British Journal of Pharmacology (1995) 115, 1038–1042

Endothelial modulation of vasoconstrictor responses to endothelin-1 in human placental stem villi small arteries

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1 The aim of this study was to assess the role of endothelial cells in the modulation of vasocontractile responses to endothelin-1 (ET-1) of human placental vasculature.

2 Isolated stem villi small arteries (diameter = 170–250 μm) were obtained from healthy parturients who underwent caesarean surgery during the 39th week of pregnancy for cephalo-pelvic disproportion. Isometric tension was measured in vascular rings mounted in a myograph system and challenged with ET-1 (10^{-12} to 10^{-6} M).

3 The vasocontractile response to ET-1 was significantly ($P<0.0001$) increased in endothelial-denuded (active tension = 1156 ± 214 mN mm^{-1}) as compared with endothelial-preserved vascular rings (active tension = 458 ± 48 mN mm^{-1}). This difference was significantly ($P<0.05$) but only partly abolished by the NO synthase inhibitor N^{ω}-nitro-L-arginine (L-NOARG, 10^{-4} M).

4 In endothelial-preserved rings submaximally precontracted with 5-hydroxytryptamine (10^{-6} M), ET-1 (10^{-12} to 10^{-9} M) induced dose-dependent relaxation (maximum relaxation = 70 ± 7%) at 10^{-9} M, which was followed, at higher doses (10^{-8} to 10^{-6} M), by a contraction. In contrast, no relaxation was seen in endothelial-denuded rings. The relaxation in rings with endothelium was significantly ($P<0.001$) reduced by L-NOARG (10^{-4} M). Moreover, it was totally abolished by combined pretreatment with L-NOARG (10^{-4} M) and the sulphonylurea glibenclamide (10^{-5} M).

5 In conclusion, endothelial cells modulate the vascular responses to ET-1 through the release of NO and a substance acting on the ATP-sensitive K^+ channel of smooth muscle of stem villi small arteries from healthy parturients.

Keywords: Nitric oxide; endothelium-derived hyperpolarizing factor; glibenclamide; ATP-sensitive K^+ channels

Introduction

Pharmacological responses of isolated blood vessels to various vasoactive agonists are modulated by endothelial cells which can release both dilator and constrictor substances. Dilator substances include nitric oxide (NO) and an unidentified endothelium-derived hyperpolarizing factor (EDHF). NO activates the soluble enzyme guanylate cyclase, whereas EDHF hyperpolarizes vascular smooth muscle by opening membrane K^+ channels (Taylor & Weston, 1988). Although recent evidence suggests that NO also causes hyperpolarization (Bolotina *et al.*, 1994), as does EDHF, it is still debated as to whether NO and EDHF are the same or two different compounds (Tare *et al.*, 1990).

Constrictor substances are mainly represented by a family of structurally related 21 amino-acid peptides, termed endothelin (ET) (Yanagisawa *et al.*, 1988), which includes three different isoforms, ET-1, ET-2 and ET-3 (Inoue *et al.*, 1989). The vasoactive properties of ET-1 are complex, depending in part on the dose and route of administration, initial blood pressure in the experimental animal used, and the type of vascular bed (De Nucci *et al.*, 1988; Lippton *et al.*, 1989). ET-1 produces potent and long-lasting vasoconstriction in a large variety of vascular beds from several species (Sakata *et al.*, 1989; Yanagisawa & Masaki, 1989), including man (Maggi *et al.*, 1989). In addition, ET-1 exerts potent mitogenic effects on vascular smooth muscle and mesangial cells (Komuro *et al.*, 1988; Simonson *et al.*, 1989). It is therefore suggested that ET-1 might be an important pathogenic factor for vascular remodelling and vasospasm (Lerman *et al.*, 1991). By contrast, ET-1 also causes vasorelaxation by inducing endothelial release of prostacyclin (De Nucci *et al.*, 1988), NO (Tod & Cassin, 1992; Warner *et al.*, 1989), and EDHF (Nakashima & Vanhoutte, 1993). It is also likely that part of the vasodilator effect of ET-1 is mediated by activation of K^+ channels on vascular smooth muscle, as these effects are inhibited by various K^+ channel blockers (Hasunuma *et al.*, 1990; Lippton *et al.*, 1991).

