 5.3; CTP at 7.3, 6.3 and 5.3; GTP at 6.3 and 5.3. Tone of the preparations was raised with 50 nM $PGF_{2\alpha}$ in the absence of L-NAME (basal perfusion pressure 42 mmHg) and 30 nM $PGF_{2\alpha}$ in the presence of L-NAME (tone did not return fully to baseline during washout).

Contractile responses to UTP as well as ATP were unaffected by α,β-meATP (10 μM), raising the possibility that these nucleotides act at a common receptor. However, it is also possible that ATP and UTP act at distinct receptors, with uridine nucleotide-specific receptors mediating the responses to UTP (von Kügelgen *et al*., 1987; Saiag *et al*., 1990, 1992; Ralevic & Burnstock, 1991). Differentiation between these two possibilities requires the discovery of selective antagonists.

The failure of PPADS at 100 μM (a concentration 100 fold greater than that obtained for its IC_{50} value at P2X receptors in other tissues) to block responses to α,β-meATP and ATP is not consistent with the expected pharmacological profile of $P2X_1$ receptors, the only P2X subtype shown to date to be expressed in smooth muscle. Insensitivity to PPADS has been described for $P2X_4$ and $P2X_6$ receptors; all the other subtypes are antagonized by PPADS (Buell *et al*., 1996; Collo *et al*., 1996). Resistance to desensitization is characteristic of $P2X_2$, $P2X_4$, $P2X_5$ and $P2X_6$ receptors and the heteropolymer $P2X_2/P2X_3$ (Collo *et al*., 1996; Evans & Surprenant, 1996; North, 1996; Garcia-Guzman *et al*., 1997). Apparently more rapid desensitization of the human $P2X_4$ receptor (Garcia-Guzman *et al*., 1997) compared to the rat $P2X_4$ receptor (Buell *et al*., 1996) suggests that there may be species differences within P2X receptor subtypes which may also be the case with the other P2X receptors. With the exception of $P2X_2/P2X_3$, these receptors are relatively insensitive to α,β-meATP. Thus, placental smooth muscle P_2 receptors are dissimilar to all of the above recombinant receptors and it may be that heteropolymerization accounts for the atypical pharmacological profile.

Removal of the endothelium did not impair constrictor responses to UTP, ATP and α,β-meATP, indicating that the P_2 receptors are present on the vascular smooth muscle. L-NAME and endothelium removal significantly increased the tone of the preparations, suggesting basal release of NO from the endothelium. L-NAME did not significantly affect responses to ATP and α,β-meATP, suggesting that NO does not modulate contractile responses of human placental cotyledons under the conditions of the present study.

At raised tone, responses to the nucleotides were abolished following removal of the endothelium and were blocked by L-NAME, indicating that vasodilation is mediated by endothelial NO. Responses to the NO donor SNP have been found to be augmented after these treatments in other preparations (Shirasaki & Su, 1985; Ralevic *et al*., 1991).

In the present study, vasodilator responses to 2MeSATP and ADP were similar both in their maximum effect and potency, whereas responses to ATP were less potent and efficacious, differing from the typical agonist profile of vascular $P2Y_1$ receptors (2MeSATP > ATP > ADP). Responses to UTP were similar to those of ATP, consistent with the presence of a distinct receptor, possibly a $P2Y_2$ receptor. An ADP-specific P2Y receptor (activated by 2MeSATP but weakly or not at all by ATP) coexisting with the $P2Y_2$ receptor has also been described in osteoblastic cells (Reimer & Dixon, 1992), hepatocytes (Dixon *et al*., 1995), rat brain microvascular endothelial cells (Frelin *et al*., 1993; Feolde *et al*., 1995; Webb *et al*., 1996) and on the endothelium of the rat mesenteric arterial bed (Ralevic & Burnstock, 1996). Molecular evidence suggests that this is a $P2Y_1$ receptor rather than a further P2Y receptor subtype (Webb *et al*., 1996).

$P2Y_4$, a receptor strongly expressed in human placenta (Communi *et al*., 1996) had a different agonist profile to the receptors characterized in the present study. It is possible that the placental $P2Y_4$ receptor is not a vascular receptor.

The suggestion of distinct receptors for ADP and ATP/UTP may be significant with respect to the physiological sources of these nucleotides. The placental vasculature is not innervated, excluding the possibility that ATP is released as a cotransmitter from perivascular nerves. Thus, the role of endothelial cells, trophoblasts, platelets and other formed elements of blood as sources of these nucleotides assumes greater significance. Vasomotor control of foetoplacental blood flow is of particular clinical interest since impaired umbilical artery perfusion is associated with a high risk of perinatal death (Alfirevic & Neilson, 1995). Delineating the role of purines in this vascular bed may lead to possible therapeutic methods of improving placental blood flow.

Despite the obstacles briefly discussed above we want to draw several tentative conclusions from the present study. There are at least two types of P2 receptors on the vascular smooth muscle of human term placenta, neither of which is similar to the classic smooth muscle $P2X_1$ receptor, based on resistance to desensitization and insensitivity to PPADS. One of these is a P2X receptor which mediates vasoconstriction to α,β-meATP, and the other a distinct P2 receptor which mediates constriction to ATP. There may be a third subtype which mediates constriction to UTP. On the endothelium, $P2Y_2$ re-

ceptors mediate vasodilatation to ATP and UTP via NO. 2MeSATP and ADP also mediate endothelium-dependent, NO-dependent relaxation, by a distinct P2Y receptor.

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