U46619-mediated vasoconstriction of the fetal placental vasculature *in vitro* in normal and hypertensive pregnancies

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Objectives **To measure in-vitro responses to the thromboxane A_2 (TxA_2) mimetic U46619 in the fetal placental vasculature of human placentae from normotensive women and those with pre-eclampsia. Furthermore, to compare fetal vascular responses to endothelin-1, 5-hydroxytryptamine, potassium chloride (KCl) and prostacyclin (PGI_2) in placentae from normal or pre-eclamptic pregnancies.**

Methods **Single placental lobules of intact placentae were bilaterally perfused *in situ* (fetal and maternal) with constant flows of Krebs' solution. Changes in fetal arterial perfusion pressure during intra-arterial infusion of vasoactive agents were recorded. Fetal placental vasoconstrictor concentration response curves were obtained to U46619 (0.01–300 nmol/l), endothelin-1 (0.4–160 nmol/l), KCl (3–300 mmol/l) and 5-hydroxytryptamine (0.03–30 μmol/l). In addition, vasodilator concentration response curves were obtained for PGI_2 (1.2–350 nmol/l) in the fetal placental circulation during submaximal increases in perfusion pressure with prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$; 0.7–2.0 μmol/l).**

Results **The maximum increase in perfusion pressure caused by U46619 in placentae from normotensive women was 194 ± 25 mmHg. The maximum response to U46619 was significantly reduced in the placentae from women with pre-eclampsia (104 ± 21 mmHg). In contrast, there were no differences in constrictor responses to endothelin-1, 5-hydroxytryptamine and KCl, or in dilator responses to PGI_2 in placentae obtained from either normotensive women or those with pre-eclampsia.**

Conclusion **TxA_2 receptor-mediated vasoconstriction is reduced in the fetal vasculature of placentae from women with pre-eclampsia, possibly to compensate for the increased levels of TxA_2 seen in these conditions.**
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Introduction

Pre-eclampsia is a common medical complication of pregnancy and is a major contributor to maternal and perinatal mortality and morbidity [1]. It is a multisystem disorder characterized by a marked increase in maternal blood pressure from mid-gestation, and is associated with proteinuria, oedema and reduced uteroplacental blood flows. The pathogenesis of pre-eclampsia is unclear, but it appears to be a disease of placental origin. It only develops in the presence of the placenta and is rapidly resolved after its removal [2,3].

There are marked changes in the maternal vasculature during pre-eclampsia, including increased responsiveness to vasopressors and generalized vasoconstriction [3]. In addition, pre-eclampsia may be associated with increases in maternal plasma concentrations of thromboxane A_2 (TxA_2), a potent vasoconstrictor and activator of platelet aggregation, and reductions in prostacyclin (PGI_2), which opposes the actions of TxA_2 [4]. Thromboxane A_2 and PGI_2 are biologically important prostanoids derived from arachidonic acid by the catalytic action of the enzymes cyclo-oxygenase-1 and 2 [4]. The human placenta produces both PGI_2 and TxA_2 [4,5] and during pre-eclampsia an imbalance of the two prostanoids may occur. During pre-eclampsia the production of placental TxA_2 is increased from the trophoblast, decidua and amnion [5–7] in addition to the occurrence of local release from platelets [8]. Furthermore, pre-eclamptic placental tissue *in vitro* has a reduced capacity to produce PGI_2 [6]. Thus, the ratio of TxA_2 to PGI_2 in the placenta has been estimated to increase from 1 : 1 in normal pregnancies to >7 : 1 in

women with pre-eclampsia [5–7]. The balance between placental TxA_2 and PGI_2 may therefore be shifted during pre-eclampsia to conditions that favour fetal placental vasoconstriction.

A characteristic of the fetal placental circulation in normal pregnancy is its low impedance to blood flow; increased fetal placental resistance may be a feature of pre-eclampsia. Hence, since the placenta is devoid of nerves [9], alterations in humoral mediators such as TxA_2 or vascular structures, or both, must contribute to the elevation in placental vascular resistance associated with pre-eclampsia. The evidence that increased synthesis of TxA_2 could be clinically important in pre-eclamptic patients led to the CLASP (Collaborative Low-Dose Aspirin Study in Pregnancy) randomized trial of low-dose aspirin as a prophylactic treatment [10]; however, its effectiveness was limited. Low-dose aspirin inhibits the cyclo-oxygenase activity of the enzyme prostaglandin H synthase. This selectively and irreversibly suppresses the synthesis of maternal platelet TxA_2 without inhibiting the production of vascular endothelial PGI_2 [6].

