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Vascular actions of purines in the foetal circulation of the human placenta

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1 The vasoactive effects of adenosine triphosphate (ATP), adenosine and other purines in the foetal circulation of the human placenta were examined. Single lobules of the placenta were bilaterally perfused *in vitro* with Krebs buffer (maternal and foetal sides 5 ml min^{-1} each, 95% O_2:5% CO_2, 37°C). Changes in foetal vascular tone were assessed by recording perfusion pressure during constant infusion of each purine. To allow recording of the vasodilator effects, submaximal vasoconstriction was induced by concomitant infusion of prostaglandin $F_{2\alpha}$ (0.7–2.0 μmol l^{-1}).

2 ATP (1.0–100 μmol l^{-1}) usually caused concentration-dependent reductions in perfusion pressure. However, biphasic with initial transient increases, or only increases in pressure were sometimes observed. Falls in pressure caused by ATP were significantly reduced by addition to the perfusate of N^G-nitro-L-arginine (L-NOARG) (100 μmol l^{-1}) but not N^G-nitro-D-arginine (D-NOARG) (100 μmol l^{-1}). They were not influenced by addition of indomethacin (10 μmol l^{-1}) or L-arginine (100 μmol l^{-1}).

3 Adenosine (0.01–1.0 mmol l^{-1}) consistently caused concentration-dependent reductions in perfusion pressure, this effect not being influenced by indomethacin. L-NOARG, but not D-NOARG, reduced the potency of adenosine approximately three fold. L-Arginine, but not D-arginine enhanced its potency by a similar amount.

4 2-Methylthio-ATP, a selective P_{2y} agonist was approximately 50 times more potent than ATP as a vasodilator agent, always causing decreases in perfusion pressure.

5 β-γ-Methylene ATP, a selective P_{2x} agonist, was approximately 100 times more potent than ATP as a vasoconstrictor, but only caused transient increases in perfusion pressure.

6 The rank order of vasodilator potencies of a selection of adenosine receptor agonists was, 2-chloroadenosine>>5-(N-cyclopropyl)-carboxamidoadenosine, >5-N-ethylcarboxamidoadenosine, >2-chloro-N^6-cyclopentyladenosine, >CGS-21680>N^6-cyclohexyladenosine = adenosine. Vasodilatation due to adenosine was inhibited by the P_1-A_2 receptor antagonist 3,7-dimethyl-1-propargylxanthine (DMPX).

7 These results suggest that ATP may cause an endothelium-dependent vasodilatation in the foetal vessels of the human placenta via activation of a P_{2y} receptor linked to the formation of nitric oxide (NO). Vasodilatation caused by ATP may mask an accompanying vasoconstrictor effect mediated, via a P_{2x} receptor, in the villous vascular smooth muscle. Adenosine acting on P_1-A_2 receptors, which are also present in the foetal vasculature, may require synergistic interaction with NO to achieve a maximal vasodilator response.

Keywords: Purinoceptors; ATP; adenosine; placenta; villous vessels; nitric oxide; endothelium

Introduction

The cardiovascular effects of adenine and adenosine nucleotides were first reported by Drury & Szent-Györgyi (1929). Subsequently adenosine was reported to produce dilatation in all vascular beds studied except the kidney and placenta (Kenakin & Pike, 1987; Olsson & Pearson, 1990). In the foetal circulation of the human placenta, adenosine has been associated with vasoconstriction during hypoxia although the exact mechanism causing the vasoconstriction remains to be determined (Kitagawa *et al.*, 1987). Large amounts of adenosine are released into the foetal effluent from the placenta perfused with Krebs solution *in vitro* in response to hypoxia, with concomitant foetal vasoconstriction. It has been suggested that adenosine may participate in this response because it can be blocked by the adenosine antagonist, theophylline (Howard *et al.*, 1987; Slegel *et al.*, 1988). Importantly, these studies were performed using placentae with basal foetal vascular tone, which is normally very low (Boura & Walters, 1991). Detection of any vasodilator responses to adenosine under these conditions would be difficult.

Activation of endothelial P_{2y} purinoceptors by ATP results in endothelium-dependent dilatation of the majority of blood vessels (Kennedy & Burnstock, 1985; Kennedy *et al.*, 1985; Houston *et al.*, 1987; Olsson & Pearson, 1990; Mathie *et al.*,1991; Ralevic *et al.*, 1991). The vasodilatation may be mediated through release of either prostacyclin (PGI_2) or NO or perhaps a combination of the two (Carter *et al.*, 1988; Mathie *et al.*, 1991; Martin *et al.*, 1991). However, ATP-induced vasodilatation is not endothelium-dependent in all vessels (Kennedy & Burnstock, 1985; Mathieson & Burnstock, 1985). The mechanism of ATP-induced vasodilatation is variable and appears to be vessel- and species-specific.

Hence the present study was undertaken to examine the vasoactive effects of adenosine and ATP in the submaximally preconstricted foetoplacental circulation *in vitro*, a situation which may reveal possible vasodilator as well as vasoconstrictor effects. A high oxygen tension was also maintained in the perfusing fluid in order to inhibit placental release of adenyl purines which might have influenced responses to the exogenously administered substances. Efforts were also made to characterize the types of purinoceptor present in the foetoplacental vasculature and to determine whether any vasodilator effects of ATP or adenosine were dependent on formation of prostaglandins or NO.

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Methods

Collection of placentae

Placentae were obtained within 20 min of vaginal or Caesarean delivery from women (aged 17–42 years) who had normal uncomplicated pregnancies. Some, but not all, of the patients had received one or more of the following drugs during labour, oxytocin (2 iu over 6–8 h), pethidine hydrochloride (100 mg, i.m.), promethazine maleate (12.5–25.0 mg, i.m.) or inhaled 70% N_2O and 30% O_2. These drugs have no apparent effects on responses of the foetal vascular tissues under the conditions used (Mak *et al.*, 1984). Placentae from women with blood pressures of $>140/90$ mmHg or who had experienced an increase of >20 mmHg diastolic pressure during pregnancy were not used, nor were those from women who smoked more than 10 cigarettes per day.

