Human Reproduction vol.12 no.1 pp.167–172, 1997

Endothelial nitric oxide synthase in placental villous tissue from normal, pre-eclamptic and intrauterine growth restricted pregnancies*

L.Myatt[1,3], A.L.W.Eis[1], D.E. Brockman[1], I.A.Greer[2] and F.Lyall[2]

[1]Department of Obstetrics and Gynecology, Pediatrics and Molecular and Cellular Physiology, Perinatal Research Institute, University of Cincinnati College of Medicine, PO Box 670526, Cincinnati, OH 45267, USA and [2]Department of Obstetrics and Gynaecology, University of Glasgow, Glasgow Royal Infirmary, 10 Alexandra Parade, Glasgow G31 2ER, UK

[3]To whom correspondence should be addressed

Nitric oxide (NO) regulates blood flow in the human placenta. As increased resistance to blood flow is seen in the fetal–placental vasculature in pregnancies complicated by pre-eclampsia and/or intrauterine growth restriction (IUGR), we examined expression of endothelial nitric oxide synthase (eNOS) in these placentas. Placental villous tissue sections were obtained from normotensive control (n = 5), IUGR alone (n = 5) or pre-eclamptic (with or without IUGR) (n = 9) patients, immunostained for eNOS and scored for localization, type (punctate or diffuse) and intensity of eNOS staining in syncytiotrophoblast and placental vessels. The significance of differences was calculated using the Mann–Whitney *U*-test. No differences in intensity or type of immunostaining in syncytiotrophoblast were seen. Placentas from patients with pre-eclampsia with or without IUGR had a significantly more basal distribution of eNOS in syncytiotrophoblast. eNOS immunostaining was absent in terminal villous capillary and faint in stem villous vessel endothelium of normal placentas, but was intense in the endothelium of both of these types of vessels in the IUGR and pre-eclampsia groups, with significantly greater staining seen in stem vessels of patients with IUGR alone. This increased eNOS expression and hence increased NO production in the fetal–placental vasculature may be an adaptive response to the increased resistance and poor perfusion in these pathological pregnancies.

Key words: endothelium/nitric oxide/placenta/pre-eclampsia/trophoblast

Introduction

Pre-eclampsia is a leading cause of maternal and perinatal morbidity and mortality (Roberts, 1994). It is often associated with fetal growth restriction and affects up to 7% of pregnant women. The aetiology of this disease remains poorly understood. It is unique to pregnancy, but occurs even in the absence of a fetus and resolves with termination of pregnancy and removal of the placenta, and appears to be associated with a placental pathology (Redman, 1991). There is also widespread and increasing evidence for endothelial cell injury or dysfunction in pre-eclampsia (Roberts *et al.*, 1991), perhaps resulting in the generalized vasospasm and poor perfusion seen in many organs. The production by the endothelium of potent vasodilators such as prostacyclin and endothelial-derived relaxing factor, or nitric oxide, has led to the popular theory that the vascular manifestations of pre-eclampsia can be ascribed to deficient production of these mediators. Indeed, inhibition of nitric oxide synthesis in pregnant rats results in a pre-eclampsia-like condition (Buhimschi *et al.*, 1995); an effect that can be reversed by treatment with the vasodilator calcitonin gene-related peptide (Yallampalli *et al.*, 1996). Although a generalized reduction of prostacyclin synthesis is well described in pre-eclampsia (Goodman *et al.*, 1982), the evidence for reduction in nitric oxide synthesis is less convincing (Cameron *et al.*, 1993; Seligman *et al.*, 1994; Lyall *et al.*, 1995).

The nitric oxide radical (NO) is generated from the metabolism of L-arginine by the enzyme nitric oxide synthase (NOS) (Moncada and Higgs, 1993), of which there are three isoforms (Lowenstein *et al.*, 1994). Nitric oxide acts via binding to haem-containing proteins, thus altering their activity (Tsai, 1994). For example, NO from endothelial cells diffuses to underlying smooth muscle where it activates guanylate cyclase, increases cGMP formation and leads to vascular relaxation (Waldman and Murad, 1988). We have described the action of NO in the human fetal–placental vasculature (Myatt *et al.*, 1991, 1992) and mapped the distribution of the endothelial or type III NOS isoform (eNOS) in the vasculature and trophoblast of the human placenta (Myatt *et al.*, 1993).