Although the relative changes in levels of various vasoactive mediators such as TxA_2 during pre-eclampsia have been established, relatively few studies have examined the implications of these changes on fetal placental vascular reactivity. We hypothesize that placentae obtained from women whose pregnancies are complicated by hypertension may have altered vascular reactivity to various autacoids such as TxA_2 and PGI_2. Therefore, the present study was designed to measure responses *in vitro* to the TxA_2 mimetic U46619, in the fetal vasculature of placentae from normotensive women and in those from women with pre-eclampsia. In order to determine the specificity of this effect, fetal placental vascular responses to endothelin-1, 5-hydroxytryptamine, KCl, and PGI_2 in placentae from women with either normotensive or hypertensive pregnancies were also examined.

Materials and methods

Collection of placentae

All experiments were formally approved by the John Hunter Hospital and the University of Newcastle Human Ethics Committees and written informed consent was obtained from women donating their placentae after delivery. Placentae were collected immediately after vaginal delivery or caesarean section from women aged 17–42 years. Women may have received one or more of the following drugs during labour: oxytocin (two intravenous units over 6–8 h), pethidine hydrochloride (100 mg intramuscularly), or inhaled 70% N_2O and 30% O_2. Women with pre-eclampsia may have also received some or a combination of the following drugs: labetalol, hydralazine and α-methyldopa. When these drugs are used therapeutically, they have no effect on placental vascular tone *in vitro* at concentrations likely to be present in maternal blood [11–13]. Placentae from women who were smokers or who had taken aspirin or corticosteroids were not used.

Selection of placentae from women with hypertensive pregnancies

Pre-eclampsia was defined as hypertension in combination with proteinuria, occurring after 20 weeks gestation in women with no previous history of hypertension or proteinuria. Hypertension is defined as one diastolic blood pressure reading of at least 110 mmHg or two consecutive blood pressure readings (Korotkov Phase IV) of at least 90 mmHg not less than 4 h apart. Proteinuria was defined as a reading of 1+ using a Dipstix® (Bayer Diagnostics, Sydney, Australia) proteinuria device on two consecutive occasions not less than 4 h apart, subsequently confirmed by 24-h urine analysis. Transient proteinuria associated with pregnancy-induced hypertension in the late third trimester or associated with labour was not considered indicative of pre-eclampsia.

Placental lobule perfusion

A suitable paired artery and vein, typically third or fourth branches of the chorionic plate vessels, leading to a peripheral 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 chorionic plate, was connected to a Gilson Minipuls 2 peristaltic pump (Gilson Medical Electronics, Villiers-le Bel, France) and the lobule of the intact placentae was perfused *in situ* with Krebs' solution at 37°C equilibrated with 95% O_2 and 5% CO_2, containing (in mmol/l): 97.0 NaCl; 24.0 $NaHCO_3$; 3.0 KCl; 1.2 KH_2PO_4; 1.89 $CaCl_2$; 1.0 $MgSO_4$; 5.5 D-glucose (pH 7.3). The maternal side of the lobule was perfused under similar conditions to those used for the fetal circulation via a cannula inserted into the intervillous space through a remnant of a spiral arteriole in the placental basal plate. Each lobule was initially perfused at 1 ml/min for 5 min and thereafter with a constant flow rate of 5 ml/min into both fetal and maternal circulations. Placentae were bathed in Krebs' solution at 37°C throughout the experiment. Changes in fetal placental perfusion pressure were monitored using a Gould P23 pressure transducer (Gould Instruments, Cleveland, Ohio, USA) interfaced to a JRAK (Melbourne, Australia) amplifier and recorded on a W+W 330 chart recorder (Basel, Switzerland).