Placental lobule perfusion

Placental lobules were perfused by a technique originally described by Penfold *et al.* (1981) as modified by Mak *et al.* (1984). A suitable paired artery and vein, typically third or fourth branches of the chorionic plate vessels, to a peripheral placental lobule were chosen. The artery was cannulated with plastic tubing and the vein cut at a convenient point to allow blood and perfusate to escape. The cannula, which was inserted to the point where the artery disappeared below the surface of the chorionic plate, was connected to a Gilson Minipuls 3 (Gilson Medical Electronics, Villiers-le Bel, France) peristalic pump and the lobule perfused with Krebs solution containing (mmol l^{-1}): NaCl 97.0, $NaHCO_3$ 24.0, KCl 3.0, KH_2PO_4 1.2, $CaCl_2$ 1.89, $MgSO_4$ 1.0, D-glucose 5.5, pH 7.3) maintained at 37°C and gassed with 95% O_2:5% CO_2. The oxygen tension of the perfusing fluid was 400–500 mmHg. Each lobule was initially perfused at 1 ml min^{-1} for 5 min and thereafter with a constant flow rate of 5 ml min^{-1}. A venous cannula was inserted only after all visible blood had been flushed out of the lobule. The maternal side of the lobule was also perfused with Krebs solution under similar conditions to those used for perfusion of the foetal circulation. Perfusate was delivered into the maternal side of the placenta by two cannulae inserted into the spiral arterioles of the basal plate. Placentae were bathed in Krebs solution at 37°C.

Experimental design

Changes in foetoplacental vascular resistance were monitored by recording the inflow pressure to the lobule, with a Gould Statham P23D transducer (Cleveland, Ohio, U.S.A.), connected via a T-junction to the foetal arterial perfusion line. Signal conditioning and amplification was performed by a J-RAK PA-2 module (Melbourne, Australia) and displayed on a Kontron 330 flat-bed recorder (Eching, Germany). Inflow pressure at the start of perfusion was typically 80–100 mmHg, declining to a stable baseline pressure between 20–40 mmHg within a period of 1 h. Drug infusions were not started until a stable baseline pressure was achieved. Preparations having baseline pressures greater than 60 mmHg were discarded. Vasoconstriction was induced in the foetal circulation with $PGF_{2\alpha}$ (0.7–2.0 μmol l^{-1}) infused into the arterial cannula with a Gilson Minipuls 3 peristaltic pump. The concentration was adjusted so that a stable pressure of 100–120 mmHg was maintained prior to establishing concentration-responses curves to the purines.

Effects of adenosine and ATP

Adenosine, ATP or their analogues were infused into the foetal circulation in a logarithmic series of gradually increasing concentrations, with a third Gilson Minipuls 3 pump, at flow rates between 5–250 μl min^{-1}. Starting with a concentration causing a threshold effect, the concentration was increased by approximately 0.5 $\log_{10}$ intervals, after each effect obtained became constant. The highest concentration used was either that causing a maximal response or that which could be achieved due to the constraint of lack of sufficient solubility in the solution being infused. Concentration-response curves were obtained to both adenosine and ATP in the same placenta, the order of administration of the purines being alternated between successive experiments. Responses were expressed as the percentage change in the $PGF_{2\alpha}$-induced pressure (maximum pressure minus basal pressure) obtained prior to the start of infusion of the purine.

Effects of other purines

Further experiments also using cross-over designs were conducted to compare either adenosine or ATP with other various agonists. The order of administration of agonist and endogenous ligand was alternated in successive experiments. Cumulative concentration-response curves were obtained with the exception of that for the constrictor effects of ATP and β-γ-methylene-ATP which were transient. In these instances, an increased concentration of each agonist was administered only after a stable baseline perfusion pressure had been re-established. When various agonists were dissolved in any vehicle other than distilled water or Krebs solution, the vehicle was examined independently for possible vascular effects.

Indirect effects of adenosine and ATP

The contribution of prostanoids or NO to the vasodilator effects of adenosine and ATP were examined by use of the cyclo-oxygenase inhibitor indomethacin (10 μmol l^{-1}) and the NO synthase inhibitor N^G-nitro-L-arginine (L-NOARG 100 μmol l^{-1}) (Mulsch & Busse, 1990). For the latter series of experiments N^G-nitro-D-arginine (D-NOARG) was used in the same concentration, for control purposes, in further preparations. L-Arginine (100 μmol l^{-1}), the precursor of NO (Palmer *et al.*, 1988) was also used in attempts to enhance any NO-mediated vasoactivity shown by either adenosine or ATP and compared with the effect of D-arginine as a control A crossover design was used to study their effects. Concentration-response curves in the presence of L-arginine and D-arginine were obtained either before or after their respective control curves had been obtained. Indomethacin, L-NOARG, D-NOARG, L-arginine and D-arginine were administered in the perfusion fluid and delivered to both foetal and maternal circulations for 60 min before establishment of concentration-response curves to the purine being studied. Concentration-response curves in the presence of indomethacin and L-NOARG were obtained after control curves.

Effects of antagonists at P_1-A_1 and P_1-A_2 receptors on response to adenosine

To study the effects of either P_1-A_1 or P_1-A_2 receptor blockade on vasodilator responses to adenosine, XAC (Rossi *et al.*, 1987) and DMPX (Seale *et al.*, 1988) were used respectively. A control concentration-response curve was determined in groups of 3–4 placentae, each concentration being infused until the effect became constant; 30 min after commencing infusion of the antagonist (80 μmol l^{-1}) a second concentration-response curve to adenosine was obtained. The curves, in the absence and presence of antagonist, were compared. Comparisons were also made with a series of control curves to adenosine obtained over 0.5–4.0 h in the absence of antagonist on both occasions.