Using Doppler flow velocity waveforms, it has been reported that resistance to blood flow increases in the fetal–placental vascular tree in pregnancies complicated by either pre-eclampsia and/or intrauterine growth restriction (IUGR) (Trudinger, 1985). This increase has been ascribed to altered vascular reactivity (perhaps dependent on NO synthesis and action) or to alterations in vascular architecture in the placental villous tree (Giles *et al.*, 1985). We have previously described eNOS protein expression in the pre-eclamptic placenta using immunohistochemistry (Ghabour *et al.*, 1995). In contrast to normotensive placentas, eNOS immunostaining was seen in small terminal villous vessels with underlying smooth muscle in pre-eclamptic placentas. It is possible that this expression of eNOS may be secondary to vascular alterations that occur in pre-eclamptic placentas, but may impinge upon regulation of

*Presented in part at the 42nd Annual Meeting of the Society for Gynecologic Investgation, Chicago, March 15–18, 1995.

placental vascular reactivity by the underlying smooth muscle and hence blood flow. In support of this, we have also recently shown that concentrations of nitrate, a breakdown product of NO, were significantly higher at delivery in umbilical venous blood from both pre-eclamptic (Lyall *et al.*, 1995) and IUGR (Lyall *et al.*, 1996) pregnancies compared with controls.

In view of the probable role of NO in regulating blood flow within both the utero–placental and fetal–placental circulations and the preliminary evidence for alterations in NO synthesis in pathological pregnancies (Lyall *et al.*, 1995, 1996), we have extended our previous study and compared the distribution and intensity of eNOS immunostaining in placentas of normal pregnancies and pregnancies complicated by IUGR and pre-eclampsia with or without IUGR.

Materials and methods

Tissues were collected from three groups of patients: (i) normotensive pregnant controls delivered in the third trimester of pregnancy for a maternal indication ($n = 5$); (ii) IUGR, defined as a birthweight less than the fifth percentile using Scottish birthweight tables and Doppler ultrasound of the umbilical artery demonstrating absent end diastolic flow velocity and reduced amniotic fluid volume ($n = 5$); and (iii) pre-eclampsia, defined as a blood pressure of >140/90 mm Hg, and with proteinuria >300 mg/l in a 24 h urine collection with or without IUGR ($n = 9$). In the control group, the indications for delivery were elective delivery for a previous history of preterm abruption ($n = 1$), emergency Caesarean section for ruptured membranes ($n = 1$), placenta praevia ($n = 1$) and antepartum haemorrhage ($n = 2$). In all patients, birthweight, placental weight, gestational age, mode of delivery and smoking habits were recorded. None of the women studied had any evidence of active or potentially infectious processes such as urinary tract infection or chorioamnionitis. Placentas were collected immediately following vaginal delivery or Caesarean section and blocks of villous tissue (1×1×1 cm) were dissected from beneath the basal plate and flash-frozen in liquid nitrogen. Tissue blocks were then kept at –70°C until processed. The study was approved by the ethical committee of the Glasgow Royal Infirmary, UK, and by the Institutional Review Board, University of Cincinnati Medical Center, USA.

Tissue sections were cut at 7 μm on a cryostat and immunostained with a monoclonal antibody H32 against eNOS with fluorescein-linked second antibody (Myatt *et al.*, 1993). This primary antibody does not cross-react with the other isoforms of NOS (type I and II). Sections were first blocked in phosphate-buffered saline (PBS) containing 0.5% bovine serum albumin (BSA) (GIBCO, Grand Island, NY, USA) and 2.5% normal goat serum (GIBCO) for 15 min at room temperature. Blocked tissue sections were then incubated with primary eNOS monoclonal antibody for 3 h at room temperature. Control sections were incubated without primary antibody. Tissue sections were rinsed six times in PBS and incubated for 1 h at room temperature with fluorescein isothiocyanate (FITC)-conjugated goat anti-mouse immunoglobulin (Ig)G in blocking solution. Sections were rinsed again six times in PBS and mounted with 25 mg/ml 1,4-diazobicyclo-(2,2,2)-octane (DABCO) (Sigma, St Louis, MO, USA) in PBS:glycerol (1:9).

Five fields on each section were examined by one investigator who was blinded to the tissue identity and scored (1–3 points) to form a semi-quantitative analysis for intensity, type (punctate or diffuse) and location (apical or basal) of syncytial staining and for intensity of villous vascular staining. The mean value for each patient was then determined. Patient groups were compared using the Mann–Whitney *U*-test for non-parametric data.