The foeto-placental vasculature is not innervated (Fox & Khong, 1990). Modulation of its tone thus relies entirely on local and/or circulating vasoactive factors. Specific and high affinity binding sites for ET-1 have been characterized on smooth muscle of foeto-placental vasculature (Robaut *et al.*, 1991; Mondon *et al.*, 1993). As ET-1 immunoreactivity is detected in endothelium of placental vessels and in villi syncytiotrophoblast (Ferré *et al.*, 1993), a paracrine and autocrine action of the peptide in placental physiology is likely. Whilst it is known that ET-1 exerts potent vasoconstrictor effects on foeto-placental blood vessels (Wilkes *et al.*, 1990; Gude *et al.*, 1991; MacLean *et al.*, 1992; Myatt *et al.*, 1992; Sabry *et al.*, 1995), the modulatory role of endothelial cells on ET-1-induced vasoconstriction in this particular vascular bed is as yet uncertain. As the placenta plays a pivotal role in nutrient transport and oxygen delivery from the mother to the foetus, it is important to know whether endothelial dysfunction leads to increased vasoconstriction of foeto-placental vessels to ET-1. We therefore examined the influence of the endothelium and, in particular two of its products, NO and EDHF, on the vascular responses to ET-1. We have chosen to test placental stem villi small arteries in this study as these vessels are believed to contribute to the establishment of the foeto-placental resistance in man (Kaufmann, 1982; Leiser *et al.*, 1991).

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Methods

Tissue preparation

Placentae were collected immediately after elective caesarean section from healthy parturients in whom cephalopelvic disproportion had been diagnosed. The caesarean section was performed whilst the patients were under thiopentone sodium succinylcholine anaesthesia, and it took place during the 39th week of pregnancy, outside the labour period. No preoperative medication except for atropine was given. All parturients underwent normal pregnancy and non had evidence of pulmonary, renal or cardiovascular disorders.

Immediately after delivery, pieces of placenta were excised between the decidual and chorionic plates. The tissue was then immersed in a receptacle filled with preoxygenated Krebs solution and was transported to the laboratory for dissection. Stem villi small arteries were carefully isolated from placenta tissue, cleaned under the light microscope, and cut into rings 2 mm in length and 170–250 μm internal diameter. In some vascular rings, the endothelium was carefully removed by gently rubbing the intimal surface with a knotted human hair (Osol *et al.*, 1989). After dissection, placental stem villi small arteries were mounted under the microscope as vascular rings by threading the vessels onto two tungsten wires (25 μm diameter). The wires were secured to supports connected to a force transducer, on the one hand, and a micrometer on the other (Mulvany & Halpern, 1977). Dissection and mounting of the rings were performed in Krebs solution at room temperature. The composition of the Krebs solution was as follows (in mM: NaCl 118, $NaHCO_3$ 25.5, KCl 5.9, $NaHPO_4$ 1.2, $MgSO_4$ 1.2, $CaCl_2$ 2.5 and glucose 5.6). The buffered solution was bubbled with 95% O_2 and 5% CO_2 and maintained at 37°C by a recirculating heater system.

Once mounted, the vessels were warmed at 37°C and allowed to equilibrate in Krebs solution for 15 min, with an initial internal circumference set to give a slight wall tension of 0.2 mN mm^{-1}. The resting tension-internal circumference relationship was then determined. From this, we applied the Laplace law to calculate the effective pressure, i.e. the pressure necessary to extend the vessel to the measured internal circumference (Mulvany & Halpern, 1977):

$$\text{effective pressure} = \frac{\text{wall tension} \times 2\pi}{\text{internal circumference}}$$

The corresponding curve of effective pressure as a function of internal circumference was then constructed using a computer programme (Shart, AD Instruments Ltd, London). Then, the rings were set to an internal circumference equivalent to 90% of what they would have if relaxed *in situ* under transmural pressure of 80 mmHg. The latter was estimated as in the *in vivo* distension pressure of human stem villous arterioles (Wilkin, 1958). It is known that near-maximal active wall tension is developed when the ring is set at this internal circumference (Mulvany & Aalkjaer, 1990). After completion of the progressive stretching procedure to determine the normalized internal circumference, each individual ring was allowed to equilibrate for 1 h.