Drugs and chemicals

Chemicals used in the Krebs solution were of analytical grade (Analar BDH, Australia). Adenosine, ATP (Boeh-

ringer Mannheim, Germany). β-γ-methylene ATP, L-arginine, D-arginine, N^G-nitro-L-arginine (L-NOARG), N^G-nitro-D-arginine (D-NOARG), (Sigma, St Louis, U.S.A.), 2-methylthio-ATP, N^6-cyclohexyladenosine (CHA), 3,7-dimethyl-1-propargylxanthine (DMPX), 8-[4-[[[[(2-aminoethyl)amino]carbonyl]methyl]oxy]phenyl]-1,3-dipropylxanthine (XAC) (Research Biochemicals, Natick, U.S.A), CGS-21680 (2-[4-(-2-carboxyethyl)-phenethylamino]-5'-N-ethylcarboxamido adenosine HCl) (Ciba Geigy, Pharmaceuticals Division, Summit, New Jersey, U.S.A.), 2-chloroadenosine (2-CADO) and 2-chloro-N^6-cyclopentyladenosine (2-CCPA) (Research Biochemicals) were dissolved in distilled water. 5'-N-ethylcarboxamidoadenosine (5-NECA) and 5'-(N-cyclopropyl)-carboxamidoadenosine (5-CPCA) (Research Biochemicals) were dissolved in 0.02 mol l^{-1} HCl. Indomethacin (Sigma, St Louis, U.S.A.) was dissolved in 5% $NaHCO_3$. Prostaglandin $F_{2\alpha}$ was supplied as its trometamol salt (Dinoprost, Upjohn, Sydney, Australia) at a concentration of 5 mg ml^{-1} in sterile distilled water and diluted as required in distilled water.

Statistical analysis

All values are expressed as means (± s.e.mean) unless otherwise stated. Linear regression analysis was performed on all concentration-response cruves using Minitab (Pasadena, U.S.A.). Differences in the linear portions of the curves were compared and tested for significant displacement and parallelism, as described by Bowman & Rand (1980). Unless otherwise stated a probability value of <0.05 was considered significant. Non-parallel curves were tested for differences by two-way analysis of variance. Pairwise comparisons were made by use of Student's paired t test where indicated.

Results

Effects of adenosine and ATP

Both ATP and adenosine caused concentration-dependent reductions in perfusion pressure when infused into isolated placental lobules. These effects developed slowly, maximal responses to each being seen 20–30 min after the start of infusion. Adenosine was significantly less potent in reducing perfusion pressure than ATP (Figure 1). It consistently reduced the perfusion pressure in every placenta examined ($n = 48$). In contrast, ATP caused variable effects. Usually ATP produced concentration-dependent falls in perfusion pressure ($n = 16$). In others ($n = 5$) only concentration-dependent transient increases in pressure occurred. Additionally, biphasic effects due to ATP were observed in further preparations, with an initial rise in pressure being followed by a fall ($n = 8$). Reductions in perfusion pressure at low concentrations but increases in response to higher concentrations also occurred ($n = 5$). For comparison of the relative vasodilator potencies of ATP and adenosine (Figure 1) data were used only from those placentae responding solely by vasodilatation to ATP.

Control experiments were performed to determine whether responses to ATP or adenosine changed with time, because of the slow responses to each concentration of the purines. Following establishment of an initial concentration-response curve to either ATP or adenosine, subsequent concentration-response curves were obtained over a period of 0.5–4.0 h. No statistically significant differences from their respective controls were observed in responses to either adenosine or ATP following these time intervals (data not shown).

Effects of other purines

The selective P_{2y} receptor agonist, 2-methylthio-ATP, caused concentration-dependent reductions in perfusion pressure (Figure 2) and was significantly more potent than ATP. A comparison of the curve for 2-methylthio-ATP with that for ATP showed that 2-methylthio-ATP was approximately 50 times more potent (Table 1a). Unlike ATP, 2-methylthio-ATP caused only reductions in perfusion pressure in all preparations ($n = 6$). However, the differences found between the vasodilator potencies of ATP and the other purines could fail to reflect accurately their true relative activities. The data used to assess the potency of ATP were obtained from placentae responding only by vasodilatation. Nevertheless its vasoconstrictor effect, seen more prominently in other preparations, could have concomitantly opposed to variable extents the vasodilator responses recorded, thus reducing its apparent vasodilator potency.

The selective P_{2x} receptor agonist, β-γ-methylene ATP, exclusively produced concentration-dependent increases in perfusion pressure (Figure 2). No vasodilator responses to this agonist were observed ($n = 6$). The increases in perfusion pressure caused by β-γ-methylene ATP or ATP were transient and showed fade, maximum responses being achieved approximately 5 min after starting infusion. Compared to ATP, β-γ-methylene-ATP was approximately 100 times more potent as a vasoconstrictor agent (Table 1a). The maximum effective concentration of β-γ-methylene ATP could not be determined, high concentrations causing very large increases in perfusion pressure (>200 mmHg) and complete cessation of the venous outflow, suggesting that leakage was occurring between the foetal and maternal compartments.

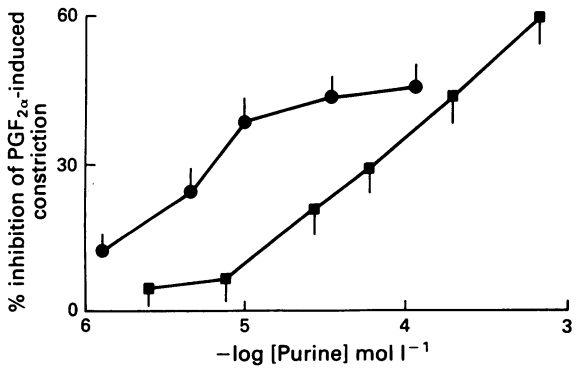

Figure 1 Concentration-response curves for the vasodilator effects of ATP (●) and adenosine (■) in blood vessels of the human placenta. Each point is the mean ± s.e.mean of at least 16 determinations. Curves do not differ significantly from parallelism and are significantly displaced, $P<0.05$.