Table I. Patient characteristics. Values are given as means ± SD

Group	Control ($n = 5$)	IUGR ($n = 5$)	Pre-eclampsia ($n = 9$)
Age (years)	32.6 ± 4.2	31.0 ± 4.2	26.1 ± 6.7
Smoker	5/5	3/5	3/9
Caesarean section	5/5	5/5	8/9
Gestational age (weeks)	30.9 ± 1.7	35.5 ± 1.3[a]	31.9 ± 3.5
Birth weight (g)	2060 ± 600	1570 ± 231	1660 ± 1080
Placental weight (g)	518 ± 118	326 ± 121[b]	375 ± 145
Placental/birth weight ratio	0.26 ± 0.06	0.22 ± 0.13	0.27 ± 0.10

IUGR = intrauterine growth restriction.
[a]$P < 0.0025$ compared with control (Student's *t*-test).
[b]$P < 0.05$ compared with control (Student's *t*-test).

Results

Data on patient characteristics are shown in Table I. The majority of patients were smokers and 18 out of 19 patients were delivered by Caesarean section. While the mean gestational age of the IUGR group was 35 weeks, the gestational ages of the control and pre-eclampsia groups were significantly less at 31 weeks. Mean birthweights were not significantly different between the groups, although were lower in the pathological pregnancies, while placental weights were significantly lower than controls in the IUGR group. This emphasizes the severity of the disease.

Positive immunostaining for eNOS was seen in the syncytiotrophoblast of every section from each group of patients (Figures 1, 2, 3). When the sections were scored by a blinded observer, no differences in the intensity or type (punctate or diffuse) of staining was seen (Table II). However, in the placental sections from patients with pre-eclampsia (Figures 1b, 3a,b), a significantly more basal distribution of eNOS in syncytiotrophoblast was seen ($P < 0.05$, Table II) when compared with the placentas of the control pregnancies (Figure 1a). In the absence of primary antibody, no eNOS immunostaining was seen (Figure 1c). Comparison of the effect of IUGR with control showed no significant effect on the localization of eNOS in syncytiotrophoblast.

In placental sections from the control pregnancies, there was no eNOS immunostaining present in the terminal villous capillaries and only faint staining in the endothelium of the stem villous vessels (Figure 2c). However, very intense staining of the terminal villous vessel and stem villous vessel endothelium was apparent in the placentas of patients with IUGR (Figure 2a,b). No immunostaining was apparent in the absence of primary antibody (Figure 2d). When tissue from pre-eclamptic pregnancies, with or without IUGR, was examined, strong immunostaining of endothelium in stem villous vessels and in some terminal villous vessels was seen (Figure 3b). The intensity of stem villous vessel endothelium eNOS staining was quantified following scoring by the blinded observer (Table III). The staining intensity in stem villous vessel endothelium appeared to be greater in both groups of patho-

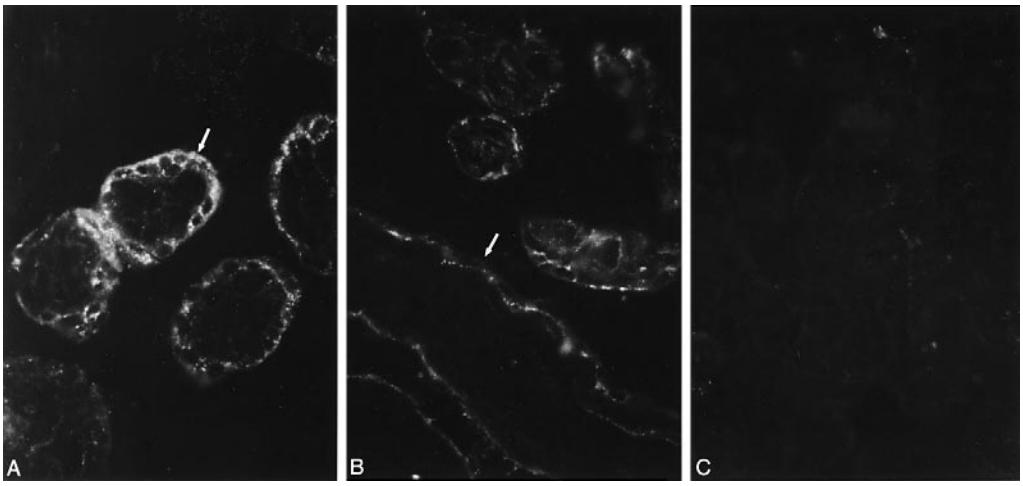

Figure 1. Immunofluorescent staining of villous tissue from (**A**) control normotensive (33 weeks), (**B**) pre-eclamptic pregnancy (37 weeks), both with endothelial nitric oxide synthase (eNOS) antibody, or (**C**) control normotensive pregnancy with no primary antibody. Secondary antibody was conjugated to fluorescein isothiocyanate (FITC). Contrast the diffuse syncytial eNOS staining (arrow) in the control pregnancy (**A**) with the basal distribution of eNOS (arrow) in the pre-eclamptic pregnancy (**B**). Original magnification ×500.