Experimental design

Upon obtaining a stable baseline pressure, typically 20–40 mmHg, U46619 was infused via a peristaltic pump (Gilson, Minipuls 3) in a series of increasing semi-logarithmic concentrations (0.01–300 nmol/l) until

a maximum increase in perfusion pressure had been obtained. The infusion rate of U46619 was not increased until an equilibrium response had occurred to the previous concentration. The threshold concentration of U46619 was defined as the minimum concentration required to cause an increase in perfusion pressure. In a separate series of experiments, endothelin-1 (0.4–160 nmol/l), 5-hydroxytryptamine (0.03–30 μmol/l) or KCl (3–300 mmol/l) were infused in a similar manner to U46619. Each agonist was examined in a single lobule only. Experiments with different agonists were often examined in individual lobules of the same placenta. In these cases, lobules were selected to be located as far apart as possible.

For studies of the vasodilator actions of PGI_2, because of the low basal perfusion pressure of the fetal placental circulation *in vitro*, it was necessary to induce a submaximal increase in perfusion pressure with prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$; 0.7–2.0 μmol/l), continuously infused into the arterial cannula using a Gilson Minipuls 3 peristaltic pump. The concentration of $PGF_{2\alpha}$ was adjusted to give a stable submaximal perfusion pressure of 90–120 mmHg before obtaining each concentration response curve to PGI_2, which was infused by peristaltic pump (Gilson Minipuls 3) in a gradually increasing semi-logarithmic series of concentrations (1.2–350 nmol/l) into the arterial cannula. The rate of PGI_2 infusion was only increased when a stable reduction in perfusion pressure had been obtained at the previous concentration.

Drugs and chemicals

Chemicals used for the Krebs' solution were of analytical grade (BDH, Port Fairy, Australia). U46619 (9,11, -dideoxy-11α, 9α, epoxymethano prostaglandin $F_{2\alpha}$; Upjohn, Kalamazoo, Michigan, USA) was supplied at a concentration of 10 mg/ml in ethanol and further dilutions were made in physiological saline (0.9% NaCl w/v). Synthetic human endothelin-1, (Calbiochem-Novabiochem, Sydney, Australia) was diluted in 0.05 mol/l acetic acid (Analar; BDH) to a concentration of 3 mmol/l and further diluted in distilled water as required. 5-Hydroxytryptamine (creatinine sulphate complex; Sigma, St Louis, Missouri, USA) was dissolved in distilled water to a working concentration of 10 mmol/l immediately before each experiment and diluted as required. Prostaglandin $F_{2\alpha}$ was supplied as its trometamol salt (Dinoprost; Upjohn, Sydney, Australia) at a concentration of 5 mg/ml in sterile distilled water and diluted as required in distilled water. PGI_2 was supplied as the sodium salt (Cayman Chemical, Ann Arbor, Michigan, USA), and dissolved in 50 μmol/l phosphate buffered saline (pH 9.5) and kept at 4°C under N_2 during infusion.

Statistical analysis

All values are expressed as means ± SEM unless stated otherwise. Linear regression analysis was performed on all concentration response curves using a custom designed macro-analysis program based on the Excel V5.0 spreadsheet (Microsoft, Redmond, Washington, USA.). Differences in the linear portions of the concentration response curves to the various agonists examined were compared and tested for significant displacement and deviation from parallelism as described by Bowman and Rand [14]. $P < 0.05$ was considered statistically significant unless stated otherwise. Non-parallel curves, and multiple comparisons of means were tested for differences using analysis of variance (ANOVA). Single comparisons were made using the Mann–Whitney U or Student's unpaired *t* tests as appropriate, where indicated (Minitab, Pasadena, California, USA).