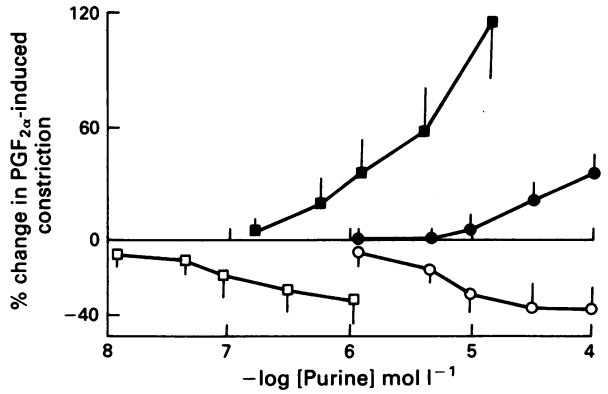

Figure 2 Concentration-response curves for vasoconstriction caused by ATP (●) and β-γ-methylene-ATP (■) and vasodilatation caused by ATP (○) and 2-methylthio-ATP (□). Each point is the mean ± s.e.mean of at least 6 determinations. The vasodilator response curves to ATP and 2-methylthio-ATP do not differ significantly from parallelism and are significantly displaced, $P<0.05$. The vasoconstrictor response curves to ATP and β-γ-methylene-ATP do not differ significantly from parallelism and are significantly displaced, $P<0.05$.

2-CADO, an agonist with activity at both P_1-A_1 and P_1-A_2 receptors (Olsson & Pearson, 1990), was the most potent purine examined for effects mediated by P_1 receptors, causing reductions in perfusion pressure. As a vasodilator agent, 2-CADO was approximately 40 times more potent than adenosine, as indicated by comparison of the linear portions of their respective concentration-response curves (Table 1c).

5-CPCA, a selective P_1-A_2 receptor agonist (Daly, 1982) also caused concentration-dependent reductions in perfusion pressure. Comparison of the regression lines obtained indicated that 5-CPCA was approximately 7 times more potent than adenosine (Table 1c). 2-CCPA, a selective P_1-A_1 receptor agonist (Lohse *et al.*, 1988) was found to be 4 times more potent as a vasodilator than adenosine in the placenta (Table 1c). 5-NECA, a non-selective adenosine agonist (Olsson & Pearson, 1990) also caused reductions in perfusion pressure. Comparison of the concentration-response curve obtained with that for adenosine indicated that 5-NECA was approximately 5 times more potent than adenosine (Table 1c). CGS-21680, a selective P_1-A_2 agonist (Olsson & Pearson, 1990) was approximately 2.5 times more potent than adenosine in causing dilatation, the difference being significant (Table 1c).

CHA, a selective P_1-A_1 receptor agonist (Olsson & Pearson, 1990) produced concentration-dependent reductions in perfusion pressure, but the concentration-response curve was not significantly different from that of adenosine (Table 1c).

Indirect effects of adenosine and ATP

Indomethacin (10.0 μmol l^{-1}) had no effect on the vasodilator activities of adenosine and ATP ($n = 6$) (Table 1b). L-NOARG (100 μmol l^{-1}) significantly inhibited ATP-induced reductions in perfusion pressure (Figure 3; $n = 6$) and had a smaller, but statistically significant inhibitory effect on adenosine-induced falls in perfusion pressure (Figure 4; $n = 6$). In further preparations, reductions in perfusion pressure caused by ATP (1.34–436 μmol l^{-1}) or adenosine (2.5–823 μmol l^{-1}) were not significantly affected by infusion of D-NOARG (100 μmol l^{-1}) ($n = 5$ and 4 respectively). L-NOARG had no effect on the increases in perfusion pressure caused by ATP, when they occurred. In instances when biphasic responses to ATP were observed initially, during infusion of L-NOARG relaxant responses were absent ($n = 3$). Infusion of L-NOARG in a number of preparations

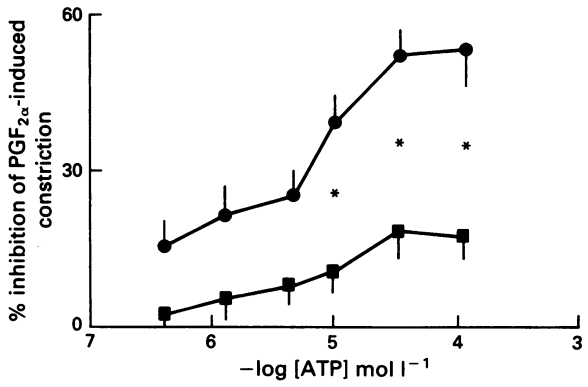

Figure 3 Effects of N^G-nitro-L-arginine (L-NOARG) (100 μmol l^{-1}) on ATP-mediated vasodilatation in foetal vessels. Concentration-response curves (± s.e.mean) to ATP in the absence (●) and presence (■) of L-NOARG. Curves differ from parallelism and are significantly displaced, $P < 0.05$. *$P < 0.05$, Student's t test between mean responses to the same concentration of agonist.

Table 1 Effects and relative potencies of purines in the foetal circulation of the placenta

a

Agonist	*Vasodilatation*	*Vasoconstriction*	*Potency* (cf. ATP)
ATP	+	+	1.0
Adenosine	+		0.16 (0.15–0.17)*
2-methylthio-ATP	+		50 (36–70)*
β-γ-methylene ATP		+	112 (87–144)*

*Significantly different from ATP (95% confidence limits)
(+ vasoactive effect, refer to text)

b

Agonist	*Potency* (cf. control)
ATP + L-arginine 100 μmol l^{-1}	1.3 (0.4–7.8)
ATP + L-NOARG 100 μmol l^{-1}	inhibited (see Fig 2b)
ATP + indomethacin 10 μmol l^{-1}	1.4 (0.7–3.8)
Adenosine + L-arginine 100 μmol l^{-1}	3.01 (2.52–3.56)*
Adenosine + L-NOARG 100 μmol l^{-1}	0.38 (0.27–0.52)*
Adenosine + indomethacin 10 μmol l^{-1}	2.4 (0.5–4.0)

*Significantly different from control (95% confidence limits)

c

Agonist	*P_1-A_2*	*P_1-A_1*	*Potency* (cf. Adenosine)
Adenosine	+	+	1.0
2-CADO	+	+	38.9 (35.5–42.6)*
5-CPCA	+		7.2 (6.4–8.1)*
5-NECA	+	+	5.2 (4.6–6.6)*
2-CCPA		+	3.8 (3.4–4.3)*
CGS-21680	+		2.4 (2.1–2.7)*
CHA		+	1.5 (1.0–2.4)