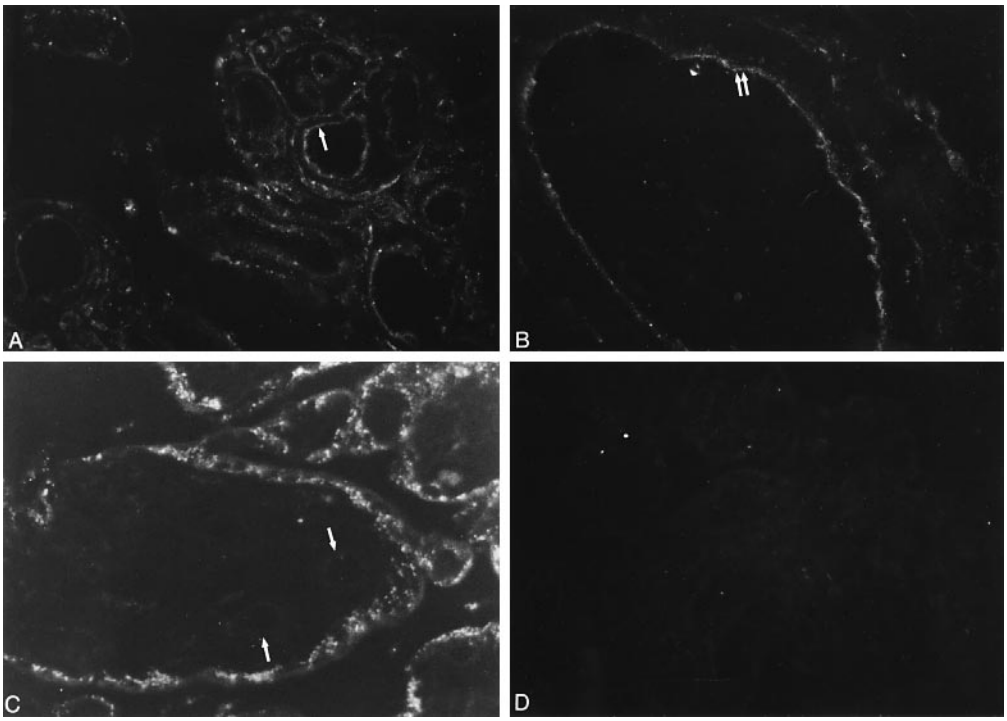

Figure 2. Immunofluorescent eNOS staining of villous tissue from a 35 week pregnancy with intrauterine growth restriction (IUGR) (**A**, **B**), a 38 week control normotensive pregnancy (**C**) or the IUGR pregnancy with no primary antibody (**D**). Secondary antibody was conjugated to fluorescein isothiocyanate (FITC). Intense staining of endothelial cells is seen in the terminal villous vasculature (**A**, single arrow) and stem villous vessels (**B**, double arrow) of the IUGR tissue in contrast to the absence of endothelial staining in the control tissue (**C**, single arrow). Original magnification ×500.

logical placentas. Using non-parametric analysis, immunostaining was significantly stronger in placentas of patients with IUGR compared with controls ($P < 0.05$, Table III). When this was done for the effect of pre-eclampsia, it was not found to be statistically significant.

Discussion

Our previous studies have demonstrated the pharmacological action of NO in the human fetal–placental vasculature *in vitro* (Myatt *et al.*, 1991, 1992). NO appears to contribute both to maintenance of basal vascular tone and to attenuate the action of vasoconstrictors such as endothelin (ET-1) and thromboxane. We have demonstrated that the eNOS isoform is found in the endothelium of the umbilical, chorionic plate and stem villous vessels, but not in the terminal villous capillary endothelium of the normotensive placenta (Myatt *et al.*, 1993). Pre-eclampsia is described as a state of endothelial dysfunction (Roberts *et al.*, 1991) in both maternal and fetal circulations. Evidence has been presented of altered prostaglandin I_2 (PGI_2) (Remuzzi