Experimental protocol

After an initial challenge with KCl (60 mM), all vessels were washed and allowed to rest until the tension again reached the baseline. Vasoconstrictor effects were tested by challenging rings, both with and without endothelium, with cumulative concentrations of ET-1 (10^{-2} to 10^{-6} M). Vasorelaxant effects of ET-1 (10^{-12} to 10^{-6} M) were tested in rings with endothelium submaximally precontracted with 5-HT (10^{-6} M), a potent vasoconstrictor agonist for the foeto-placental vasculature (Sabry *et al.*, 1995). Some vessels were incubated with N^{ω}-nitro-L-arginine (L-NOARG; 10^{-4} M) for 30 min to inhibit NO synthesis. Others were incubated with both L-NOARG and the sulphonylurea glibenclamide (10^{-5} M), for 20 min to inhibit both NO production and activity of the ATP-sensitive K^+ channel. All vessels were preincubated for 30 min with indomethacin (10^{-5} M) to inhibit synthesis of prostanoids. Relaxation with 10^{-6} M acetylcholine (ACh) was tested in all vascular preparations to assess the functional integrity of endothelium.

Different vascular segments were used at each time due to the long lasting effects of ET-1.

Drugs

ET-1 was obtained from Neosystem (Strasbourg, France), whereas ACh, indomethacin, L-NOARG and 5-HT were purchased from Sigma (St Louis, Missouri, U.S.A.). Glibenclamide was obtained from Hoechst (Paris, France). All drugs were freshly prepared before use. Dilution of all chemicals was carried out in distilled water except for indomethacin, which was dissolved in ethanol (final bath concentration 0.01%).

Statistical analysis

Results were expressed as active tension values (mN mm^{-1}) when comparisons were made between rings obtained from different placentae. For comparisons between rings obtained from the same placenta, results were expressed as % maximal contraction in response to ET-1 (10^{-6} M). ANOVA for repeated measurements was used to compare the effects of ET-1 in rings with and without endothelium, and to assess the effects of L-NOARG either alone or in combination with glibenclamide as compared with control rings. All data are expressed as mean ± s.e.mean. A P value of less than 0.05 was considered significant.

Results

The effects of ET-1 on the vasocontractile responses of rings with and without endothelium are illustrated in Figure 1. Removal of the endothelium resulted in a significantly greater vasoconstrictor response to ET-1, increasing the active tension from 458 ± 48 mN mm^{-1} in rings with endothelium ($n=9$) to 1156 ± 214 mN mm^{-1} in rings without endothelium ($n=7$) ($P<0.0001$) (Figure 1).

The role played by NO in modulation of the vasocontractile response to ET-1 was assessed in a separate set of experiments

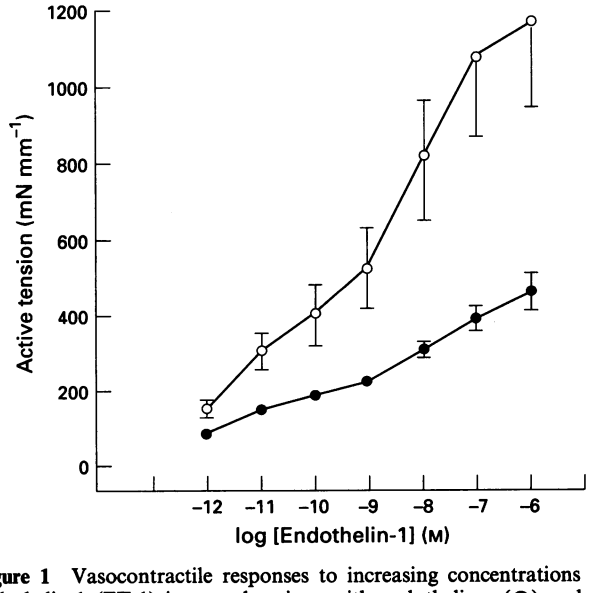

Figure 1 Vasocontractile responses to increasing concentrations of endothelin-1 (ET-1) in vascular rings with endothelium (●) and in rings where the endothelium had been removed (○). Results are expressed as means ± s.e.mean of 7 to 9 observations.