*Significantly different from adenosine (95% confidence limits)
(+ reported agonist selectivity, refer to text)

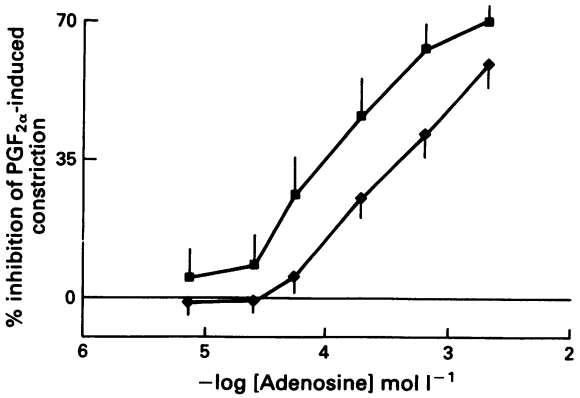

Figure 4 Concentration-dependent vasodilatation caused by adenosine in the absence (■) and presence (◆) of N^G-nitro-L-arginine (100 μmol l^{-1}). Each point is the mean ± s.e.mean of 6 determinations. Curves do not differ significantly from parallelism but are significantly displaced, $P<0.05$.

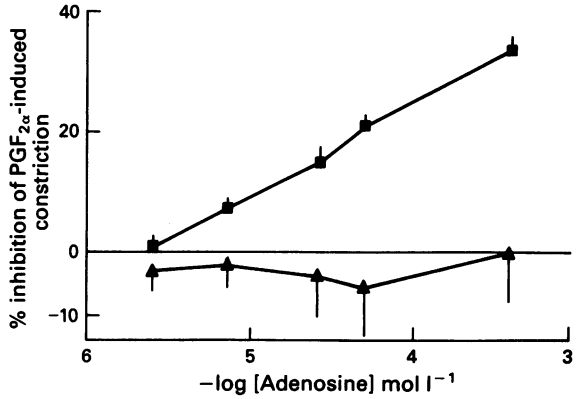

Figure 6 Vasodilatation caused by adenosine in the absence (■) and presence (▲) of the P_1-A_2 antagonist 3,7-dimethyl-1-propargylxanthine (DMPX, 80 μmol l^{-1}) ($n=4$). The curves are significantly different ($P<0.05$, two way analysis of variance) and are not parallel.

($n=3$) was followed by increases in perfusion pressure (up to 30 mmHg). This effect, however, was absent in the remainder ($n=9$). When perfusion pressures increased in the presence of L-NOARG the concentration of $PGF_{2\alpha}$ being infused concomitantly was reduced so that the pressure fell back to the basal level before infusing either ATP or adenosine.

L-Arginine (100 μmol l^{-1}), when infused through the foetal circulation for 60 min did not change the basal perfusion pressure significantly ($n=12$) and was without effect on ATP-induced reductions in perfusion pressure (Table 1b). In contrast, a significant potentiation of adenosine-induced reduction in perfusion pressure (Figure 5) was observed during infusion of this amino acid. In further experiments there was no significant change in the potency of adenosine when a similar concentration of D-arginine was infused ($n=4$).

Effects of antagonists at P_1-A_1 and P_1-A_2 receptors on responses to adenosine

Addition of DMPX, an antagonist selective for P_1-A_2 purinoceptors (Seale *et al.*, 1988) to the Krebs solution (80 μmol l^{-1}) perfusing the placental lobules markedly reduced vasodilator responses to adenosine (Figure 6). In the presence of the XAC (80 μmol l^{-1}), which has high affinity but moderate selectivity as an antagonist at P_1-A_1 receptors (Rossi *et al.*, 1987), the mean vasodilator responses to adenosine (3–300 μmol l^{-1}) were not significantly different from those of the concentration-response curve to adenosine obtained in its absence (linear regression analysis, $P>0.05$). In addition, neither curve differed significantly in position from that of the control curve to adenosine obtained when examining DMPX, as shown in Figure 6.

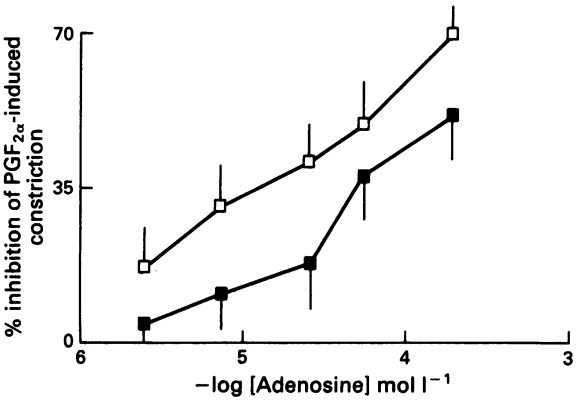

Figure 5 Concentration-dependent vasodilatation caused by adenosine in the absence (■) and presence (□) of L-arginine (100 μmol l^{-1}). Each point is the mean ± s.e.mean of 6 determinations. Curves do not differ significantly from parallelism but are significantly displaced, $P<0.05$.

Discussion

The importance of endogenous adenyl purines in the control of vascular resistance and foetal blood flow in the placenta is not known. However purines, interacting with autacoids both locally released and circulating in the umbilical blood, are likely to have important roles in the control of villous vascular tone, particularly as neural mechanisms do not influence the foetal extracorporeal vasculature (Boura & Walters, 1991). Adenosine concentrations in the placenta increase 100 fold during delivery (Sim & Maguire, 1972) and it is released from perfused placental tissues into the foetal circulation during ischaemia (Kitagawa *et al.*, 1987). Adenosine and its metabolites are also found in umbilical cord blood after delivery (Irestedt *et al.*, 1989) and during perinatal hypoxia (O'Connor *et al.*, 1981).