Results

The clinical characteristics of normotensive and hypertensive pregnancies are given in Table 1. The placentae used in this study were from either normotensive women aged 28.6 ± 1.3 years ($n = 17$), or women with pre-eclampsia aged 29.4 ± 1.8 years ($n = 16$). The gestation of the women with pre-eclamptic pregnancies (33.5 ± 0.8 weeks) was significantly shorter than that of women with normotensive pregnancies (38.1 ± 0.6 weeks; Mann–Whitney test). All women in the pre-

Table 1 **Characteristics of normotensive and pre-eclamptic pregnant women used in this study**

	Normal ($n = 17$)	Pre-eclamptic ($n = 16$)	*P*
Age (years)	28.6 ± 1.3 (18–39)	29.4 ± 1.8 (18–37)	NS
Gestation age (weeks)	38.1 ± 0.6 (33–41)	33.5 ± 0.8** (23–40)	0.01
Proteinuria (g/l)§	0 (not detected)	3 (1–3)***	0.001
SBP (mmHg)†	126 ± 2	161 ± 2**	0.01
DBP (mmHg)†	77 ± 3	102 ± 2*	0.05
Birthweight (kg)‡	3.33 ± 0.18 (2.12–4.62)	2.33 ± 0.25* (1.02–4.52)	0.01
Parity	0 (0–3)	0 (0–3)	NS

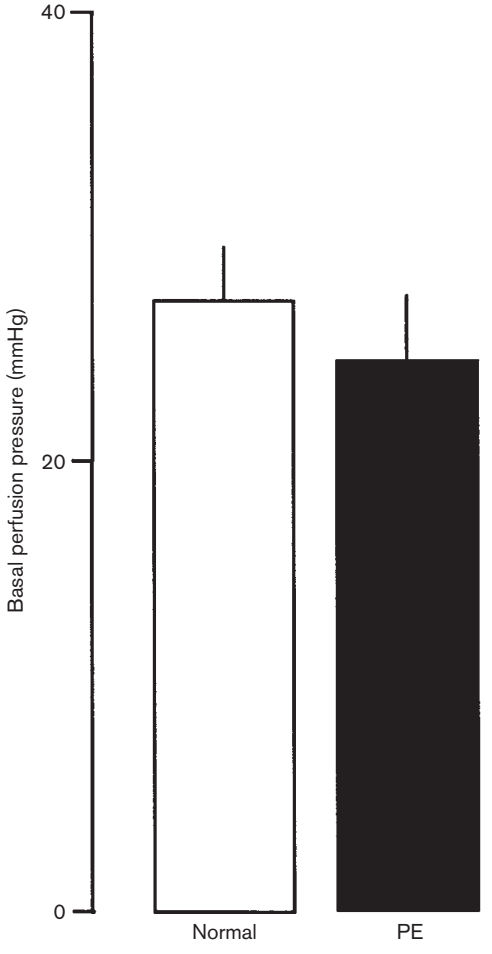
Data are presented as mean ± SEM and range (where appropriate). †Blood pressure measurements are the means of the maximum values recorded in each patient before the onset of labour. ‡Significant difference in birthweight between groups is a consequence of differences in gestational age at delivery. §All pre-eclamptic women were determined to have significant proteinuria (⩾ 0.3 g/l as determined by 24-h urine analysis). Urinary protein was not measured in normotensive women. All statistical comparisons are using the Mann–Whitney U-test. *Statistically significant. NS, not significantly different from normal; SBP, systolic blood pressure; DBP, diastolic blood pressure.

eclamptic group were shown to have proteinuria using the Dipstix® device, and were later confirmed by 24-h urine protein analysis to have measurements of >0.3 g/l. No women in the normotensive group experienced proteinuria, as indicated by the Dipstix® device.

Basal fetal arterial perfusion pressure

In the studies examining the effects of U46619, placental lobules from normotensive women had a basal arterial perfusion pressure of 28.8 ± 4.0 mmHg ($n = 6$). The basal perfusion pressure in placental lobules from women with pre-eclampsia (25.2 ± 5.3 mmHg, $n = 8$) were not significantly different from the normotensive control group (Mann–Whitney) (Fig. 1). There were also no differences between the mean weights of the placental lobules, measured after each experiment for each group (normotensive women 31.0 ± 6.2 g, pre-eclamptic women 26.1 ± 2.9 g; Mann–Whitney). No differences were observed in the basal perfusion pressure and lobule weight of the placentae used to examine the vasoactive effects of endothelin-1, 5-hydroxytryptamine, KCl or PGI_2.

Fig. 1



Basal fetal arterial perfusion pressures of isolated placental lobules from normotensive women ($n = 17$, normal) or those with pre-eclampsia ($n = 16$, PE). All values are means ± SEM.