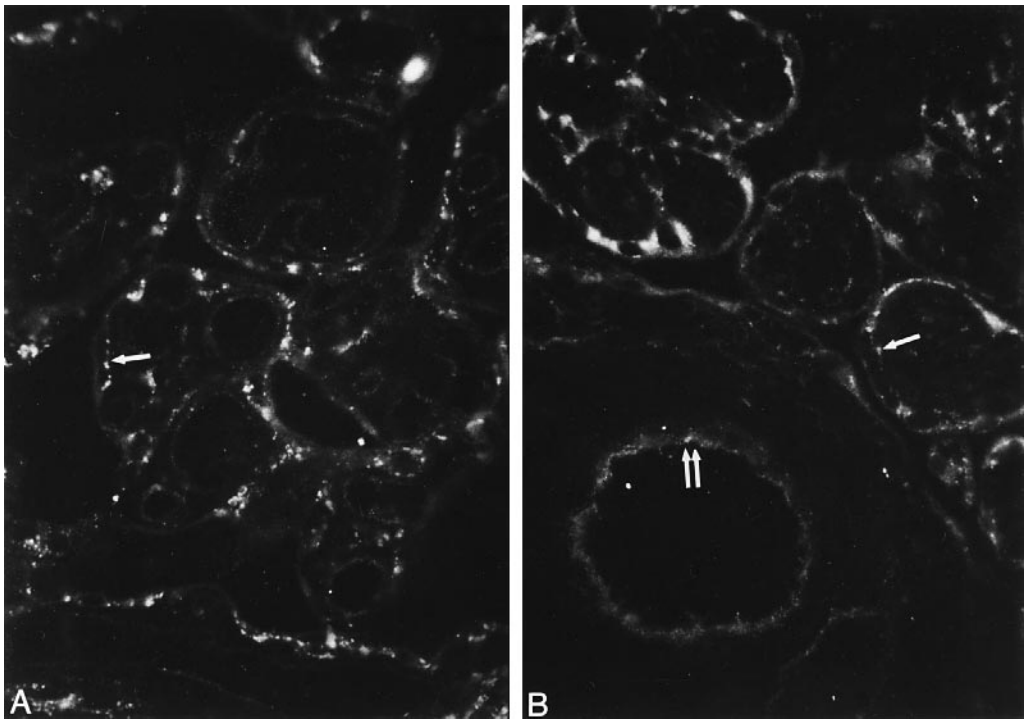

Figure 3. Immunofluorescent staining of villous tissue from (**A**) pre-eclamptic (37 weeks) and (**B**) pre-eclamptic plus intrauterine growth restriction (IUGR) pregnancy (30 weeks) with eNOS antibody and fluorescein isothiocyanate (FITC)-conjugated secondary antibody. Basal synctiotrophoblast staining (single arrow) is seen in both the pre-eclamptic (**A**) and pre-eclamptic plus IUGR (**B**) tissue. Obvious immunostaining is seen in the terminal villous vessels of both tissues (**A**, **B**) and in the stem villous vessels (double arrow) of the pre-eclamptic plus IUGR tissue (**B**). Original magnification ×500.

Table II. Endothelial nitric oxide synthase(eNOS) syncytiotrophoblast immunostaining. Mean values for five fields from each sample are shown

Group		Intensity	Localization
Control	a	2.6	2.8
	b	2.4	3.0
	c	2.0	2.2
	d	2.4	1.6
	e	3.0	2.2
Pre-eclampsia	a	2.6	1.0[a]
	b	2.2	2.4
	c	2.0	1.0
	d	2.2	1.4
	e	3.0	2.2
	f	3.0	2.0
	g	3.0	1.6
	h	1.4	1.0
	i	2.6	1.6
IUGR	a	3.0	2.8
	b	2.0	2.8
	c	2.8	2.2
	d	2.0	1.0
	e	2.4	2.0

IUGR = intrauterine growth restriction.
Intensity: 1 = low; 2 = moderate; 3 = bright.
Localization: 1 = basal; 2 = continuous; 3 = apical.
[a]Z = –2.014, $P < 0.05$ (Mann–Whitney *U*-test) for pre-eclampsia group compared with control.