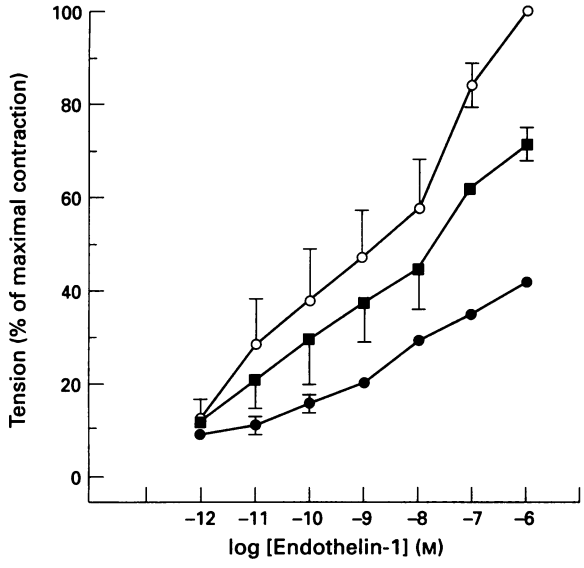

Figure 2 Vasocontractile responses to increasing concentrations of endothelin-1 (ET-1) in vascular rings with endothelium, either untreated (●) or preteated with N^{ω}-nitro-L-arginine (■), and in rings where the endothelium had been removed (○). Results are expressed as means ± s.e.mean of 3 observations.

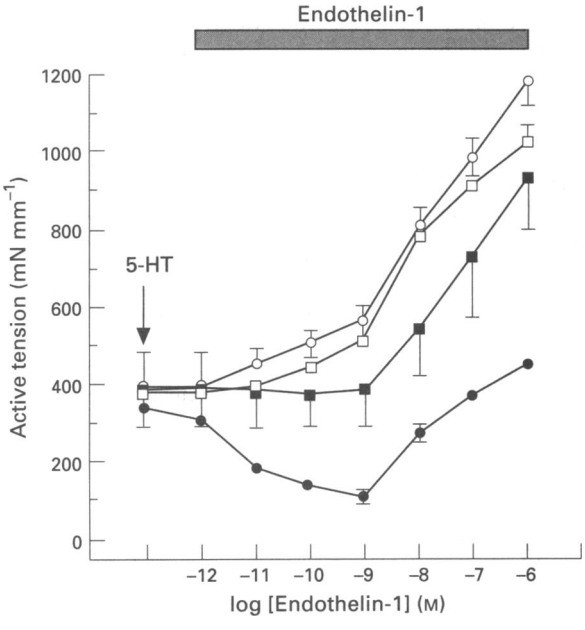

Figure 3 Effects of pretreatment with N^{ω}-nitro-L-arginine (L-NOARG) (■) and the combination of L-NOARG and glibenclamide (□) on responses to endothelin-1 in rings precontracted with 5-hydroxytryptamine (5-HT, 10^{-6} M). Pretreated rings with endothelium were compared with control rings with (●) and without (○) endothelium. Results are expressed as means ± s.e.mean of 4 observations.

where 3 rings obtained from the same placenta were tested at the same time (Figure 2). The first two rings either had their endothelium removed or left intact, whereas the third ring, in which the endothelium was preserved, was pretreated with L-NOARG (10^{-4} M). Thus, 9 rings obtained from 3 placentae were tested in this set of experiments. A significantly ($P<0.001$) greater vasocontractile response to ET-1 was again seen in rings without endothelium as compared with rings with endothelium (Figure 2). However pretreatment with L-NOARG (10^{-4} M) of rings with endothelium significantly ($P<0.05$), but incompletely, abolished the vasorelaxation (Figure 2).

In a last set of experiments, 4 rings obtained from the same placenta were studied at the same time (Figure 3). The first three rings underwent the same protocol as that previously described for Figure 2, whereas the fourth ring was pretreated with a combination of L-NOARG (10^{-4} M) and glibenclamide (10^{-5} M). Thus, 16 rings obtained from 4 placentae were tested in this set of experiments. To determine whether ET-1 had any vasorelaxant effects, increasing concentrations of ET-1 (10^{-12} to 10^{-6} M) were tested in rings submaximally precontracted with 5-HT (10^{-6} M). Rings with endothelium relaxed when low concentrations of ET-1 (10^{-12} to 10^{-9} M) were applied, eliciting maximum relaxation (70 ± 7%) at 10^{-9} M (Figure 3). This relaxation was endothelium-dependent as no relaxation was seen when the endothelium was removed. Moreover, removal of the endothelium caused a further contraction of the rings. The relaxation in rings with endothelium was partly abolished with L-NOARG (10^{-4} M) pretreatment, whereas it was totally abolished by combined pretreatment with L-NOARG (10^{-4} M) and glibenclamide (10^{-5} M) (Figure 3).

Discussion

The main results of this study indicate (i) that endothelial cells exert a modulatory effect on ET-1-induced vasoconstriction in small stem villi arteries from healthy term parturients, and (ii) that this modulatory effect is mediated by vasodilator substances which are released from endothelial cells upon ET-1 stimulation.