Fetal placental vasoconstriction

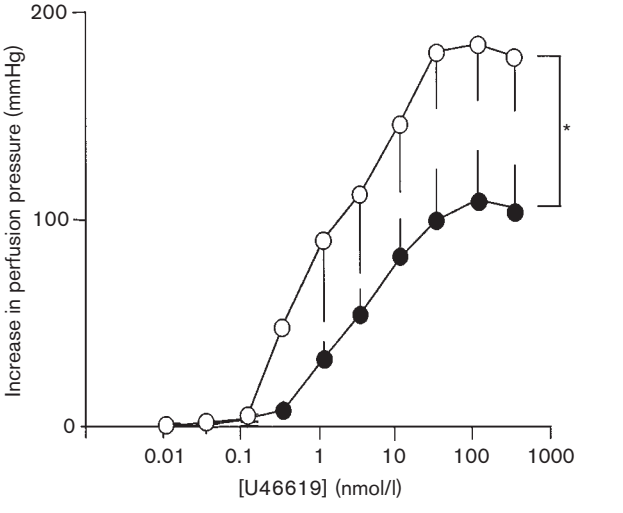
U46619

U46619 (0.01–300 nmol/l) caused a concentration-dependent increase in arterial perfusion pressure of the fetal placental circulation. The maximum increase in pressure obtained with U46619 in placentae from women with pre-eclampsia (104 ± 21 mmHg, $n = 8$) was significantly lower than that of the normotensive control group (194 ± 25 mmHg; ANOVA $n = 6$; Fig. 2). The mean threshold concentration of U46619 (0.03 nmol/l; Fig. 2) in the fetal placental circulation of placentae from normotensive women was not different from that in placentae from women with pre-eclampsia (Mann–Whitney). The effects of U46619 were examined in the placenta obtained from pre-eclamptic pregnancies ranging in gestation from 29 to 38 weeks. Similar responses to U46619 were observed in these placentae regardless of gestational age.

Endothelin-1, 5-hydroxytryptamine and potassium chloride

Endothelin-1 (0.4–160 nmol/l), 5-hydroxytryptamine (0.03–30 μmol/l) and KCl (3–300 mmol/l) all caused concentration-dependent increases in fetal placental arterial perfusion pressure. In contrast to the results with U46619, no differences were observed between

Fig. 2



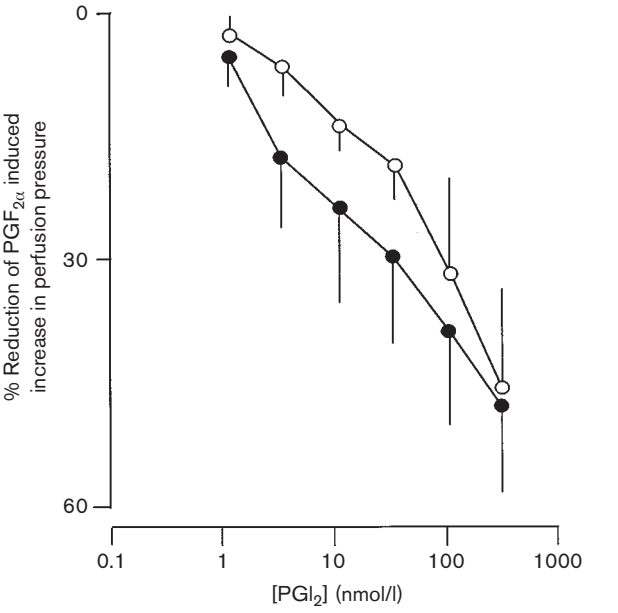
Comparison of the increases in perfusion pressure in the fetal vasculature caused by the thromboxane A_2 mimetic U46619 in placentae from normotensive women (○, n = 6), or women with pre-eclampsia (●, n = 8). $^*P < 0.05$, ANOVA regression analysis. All values are means ± SEM.

the concentration-dependent curves and the maximum responses for each of these constrictors in placentae obtained either from normotensive women or from those with pre-eclampsia (Fig. 3). However, it should be noted that the maximum responses in fetal arterial perfusion pressure for endothelin-1, 5-hydroxytryptamine and KCl were significantly less than the maximum response to U46619 in placentae from normotensive women (ANOVA, regression analysis, n = 4–6). The relative potencies of the vasoconstrictor agonists in the fetal circulation of placentae from normotensive women were in the following order: U46619 ($EC_{50} = 6.4 \pm 1.5$ nmol/l) > endothelin-1 ($EC_{50} = 11.6 \pm 4.2$ nmol/l) > 5-hydroxytryptamine ($EC_{50} = 5.9 \pm 3.1$ μmol/l) > KCl ($EC_{50} = 86.4 \pm 16.5$ mmol/l).