Table III. Endothelial nitric oxide synthase (eNOS) immunostaining in placental vessels. Mean values for five fields from each sample are shown

Group		Stem	Villous	Capillaries
Control	a	1.2	1.2	1.0
	b	1.9	1.4	1.0
	c	1.3	1.3	NS
	d	2.3	2.1	2.0
	e	1.0	1.0	NS
Pre-eclampsia	a	1.3	1.1	1.0
	b	2.7	1.5	NS
	c	2.4	2.4	1.8
	d	2.7	1.3	NS
	e	1.8	1.2	1.0
	f	1.0	1.0	1.0
	g	2.8	1.6	2.4
	h	2.9	3.0	2.8
	i	2.0	1.3	1.5
IUGR	a	2.6[a]	2.0	2.3
	b	3.0	2.0	2.0
	c	2.4	1.7	1.5
	d	2.4	1.7	2.3
	e	2.0	1.1	1.2

IUGR = intrauterine growth restriction; NS = none seen.
Intensity: 1 = low; 2 = moderate; 3 = bright.
[a]Z = –2.41, $P < 0.05$ (Mann–Whitney *U*-test) for IUGR group compared with control.

et al., 1980) and NO (Pinto *et al.*, 1991) production by umbilical vessels from pre-eclamptic pregnancies, suggestive of endothelial dysfunction.

The fetal circulation in pre-eclampsia and/or IUGR is also characterized by abnormal umbilical blood flow velocity waveforms, thought to be indicative of increased placental resistance (Trudinger *et al.*, 1985). The previllous arterioles are probably the major determinant of resistance in the placental circulation. This increased resistance may be due to altered production of or response to vasoactive agents, but also perhaps to an altered vascular anatomy. Alterations in the vascular

architecture with a reduced number of villous vessels, narrower lumens and thicker (muscular) vessel walls have been described in pre-eclampsia and/or IUGR (Giles *et al.*, 1985). Interestingly, infusion of the NO donor glyceryl trinitrate into the maternal circulation of pregnancies exhibiting such waveforms has been reported to improve the umbilical flow velocity waveforms (Giles *et al.*, 1992), suggesting that NO may cross the placenta and dilate the placental villous vasculature. However, maternal infusion of glyceryl trinitrate to either first trimester normal pregnant women or to women in the second trimester who showed abnormal uterine artery blood flow at 24–26 weeks has been shown to reduce uterine artery resistance as measured by ultrasound examination (Ramsay *et al.*, 1994). Therefore, NO donors will dilate the uterine circulation which may then trigger a response in the umbilical circulation independent of NO transfer. In-vitro perfusion studies were unable to demonstrate that NO infused into the placental intervillous space could dilate the preconstricted fetal–placental circulation (Myatt *et al.*, 1995), supporting the concept that NO does not easily cross the placenta. We have previously shown that there was no overt difference in eNOS immunostaining in the syncytiotrophoblast of the pre-eclamptic placenta when compared with controls (Ghabour *et al.*, 1995). Recently, Conrad and Davis (1995) found no difference in eNOS activity measured in villous trophoblast dissected from normotensive or pre-eclamptic placentas. These current studies confirm and extend these observations. Syncytiotrophoblast eNOS immunostaining was present in the normotensive group and in all groups of pathological placentas including pre-eclampsia and/or IUGR. There was, however, an appreciable difference between the groups in the cellular localization of immunostaining in syncytiotrophoblast, although there was no difference in intensity or type (punctate or diffuse) of immunostaining. A significantly more basal distribution of eNOS immunostaining in syncytiotrophoblast was found in placentas from patients with pre-eclampsia, with or without IUGR, compared with controls or with IUGR alone. This feature therefore appears to be specific to pre-eclampsia. As syncytiotrophoblast functions as an endothelium lining the intervillous space, this difference in cellular syncytiotrophoblast eNOS location may be analogous to other descriptions of endothelial dysfunction in pre-eclampsia. Ultrastructural differences in the trophoblast of pre-eclamptic placentas including decreased pinocytotic activity and a decrease in secretory droplets have been reported (Jones and Fox, 1980). The effect, however, on NO production by syncytiotrophoblast is unknown. There are currently no published reports of NO production by isolated syncytiotrophoblast from the pre-eclamptic placenta, although Wang *et al.* (1994) report that NO release from explants of pre-eclamptic placentas is not different from that of normal placentas.

A striking finding of the present studies was the very intense eNOS staining in terminal villous vessels and stem villous vessel endothelium of the placentas from pathological pregnancies when compared with the controls. This increased intensity, although seen in both the pathological groups was, however, only significantly greater in placentas from patients with IUGR alone. We have previously described this observation in pre-eclamptic pregnancies (Ghabour *et al.*, 1995), but our current findings suggest it is not a unique feature of pre-eclampsia. The common features linking the three groups of pathological pregnancies examined here are the abnormal umbilical flow velocity waveforms (Trudinger *et al.*, 1985) and the alterations reported in the placental vasculature in such pregnancies (Giles *et al.*, 1985). Those alterations include a smaller number of vessels in the terminal villi, a narrowing of the lumen of these vessels and thickening of the smooth muscle in the vessel walls. The increased expression of eNOS detected in these vessels by immunohistochemistry also agrees with our recent observation that concentrations of nitrate, a breakdown product of NO, are increased in umbilical venous blood from pregnancies complicated by pre-eclampsia and IUGR (Lyall *et al.*, 1995, 1996). These observations would also suggest that the altered eNOS expression observed in the villous vessels is not simply a reflection of endothelial dysfunction, for this has been described as a characteristic of pre-eclampsia (Roberts *et al.*, 1991) but not of IUGR. In the pre-eclamptic human placenta, ultrastructural changes in the terminal villous vessels have been described (Jones and Fox, 1980).