In a recent study, we have demonstrated that ET-1 is approximately 1,000 times more potent than 5-HT and phenylephrine in causing vasoconstriction of the stem villi small arteries (Sabry *et al.*, 1995). Data from the present study show that ET-1-induced vasoconstriction is even more accentuated when the endothelium is removed (Figure 1). This suggests that ET-1 stimulates the release of one (or several) vasodilator substances from the endothelium. These dilator substances act, in turn, by a braking mechanism to reduce the ET-1-induced contraction of smooth muscle of the stem villi arteries. This hypothesis is further supported by our findings that ET-1, at low concentrations (10^{-12} to 10^{-9} M), causes relaxation of vascular rings that had been submaximally precontracted with 5-HT. This relaxation is endothelium-dependent, as no relaxation was seen in rings where the endothelium had been mechanically removed. It is likely that at these relatively low concentrations, ET-1 acts mainly on endothelial cells by causing the release of endothelial vasodilator substances. Higher concentrations of ET-1 (10^{-8} to 10^{-6} M) cause vasoconstriction, probably as a result of a predominant effect of ET-1 on vascular smooth muscle.

To determine the nature of vasodilator substances which are released from endothelial cells upon ET-1 stimulation, we examined, in two separate sets of experiments, the influence of the NO synthase inhibitor L-NOARG (Ishii *et al.*, 1990; Moore *et al.*, 1990), and of the combination of L-NOARG and the ATP-sensitive K^+ channel blocker, glibenclamide. Vascular rings preincubated for 30 min with L-NOARG significantly lost their ability to relax with low concentrations of ET-1. This suggests that a major portion of relaxation induced by ET-1 was indeed mediated by NO. Our observation is consistent with reports from previous studies on the role of NO in the vasorelaxant effects of ET-1 in various vascular beds of several species (De Nucci *et al.*, 1988; Sakata *et al.*, 1989; Tod & Cassin, 1992) and extends these results to human foeto-placental vasculature.

The vasorelaxant response to ET-1 is, however, incompletely inhibited by L-NOARG, notwithstanding the fact that large amounts of this NO synthase inhibitor were used. Inefficiency of L-NOARG in completely inhibiting NO production might be one explanation. However, a more likely

explanation is that ET-1 induces the release of vasodilator substances different from NO. Several authors have suggested that EDHF could be one such substance (VanRenterghem, 1988; Brayden, 1990). The identity of EDHF remains elusive (Suzuki & Chen, 1990). However, compelling evidence suggests that this endothelial factor acts mainly on the ATP-sensitive K^+ channel (Standen *et al.*, 1989), which can be specifically blocked by agents such as the sulphonylurea glibenclamide (Quast & Cook, 1989). Because there is some evidence to suggest that NO also causes hyperpolarization of the vascular smooth muscle (Tare *et al.*, 1990), it is not clear whether EDHF can be identified with NO or whether they are two separate endothelial products. However, more recent evidence suggests that hyperpolarization of vascular smooth muscle caused by NO is mediated primarily by activation of a calcium-dependent channel (Archer *et al.*, 1994; Bolotina *et al.*, 1994), probably through an increase in guanosine 3':5'-cyclic monophosphate (cyclic GMP) and subsequent stimulation of cyclic GMP-dependent protein kinase (Archer *et al.*, 1994). It is, therefore, likely that NO and EDHF, which activate two different K^+ channels (i.e., the calcium-dependent K^+ channel and the ATP-sensitive K^+ channel, respectively), are two different compounds. Consistent with this hypothesis is our demonstration of complete inhibition of the vasodilator response to ET-1 by a combination of L-NOARG and glibenclamide. This suggests that the L-arginine-NO pathway on the one hand, and ATP-sensitive K^+ channel activation, on the other, account for all the vasodilator effects of ET-1 in human stem villi arteries.

Compelling evidence now suggests that ET-1 may play a role in the physiology and pathophysiology of the foeto-placental circulation in man. Indeed, plasma levels of ET-1 are elevated in preeclampsia as compared with normal pregnancy (Mastrogiannis *et al.*, 1991; Nova *et al.*, 1991; Clark *et al.*, 1992). Furthermore, high levels of ET-1 are closely related with biological markers of renal impairment in preeclampsia (Clark *et al.*, 1992). ET-1 levels also appear to rise late in gestation in normal pregnant women (Clark *et al.*, 1992). It is still uncertain as to whether increased ET-1 levels could indirectly (through the release of NO and EDHF) contribute to the decrease in vascular resistance, which normally occurs during late gestation (Greiss, 1966; Peeters *et al.*, 1980). Nevertheless, it is likely that functional integrity of the endothelium could affect in a critical manner the vascular responses to ET-1 in both healthy and high risk pregnancies (McQueen *et al.*, 1993).