The present study has shown that adenyl purines in the foetal circulation of the human placenta cause both vasoconstriction and vasodilatation, the relative prominence of which depended on the purine. Thus, adenosine, 2-CADO, 5-CPCA, 2-CCPA, 5-NECA, CHA and CGS -21680 caused only vasodilatation and β-γ-methylene-ATP only vasoconstriction in all placental preparations. In contrast, ATP exerted both effects, the prominence of each depending on the preparation. In particular, the vasodilatation always seen in the present study during infusion of adenosine contrasted with its reported ability to cause placental foetal vasoconstriction (Kitagawa *et al.*, 1987; Slegel *et al.*, 1988). However, this apparent anomaly is capable of explanation. In the present work the normal very low tone of the villous vasculature was increased with $PGF_{2\alpha}$ to permit any vasodilator responses to be seen. The oxygen tension of the Krebs solution was also high. In the other reported studies the tone of the vasculature was low and vasoconstrictor responses to reducing oxygen tension examined. The combined findings may have physiological implications. Release of adenosine in local areas of ischaemia in the placenta may contribute to the ensuing vasoconstrictor response, so directing blood to placental villi having higher oxygen tensions where its vasodilator effect could help sustain adequate blood flow.

Unequivocal identification of the receptors involved in mediating the vascular effects of purines is difficult, due to lack of highly specific antagonists of their actions and the ability of cells to vary the concentration of an individual

purine at receptor sites by uptake and metabolism. Nevertheless, Burnstock (1978) classified receptors for purines into two groups, P_1 and P_2, based on their relative potencies and various preparations. The rank order of potency for agonists at the P_1 receptor is adenosine > AMP > ADP > ATP, while the reverse potency order defines P_2 receptors. The latter were subsequently further divided into two subgroups, P_{2x} and P_{2y} (Burnstock & Kennedy, 1985). The P_{2y} receptor which is found on the vascular endothelium, has been linked to the production of NO (Mathie *et al.*, 1991) and prostacyclin (Carter *et al.*, 1988). The P_{2x} receptor, found on vascular smooth muscle cells,may be directly coupled to activation of calcium channels (Benham & Tsien, 1987). P_1 receptors have also been divided into two sub-groups, A_1 and A_2, primarily on their ability, when activated, to inhibit or stimulate adenylate cyclase respectively (Olsson & Pearson, 1990).

The present study demonstrated the probable existence of the P_{2x}, P_{2y} and P_1-A_2 subgroups of purine receptor in the foetal resistance vessels of the human placenta. In this respect human placental vessels appear to be similar to human subcutaneous and omental resistance vessels which also contain P_{2x}, P_{2y} and P_1 receptors (Martin *et al.*, 1991). The activity of ATP and the much higher potency of the selective agonist 2-methylthio-ATP indicates that the vasodilatation caused by ATP in the foetoplacental vascular bed is probably mainly due to activation of P_{2y} receptors. Presumably, these are located on the vascular endothelium (Kennedy *et al.*, 1985; Needham *et al.*, 1987) since ATP-induced vasodilatation appeared dependent on formation of NO, being substantially inhibited by L-NOARG. Mathie *et al.* (1991) have also shown that ATP vasodilatation in the rabbit hepatic arterial vascular bed is mediated by NO. No evidence was found for prostacyclin, or other vasodilator prostaglandins, being involved in the vasodilator effects of ATP in the placenta since the cyclo-oxygenase inhibitor indomethacin did not modify responses to ATP.

The presence of P_{2x} receptors in the resistance vessels of the foetoplacental vascular bed was indicated by the finding that the P_{2x} agonist, β-γ-methylene-ATP, was a potent constrictor agent. At times, ATP also caused vasoconstriction. Sometimes this action preceded its vasodilator action whereas in other preparations it was the sole effect. The dilator and constrictor responses to ATP suggest that it has the ability to interact with both P_{2y} and P_{2x} receptor subtypes. P_{2y} receptor-mediated vasorelaxation appeared to be the predominant effect, otherwise masking vasoconstriction mediated by P_{2x} receptors. Variability in responses to ATP, in what appeared to be identical preparations, could have been due to changes in endothelial cell function. A vascular endothelium with reduced function, due to ischaemia before perfusion or to other factors, may tend to reduce dilator responses to ATP. Support for this idea came from the observation that when biphasic responses to ATP were obtained, L-NOARG inhibited the secondary dilatation.

Formation of NO by the foetal vasculature also contributed to the vasodilator responses to adenosine. The precursor of NO, L-arginine, potentiated vasodilator responses to this purine whereas responses were reduced during inhibition of NO synthesis with L-NOARG. It may be that concomitant production of NO by the foetal vascular endothelial cells during the vasoconstriction caused by prostaglandin $F_{2\alpha}$ administration contributed to the vascular dilator response to adenosine. Basal NO release has been demonstrated in the foetal circulation of the human perfused placenta (Gude *et al.*, 1990) and endogenous NO reduces foetal vascular responses to endothelin, U44619 and 5-hydroxytryptamine (Gude *et al.*, 1993).

L-Arginine was found to potentiate vasodilatation caused by adenosine but not that caused by ATP. This finding, bearing in mind the evidence obtained indicating that NO release contributed to responses to both purines, can perhaps be related to the method used for selecting placentae. For those experiments studying the effects of L-arginine on responses to ATP, the placentae used were those that responded to ATP solely by vasodilatation, no vasoconstrictor responses being seen. Thus placental endothelial cell function in the ATP experiments was likely to be good and addition of L-arginine unlikely to increase further the output of NO. On the other hand, the variable nature of responses to ATP, dilatation and vasoconstriction, indicated possible reduced endothelial cell function in some placentae. The latter could not have been identified in the group in which adenosine was used, as this purine consistently caused vasodilatation, presumably mainly due to a direct action on the foetal vascular smooth muscle, as in other vessels (Olsson & Pearson, 1990). In the latter circumstances there could have been some depression of endothelial cell function causing less than optimal output of NO which improved when the availability of L-arginine was increased.