Fetal placental vasodilatation mediated by PGI_2

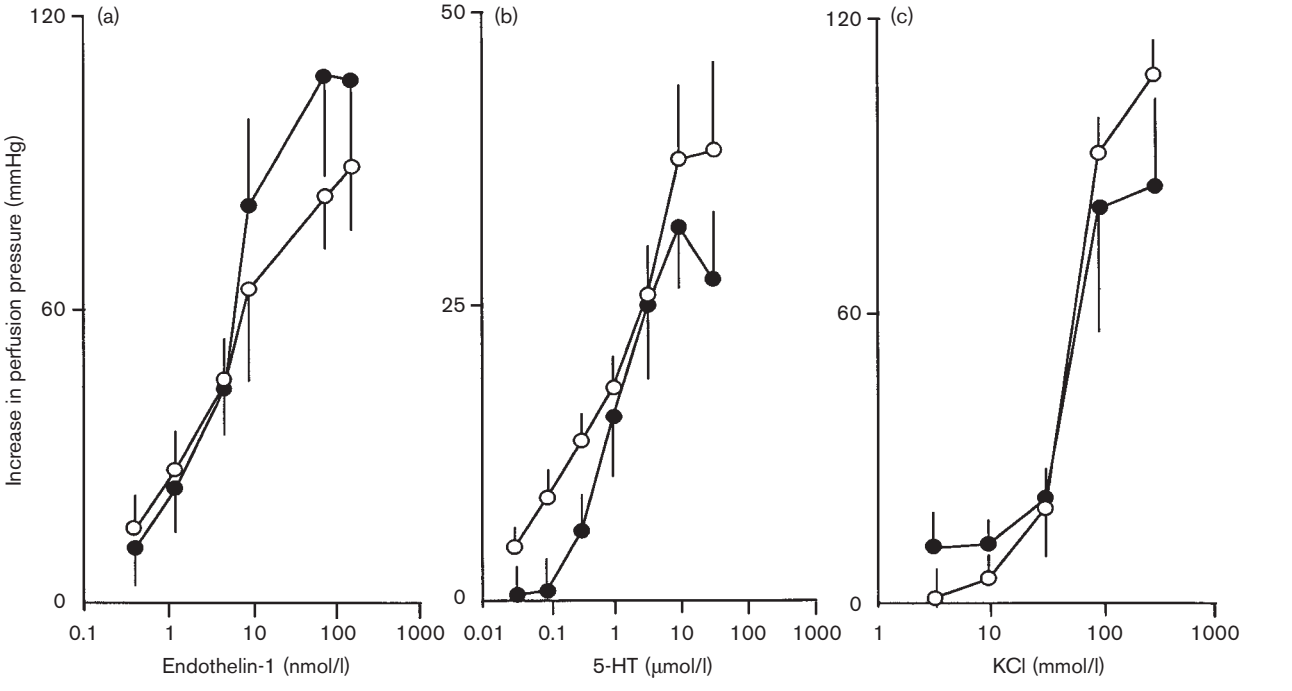
PGI_2 (1.2–350 nmol/l) caused a concentration-dependent decrease in perfusion pressure of the fetal placental vasculature constricted with $PGF_{2\alpha}$. The PGI_2-induced reduction in pressure was not significantly different in placentae from normotensive women than in placentae from women with pre-eclampsia (ANOVA, regression analysis, $n = 4–5$; Fig. 4). Basal perfusion pressures for placentae used to examine the dilator effects of PGI_2 were 30.3 ± 3.2 mmHg for normotensive women and 23.0 ± 3.7 mmHg for those with pre-

Fig. 4



Comparison of the vasodilator effects of prostacyclin (PGI_2) in the fetal circulation submaximally constricted with prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) in placentae from normotensive women (○, $n = 4$) or those with pre-eclampsia (●, $n = 4–5$). All values are means ± SEM.