The stimulus to increased eNOS expression in villous vessel endothelium is unclear. It may be indirect, resulting from vessel hypertrophy which arises from the increased transmural pressure in the vessels. Alternatively, the stimulus may be direct. The promoter for the eNOS gene has a 'shear stress' response element (Marsden *et al.*, 1992). Decreasing the diameter of villous vessels will increase shear stress over the endothelial cells which may then stimulate eNOS induction and also stimulate growth factor expression which could induce vascular smooth muscle cell growth/hypertrophy (Lyall and Deehan, 1994). This eNOS would then produce NO as a compensatory response to attempt to dilate the vessels and to lower resistance. The expression of eNOS might also be up-regulated by fetal–placental hypoxia, which complicates pre-eclampsia and IUGR. Locally-created focal ischaemia (Zhang *et al.*, 1993) has been shown by immunostaining to increase eNOS expression in rat cerebral vessel endothelium.

As pre-eclamptic and IUGR pregnancies share characteristic abnormal umbilical blood flow velocities indicative of increased placental resistance and abnormal vascular anatomy, we feel that the increased eNOS expression in their vessels is not a disease-specific feature, but perhaps an adaptive response to increased resistance and poor perfusion.

Acknowledgements

Supported in part by NIH grant HL 47860 (L.M.) and by a grant from Action Research (I.A.G. and F.L.).

References

Buhimschi, I., Yallampalli, C., Chwalisz, K. and Garfield, R.E. (1995) Pre-eclampsia-like conditions produced by nitric oxide inhibition: effects of L-arginine, D-arginine and steroid hormones. *Hum. Reprod.*, **10,** 2723–2730.

Cameron, I.T., Van Papendorp, C.L., Palmer, R.M.J. *et al.* (1993) Relationship between nitric oxide synthesis and increase in systolic blood pressure in women with hypertension in pregnancy. *Hypert. Preg.*, **12,** 85–92.

Conrad, K.P. and Davis, A.K. (1995) Nitric oxide synthase activity in placentae from women with preeclampsia. *Placenta*, **16,** 691–699.

Ghabour, M.S., Eis, A.L.W., Brockman, D.E. *et al.* (1995) Immunohistochemical characterization of placental nitric oxide synthase expression in preeclampsia. *Am. J. Obstet. Gynecol.*, **173,** 173–180.

Giles, W., O'Callaghan, S., Bossa, A. *et al.* (1992) Reduction in human fetal umbilical–placental vascular resistance by glyceryl trinitrate. *Lancet*, **340,** 856.

Giles, W.B., Trudinger, B.J., Baird, P.J. (1985) Fetal umbilical artery flow velocity waveforms and placental resistance: pathological correlation. *Br. J. Obstet. Gynaecol.*, **92,** 31–38.

Goodman, R.P., Killam, A.P., Brash, A.R. *et al.* (1982) Prostacyclin production during pregnancy. Comparison of production during normal pregnancy and pregnancy complicated by hypertension. *Am. J. Obstet. Gynecol.*, **142,** 817–822.

Jones, C.J.P. and Fox, H. (1980) An ultrastructural and ultrahistochemical study of the human placenta in maternal preeclampsia. *Placenta*, **1,** 61–76.

Lowenstein, C.J., Dinnerman, J.L. and Snyder, H. (1994) Nitric oxide: a physiologic messenger. *Ann. Intern. Med.*, **120,** 227–237.

Lyall, F. and Deehan, M.R. (1994) Effects of pressure overload on vascular smooth muscle cells. In Lyall, F. and El-Haj, A.J. (eds), *Biomechanics and Cells.* Cambridge University Press, Cambridge, UK, pp. 23–36.

Lyall, F., Young, A. and Greer, I.A. (1995) Nitric oxide concentrations are increased in the feto–placental circulation in preeclampsia. *Am. J. Obstet. Gynecol.*, **173,** 173–180.