The probable existence of P_1-A_2 receptors mediating the action of adenosine in the foetal vessels of the placenta was obtained by the finding that DMPX, an antagonist at these receptors (Seale *et al.*, 1988), inhibited responses to the purine. In contrast, a relatively high concentration of XAC had no effect on responses to adenosine, this agent being a moderately selective antagonist at P_1-A_1 receptors (Rossi *et al.*, 1987). A_2 binding sites have been identified on human placental cell membranes (Fox & Kurpis, 1983). The rank order of potencies found for the P_1 receptor agonists used also indicated that, in keeping with other studies (Edvinsson & Fredholm, 1983; Kusachi *et al.*, 1983; Leung *et al.*, 1985; Mustafa & Askan, 1985; Hutchison *et al.*, 1988) relaxation of human placental foetal vascular smooth muscle in response to purines can additionally be mediated through an A_2 receptor subtype. On the other hand, 5-NECA was not as potent as a vasodilator in the placenta as reported in other vascular preparations (Olsson & Pearson, 1990) and was found to be less potent than 2-CADO. CGS-21680 was less potent than 5-NECA and only slightly more potent than adenosine despite being reported to be a highly selective P_1-A_2 agonist in the rat brain (Jarvis *et al.*, 1989). CGS-21680 has also been found to be less potent than 5-NECA in causing relaxation of the human coronary artery *in vitro* (Makujina *et al.*, 1992). Variation in the relative potencies of the various A_2 agonists between preparations appears to be common (Olsson & Pearson, 1990).

In summary, the results of this study indicate that in foetal blood vessels in the human placenta, in the presence of high oxygen tensions, both ATP and adenosine cause vasodilatation, the magnitude of these responses being modified by changes in endothelial NO output. Vasodilatation to ATP is probably mediated by P_{2y} receptors located on the endothelium. ATP can also cause vasoconstriction probably mediated via a P_{2x} receptor, perhaps located on the vascular smooth muscle. Therefore, the overall response to ATP may be critically dependent on endothelial cell function. On the other hand, vasodilatation caused by adenosine may be predominantly mediated by a P_1-A_2 receptor and may require synergistic interaction with endogenously produced NO to exert its full effect.

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References

BENHAM, C.D. & TSIEN, R.W. (1987). A novel receptor-operated Ca^{2+} permeable channel activated by ATP in smooth muscle. *Nature*, **328**, 275–278.

BOURA, A.L.A. & WALTERS, W.A.W. (1991). Review article: autacoids and the control of vascular tone in the human umbilical-placental circulation. *Placenta*, **12**, 453–477.

BOWMAN, W.C. & RAND, M.J. (1980). In *Textbook of Pharmacology*. 2nd ed. ed. Bowman, W.C. & Rand, M.J. pp. 41.1–41.48. Oxford: Blackwell.

BURNSTOCK, G. (1978). A basis for distinguishing two types of purinergic receptors. In *Cell Membrane Receptors for Drugs and Hormones: A Multidisciplinary Approach*. ed. Staub, R.W. & Bolis, L. pp. 107–118. New York: Raven Press.

BURNSTOCK, G. & KENNEDY, C. (1985). Is there a basis for distinguishing two types of P_2-purinoceptor? *Gen. Pharmacol.*, **16**, 433–440.

CARTER, T.D., HALLAM, T.J., CUSACK, N.J. & PEARSON, J.D. (1988). Regulation of P_{2y}-purinoceptor-mediated prostacyclin release from human endothelial cells by cytoplasmic calcium concentration. *Br. J. Pharmacol.*, **95**, 1181–1190.

DALY, J.W. (1982). Adenosine receptors: targets for future drugs. *J. Med. Chem.*, **25**, 197–207.

DRURY, A.N. & SZENT-GYÖRGYI, A. (1929). The physiological activity of adenine compounds with especial reference to their action upon the mammalian heart. *J. Physiol.*, **68**, 213–237.

EDVINSSON, L. & FREEDHOLM, B.B. (1983). Characterization of adenosine receptors in isolated cerebral arteries of the cat. *Br. J. Pharmacol.*, **80**, 631–637.

FOX, I.H. & KURPIS, L. (1983). Binding characteristics of an adenosine receptor in the human placenta. *J. Biol. Chem.*, **258**, 6952–6955.

GUDE, N.M., BOURA, A.L.A., BRENNECKE, S.P., JAMAL, O., SMITH, R. & WALTERS, W.A.W. (1992). Evidence for inhibition by endothelium derived relaxing factor of thromboxane A_2 receptor mediated vasoconstriction in fetal vessels of human perfused placental lobules. *Placenta*, **13**, 597– 605.

GUDE, N.M, KING, R.G. & BRENNECKE, S.P. (1990). Role of endothelium-derived nitric oxide in maintenance of low fetal vascular resistance in placenta. *Lancet*, **336**, 1589–1590.

HOUSTON, D.S., BURNSTOCK, G. & VANHOUTTE, P.M. (1987). Different P_2 purinergic receptor sub-types of endothelium and smooth muscle in canine blood vessels. *J. Pharmacol. Exp. Ther.*, **241**, 501–506.

HOWARD, R.B., HOSOKAWA, T. & MAGUIRE, M.H. (1987). Hypoxia-induced fetoplacental vasoconstriction in perfused human placental cotyledons. *Am. J. Obstet. Gynecol.*, **157**, 1261–1266.

HUTCHISON, A.J., WEBB, R.L., OEI, H.H., GHAI, G.R., ZIMMERMAN, M.B. & WILLIAMS, M. (1989). CGS 21,680C, an A_2 selective adenosine receptor agonist with preferential hypotensive activity. *J. Pharmacol. Exp. Ther.*, **251**, 47–55.

IRESTEDT, L., DAHLIN, I., HERTZBERG, T., SOLLEVI, A. & LAGERCRANTZ, H. (1989). Adenosine concentration in umbilical cord blood of newborn infants after vaginal delivery and Cesarean section. *Pediatr. Res.*, **26**, 106–108.

JARVIS, M.F., SCHULZ, R., HUTCHISON, A.J., DO, U.H., SILLS, M.A. & WILLIAMS, M. (1989). [^{3}H]CGS-21680, a selective A_2 agonist directly labels A_2 receptors in rat brain. *J. Pharmacol. Exp. Ther.*, **251**, 888–891.

KENAKIN, T.P. & PIKE, N.B. (1987). An *in vitro* analysis of purine mediated renal vasoconstriction in rat isolated kidney. *Br. J. Pharmacol.*, **90**, 373–381.