Fig. 3



Comparison of the increases in perfusion pressure in the fetal vasculature caused by (a) endothelin-1, (b) 5-hydroxytryptamine and (c) KCl in placentae from normotensive women (○) or women with pre-eclampsia (●). All values are means ± SEM., $n = 4–6$.

eclampsia ($P > 0.05$, Mann–Whitney). The perfusion pressure was increased by infusion of $PGF_{2\alpha}$ to 106.8 ± 5.1 mmHg and 92.5 ± 9.7 mmHg in normal and pre-eclamptic placentae, respectively, which were also not significantly different (Mann–Whitney). The amount of $PGF_{2\alpha}$ required to increase the perfusion pressure to these levels was significantly different between normotensive (0.52 ± 0.08 μmol/l) and pre-eclamptic placentae (1.97 ± 0.26 μmol/l; Mann–Whitney).

Discussion

We examined the changes in fetal vascular responses mediated by various vasoactive autacoids in the bilaterally perfused placenta *in vitro* from normotensive and pre-eclamptic women. These results demonstrate for the first time that increases in perfusion pressure mediated by the TxA_2-mimetic U46619 are attenuated in placentae *in vitro* from women with pre-eclampsia, compared with placentae from women who were normotensive. Placental vascular responses to the constrictors endothelin-1, 5-hydroxytryptamine and KCl and to the dilator PGI_2 were not different in normotensive and pre-eclamptic placentae. The data suggest that there may be a compensatory down-regulatory mechanism in the fetal placental circulation in women with pre-eclampsia, which counteracts the increased placental TxA_2 levels that occur in this disease.

U46619 was found to cause a powerful increase in perfusion pressure in the fetal placental circulation *in vitro*, increasing the pressure to nearly 200 mmHg, and confirming previous studies in the human perfused placenta [15] and the human umbilical artery [16]. It has been shown that the mobilization of both intra- and extracellular calcium contributes to U46619-induced vasoconstriction in human placental umbilical arteries [17], and this may partly explain the greater maximum response compared with that induced by the constrictors endothelin-1, 5-hydroxytryptamine and KCl. U46619 acts on the G-protein-coupled thromboxane prostanoid (TP) membrane receptors to cause vascular smooth muscle vasoconstriction. Recently, two splice variants of the thromboxane receptor gene have been identified in the human placenta: TP_α [18] and TP_β [19]. It has also been shown, using *in situ* hybridization and immunocytochemistry, that placental trophoblast and decidual cells express TxA_2 receptors [20] and, using functional studies, that umbilical endothelial cells possess TxA_2 receptors [21]. Nevertheless, the TP receptor subtype(s) by which U46619 mediates vasoconstriction in the fetal placental circulation have not been fully characterized.

Others have observed that the vasoconstrictor effects of U46619 are reduced in the fetal vasculature of placentae from women with diabetic pregnancies, possibly as a result of the decreased affinity of TxA_2 receptors [22]. Increased TxA_2 production has been implicated in the pathogenesis of diabetic complications, and so may be a contributing factor to these findings [23]. Interestingly, it has been reported [24] that in a group of pre-eclamptic women, placental TxA_2 receptor affinity or density was not altered; however, the tissue source and the subtype of thromboxane receptors was not differentiated in this study.

Desensitization of TxA_2 receptors to U46619 has been reported to occur in platelets [25]. Although desensitization to TxA_2 agonists can be shown *in vitro*, suggesting that increased placental release of TxA_2 in pre-eclampsia could similarly affect the fetal vasculature, it still remains to be determined whether or not TxA_2 receptor downregulation occurs in vascular smooth muscle and other tissues *in vivo*.

As with U46619, we also observed that the vasoconstrictor effects of $PGF_{2\alpha}$ were reduced in pre-eclamptic placentae. We did not obtain full concentration response curves to $PGF_{2\alpha}$ but would expect to obtain similar results as for U46619. Similarly reduced responses to $PGF_{2\alpha}$ have been observed in arterial ring segments isolated from the placental chorionic plate [26]. It is possible, therefore, that the effects we have observed with U46619 and $PGF_{2\alpha}$ in the perfused placenta reflect a diminished response to vasoconstrictor prostanoids in general, possibly through changes in their coupling to intracellular effector mechanisms.