Lyall, F., Greer, I.A., Young, A. and Myatt, L. (1996) Nitric oxide concentrations are increased in the feto–maternal circulation in intrauterine growth restriction. *Placenta*, **17,** 165–168.

Marsden, P.A., Schappert, K.T., Chen, H.S. *et al.* (1992) Molecular cloning and characterization of human endothelial nitric oxide synthase. *FEBS Lett.*, **307,** 287–293.

Moncada, S. and Higgs, A. (1993) The L-arginine nitric oxide pathway. *N. Engl. J. Med.*, **329,** 2002–2008.

Myatt, L., Brewer, A.S. and Brockman, D.E. (1991) The action of nitric oxide on the perfused human fetal–placental circulation. *Am. J. Obstet. Gynecol.*, **164,** 687–692.

Myatt, L., Brewer, A.S., Langdon, G. *et al.* (1992) Attenuation of the vasoconstrictor effects of thromboxane and endothelin by nitric oxide in human fetal–placental circulation. *Am. J. Obstet. Gynecol.*, **166,** 224–230.

Myatt, L., Brockman, D.E., Eis, A.L.W. *et al.* (1993) Immunohistochemical localization of nitric oxide synthase in the human placenta. *Placenta*, **14,** 487–495.

Myatt, L., Brewer, A.S. and Brockman, D.E. (1995) Failure of nitric oxide from the maternal intervillous space to regulate villous vascular reactivity in the fetal circulation of the human placenta *in vitro*. *J. Soc. Gynecol. Invest.*, **2,** 258.

Pinto, A., Sorrentino, R. and Sorrentino, P. (1991) Endothelial-derived relaxing factor released by endothelial cells of human umbilical vessels and its impairment in pregnancy-induced hypertension. *Am. J. Obstet. Gynecol.*, **164,** 507–513.

Ramsay, B., De Belder, A., Campbell, S. *et al.* (1994) A nitric oxide donor improves uterine artery diastolic blood flow in normal early pregnancy and in women at high risk of preeclampsia. *Eur. J. Clin. Invest.*, **24,** 76–78.

Redman, C.W.G. (1991) Current topic: preeclampsia and the placenta. *Placenta*, **12,** 301–308.

Remuzzi, G., Marchessi, D., Zoja, C. *et al.* (1980) Reduced umbilical and placental prostacyclin in preeclampsia. *Prostaglandins*, **20,** 105–110.

Roberts, J.M. (1994) Pregnancy-related hypertension. In Creasy, R.K. and Resnik, R. (eds), *Maternal–Fetal Medicine: Principles and Practice.* W.B.Saunders, Philadelphia, USA, pp. 804–843.

Roberts, J.M., Taylor, R.N. and Goldfien, A. (1991) Clinical and biochemical evidence of endothelial cell dysfunction in the pregnancy syndrome preeclampsia. *Am. J. Hypertens.*, **4,** 700–708.

Seligman, S.P., Buyon, J.P., Clancy, R.M. *et al.* (1994) The role of nitric oxide in the pathogenesis of preeclampsia. *Am. J. Obstet. Gynecol.*, **171,** 944–948.

Trudinger, B.J., Giles, W.D., Cook, C.M. *et al.* (1985) Fetal umbilical artery flow velocity waveforms and placental resistance: clinical significance. *Br. J. Obstet. Gynaecol.*, **92,** 23–30.

Tsai, A.L. (1994) How does NO activate hemoproteins? *FEBS Lett.*, **341,** 141–145.

Waldman, S.A. and Murad, F. (1988) Biochemical mechanisms underlying vascular smooth muscle relaxation: the guanylate cyclase–cyclic GMP system. *J. Cardiovasc. Pharmacol.*, **12** (Suppl. 5), S115–S118.

Wang, Y.I., Walsh, S.W., Parnell, R. *et al.* (1994) Placental production of nitric oxide and endothelin in normal and preeclamptic pregnancies. *Hypert. Pregnancy,* **13,** 171–178.

Yallampalli, C., Dong, Y.-L. and Wimalawansa, S.J. (1996) Calcitonin gene-related peptide reverses the hypertension and significantly decreases the fetal mortality in preeclampsia rats induced by N^G-nitro-L-arginine methyl ester. *Hum. Reprod.*, **11,** 895–899.

Zhang, Z.G., Chopp, M., Zaloga, C. *et al.* (1993) Cerebral endothelial nitric oxide synthase expression after focal cerebral ischemia in rats. *Stroke*, **24,** 2016–2022.

Received on July 25, 1996; accepted on October 9, 1996