KENNEDY, C. & BURNSTOCK, G. (1985). Evidence for two types of P_2-receptor in longitudinal muscle of the rabbit portal vein. *Eur. J. Pharmacol.*, **111**, 49–56.

KENNEDY, C., DELBRO, D. & BURNSTOCK, G. (1985). P_2-receptors mediate both vasodilatation (via the endothelium) and vasoconstriction of the isolated rat femoral artery. *Eur. J. Pharmacol.*, **107**, 161–168.

KITAGAWA, H., SLEGEL, P.H. & MACGUIRE, M.H. (1987). Ischaemia induced vasoconstriction and adenosine release in perfused human placental cotyledons. *Pharmacologist*, **29**, 197.

KUSACHI, S., THOMPSON, R.D. & OLSSON, R.A. (1983). Ligand selectivity of dog coronary adenosine receptor resembles that of adenylate cyclase stimulatory $R_{(a)}$ receptors. *J. Pharmacol. Exp. Ther.*, **227**, 316–322.

LEUNG, E.C., JOHNSTON, C.I. & WOODCOCK, E.A. (1985). An investigation of the receptors involved in the coronary vasodilatory effect of adenosine analogues. *Clin. Exp. Pharmacol. Physiol.*, **12**, 515–519.

LOHSE, M.J., KLOTZ, K.N., SCHWABE, U., CRISTALLI, G., VITTORI, S. & GRIFANTINI, M. (1988). 2-Chloro-N^6-cyclopentyladenosine: a highly selective agonist at A_1 adenosine receptors. *Naunyn Schmied. Arch. Pharmacol.*, **337**, 687–689.

MAK, K.K.-W., GUDE, N.M., WALTERS, W.A.W. & BOURA, A.L.A. (1984). Effects of vasoactive autacoids on the human umbilical-fetal placental vasculature. *Br. J. Obstet. Gynaecol.*, **91**, 99–106.

MAKIJINA, S.R., SABOUNI, M.H., BHATIA, S., DOUGLAS, F.L. & MUSTAFA, S.J. (1992). Vasodilatory effects of adenosine A_2 receptor agonists CGS 21680 and CGS 22492 in human vasculature. *Eur. J. Pharmacol.*, **221**, 243–247.

MARTIN, G.N., MCG.THOM, S.A. & SEVER, P.S. (1991). The effects of adenosine triphosphate (ATP) and related purines on human isolated subcutaneous and omental resistance arteries. *Br. J. Pharmacol.*, **102**, 645–650.

MATHIE, R.T., RALEVIC, V., ALEXANDER, B. & BURNSTOCK, G. (1991). Nitric oxide is the mediator of ATP-induced dilatation of the rabbit hepatic arterial vascular bed. *Br. J. Pharmacol.*, **103**, 1602–1606.

MATHIESON, J.J.I. & BURNSTOCK, G. (1985). Purine mediated relaxation and constriction of isolated rabbit mesenteric artery are not endothelium dependent. *Eur. J. Pharmacol.*, **118**, 221–229.

MULSCH, A. & BUSSE, R. (1990). N^G-nitro-L-arginine (N^5-[imino-(nitroamino methyl]-L-orthinine) impairs endothelium dependent dilatations by inhibitory cytosolic nitric oxide synthesis from L-arginine. *Naunyn Schmied. Arch. Pharmacol.*, **341**, 143–147.

MUSTAFA, S.J. & ASKAN, A.O. (1985). Evidence suggesting an $R_{(a)}$-type adenosine receptor in bovine coronary arteries. *J. Pharmacol. Exp. Ther.*, **232**, 49–56.

NEEDHAM, L., CUSACK, N.J., PEARSON, J.D. & GORDON, J.L. (1987). Characteristics of the P_2 purinoceptor that mediates endothelial prostacyclin production by pig aortic endothelial cells. *Eur. J. Pharmacol.*, **143**, 199–209.

O'CONNOR, M.C., HARKNESS, R.A., SIMMONDS, R.J. & HYTTEN, F.E. (1981). The measurement of hypoxanthine, xanthine, inosine and uridine in umbilical cord blood and fetal scalp blood samples as a measure of fetal hypoxia. *Br. J. Obstet. Gynaecol.*, **88**, 381–390.

OLSSON, R.A. & PEARSON, J.D. (1990). Cardiovascular purinoceptors. *Physiol. Rev.*, **70**, 761–845.

PALMER, R.M.J., REES, D.D., ASHTON, D.S. & MONCADA, S. (1988). L-arginine is the physiological precursor for the formation of nitric oxide in endothelium dependent relaxation. *Biochem. Biophys. Res. Commun.*, **1553**, 1251–1256.

PENFOLD, P., DRURY, L., SIMMONDS, R. & HYTTEN, F.E. (1981). Studies on a single placental cotyledon in vitro: 1. The preparation and its viability. *Placenta*, **2**, 149–154.

RALEVIC, V., MATHIE, R.T., ALEXANDER, B. & BURNSTOCK, G. (1991). Characterisation of P_{2x} and P_{2y}-purinoceptors in the rabbit hepatic arterial vasculature. *Br. J. Pharmacol.*, **103**, 1108–1113.

ROSSI, N.F., CHURCHILL, P.C., JACOBSON, K.A. & LEAHY, A.E. (1987). Further characterization of the renovascular effects of N^6-cyclohexyladenosine in the isolated perfused rat kidney. *J. Pharmacol. Exp. Ther.*, **240**, 911–915.

SEALE, T.W., ABLA, K.A., SHAMIM, M.T., CARNEY, J.M. & DALY, J.W. (1988). 3,7-Dimethyl-1-propargylxanthine: a potent and selective *in vivo* antagonist of adenosine analogs. *Life Sci.*, **43**, 1671–1684.

SIM, M.K. & MAGUIRE, M.H. (1972). Presence of adenosine in the human term placenta: determination of adenosine content and pathways of adenosine metabolism. *Circ. Res.*, **31**, 779–788.

SLEGEL, P., KITAGAWA, H. & MAGUIRE, M.H. (1988). Determination of adenosine in fetal perfusates of human placental cotyledons using fluorescence derivatization and reversed-phase high-performance liquid chromatography. *Anal. Biochem.*, **171**, 124–134.

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