The altered mechanisms responsible for reducing blood flow in the fetal circulation of the placenta during pre-eclampsia have not been fully established. Possible changes in fetal neural influences can be disregarded, since the fetal extracorporeal vasculature is not innervated [9]. It is more likely that vasoconstriction caused by changes in vascular levels of vasoactive mediators, plus alterations in fetal vascular morphology, are responsible. Obliteration of a proportion of the tertiary stem villous arterioles [27] could be expected to increase total vascular resistance and impedance to fetal placental blood flow. However, the present data do not support any suggestion that structural changes in the fetal vessel wall were responsible for the reduced responsiveness to U46619. There were no differences in the increases in perfusion pressure obtained with endothelin-1, 5-hydroxytryptamine and KCl between the placentae from normotensive women and those of women with pre-eclampsia. Endothelin-1 causes vasoconstriction of the fetal placental circulation by activating ET_A receptors located on the vascular smooth muscle [28] while 5-hydroxytryptamine mediates its contractile effects by activation of $5\text{-}HT_2$ receptors [29]. KCl acts by causing membrane depolarization which opens voltage-operated calcium channels, resulting in vasoconstriction. Several studies have examined

the reactivity of human isolated placental arterial or venous ring preparations to endothelin-1 and 5-hydroxytryptamine in normal and hypertensive pregnancies, but the results have been inconclusive [30–33]. Furthermore, studies of the possible alterations in the placental production of these vasoconstrictors during pre-eclampsia have demonstrated no clear results [34–36]. Although the study of human placental function *in vivo* is complicated by ethical considerations, we believe such limitations are in part circumvented by the use of the human placental perfusion technique, which resembles the *in vivo* situation more closely than isolated vascular ring preparations. However, there are limitations to extrapolating findings in the perfused placental model *in vitro* to physiological events *in vivo*. The advantages and disadvantages of the perfused placenta preparation have been reviewed in detail previously [37,38].

The evidence that increased synthesis of TxA_2 could be clinically important in pre-eclamptic patients led to the CLASP randomized trial of low-dose aspirin as a prophylactic treatment for pre-eclampsia, which was subsequently found not to be effective [10]. The lack of therapeutic efficacy for aspirin in pre-eclampsia has been attributed to a number of factors. Low-dose aspirin may only inhibit maternal platelet TxA_2, the dose given being too low to obtain a prophylactic effect against pre-eclampsia because placental TxA_2 is not inhibited [39]. Maternal ingestion of low-dose aspirin should not affect the fetal placental circulation, since neither TxA_2 nor PGI_2 levels are significantly reduced in umbilical or neonatal blood or urine [40]. Our findings suggest that even if higher doses of aspirin were used, it may still be ineffective in the fetal placental circulation if the reduced vascoconstrictor response to TxA_2 demonstrated *in vitro* also occurs *in vivo*. Perhaps the use of specific TxA_2 receptor antagonists would be more clinically useful in the treatment of pre-eclampsia than blockade of TxA_2 synthesis by low-dose aspirin.

The present study has also demonstrated that fetal placental vasodilator responses *in vitro* to PGI_2 were not changed in pre-eclampsia. Others have provided evidence suggesting enhanced dilator responses mediated by the stable PGI_2 analogue iloprost, in human chorionic arterial rings from the placentae of women with pre-eclampsia [26]. Thus, although the placental production of PGI_2 may be reduced in pre-eclampsia, the vascular smooth muscle mechanisms responsible for the dilator effect are unaffected and may even be enhanced during pre-eclampsia.

In conclusion, the results of this study indicate that the TxA_2 receptor mediated increase in perfusion pressure in the fetal vasculature *in vitro* is reduced in placentae obtained from women with pre-eclampsia. A reduced vascular response to U46619 is consistent with the development in pre-eclampsia of a compensatory mechanism to counter the effects of increased placental levels of TxA_2 seen in this condition.

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