This study was supported by grants from the Mutuelle Générale de l'Éducation Nationale and from the Délégation à la Recherche Clinique, Assistance Publique-Hôpitaux de Paris.

References

ARCHER, S.L., HUANG, J.M.C., HAMPL, V., NELSON, D.P., SHULTZ, P.J. & WEIR, E.K. (1994). Nitric oxide and cGMP cause vasorelaxation by activation of a charybdotoxin-sensitive K channel by cGMP-dependent protein kinase. *Proc. Natl. Acad. Sci. U.S.A.*, **91**, 7583–7587.

BOLOTINA, V.M., NAJIBI, S., PALACINO, J.J., PAGANO, P.J. & COHEN, R.A. (1994). Nitric oxide directly activates calcium-dependent potassium channels in vascular smooth muscle. *Nature*, **368**, 850–853.

BRAYDEN, J.E. (1990). Membrane hyperpolarization is a mechanism of endothelium-dependent cerebral vasodilatation. *Am. J. Physiol.*, **259**, H668–H673.

CLARK, B.A., HALVORSON, L., SACHS, B. & EPSTEIN, F.H. (1992). Plasma endothelin levels in preeclampsia: elevation and correlation with uric acid levels and renal impairment. *Am. J. Obstet. Gynecol.*, **166**, 962–968.

DE NUCCI, G., THOMAS, R., D'ORLEANS-JUSTE, P., ANTUNES, E., WALDER, C., WARNER, T.D. & VANE, J.R. (1988). Pressor effects of circulating endothelin are limited by its removal in the pulmonary circulation and by the release of prostacyclin and endothelium-derived relaxing factor. *Proc. Natl. Acad. Sci. U.S.A.*, **85**, 9797–9800.

FERRÉ, F., MONDON, F., MIGNOT, T.M., CRONIER, L., CAVERO, I., ROSTÈNE, W. & MALASSINÉ, A. (1993). Endothelin-1 binding sites and immunoreactivity in the cultured human placental trophoblast: evidence for an autocrine and paracrine role for endothelin-1. *J. Cardiovasc. Pharmacol.*, **22** (Suppl. 8), S214–S218.

FOX, S.B. & KHONG, T.Y. (1990). Lack of innervation of human umbilical cord: an immunohistological and histochemical study. *Placenta*, **11**, 59–62.

GREISS, Jr, F.C. (1966). Pressure-flow relationship in the gravid uterine vascular bed. *Am. J. Obstet. Gynecol.*, **96**, 41–47.

GUDE, N.M., KING, R.G. & BRENNECKE, S.P. (1991). Endothelin: release by and potent constrictor effect on the fetal vessels of human perfused placental lobules. *Reprod. Fertil. Dev.*, **3**, 495–500.

HASUNUMA, K., RODMAN, D.M., O'BRIEN, R.F. & MCMURTRY, I.F. (1990). Endothelin 1 causes pulmonary vasodilatation in rats. *Am. J. Physiol.*, **259**, H48–H54.

INOUE, A., YANAGISAWA, M., KIMURA, S., KASUYA, Y., MIYAUCHI, T., GOTO, K. & MASAKI, T. (1989). The human endothelin family: three structurally and pharmacologically distinct isopeptides predicted by three separate genes. *Proc. Natl. Acad. Sci. U.S.A.*, **86**, 2863–2867.

ISHII, K., CHANG, B., KERWIN, J.F., HUANG, Z.J. & MURAD, F. (1990). N^{ω}-nitro-L-arginine: a potent inhibitor of endothelium-derived relaxing factor formation. *Eur J. Pharmacol.*, **176**, 219–223.

KAUFMANN, P. (1982). Development and differentiation of the human placental villous tree. *Bibl. Anat.*, **22**, 29–39.

KOMURO, I., KURIHARA, H., SUGIYAMA, T., TAKAKU, F. & YAZAKI, Y. (1988). Endothelin stimulates c-fos and c-myc expression and proliferation of vascular smooth muscle cells. *FEBS Lett.*, **238**, 249–252.

LEISER, R., KOSANKE, G. & KAUFMANN, P. (1991). In *Placenta Basic Research for Clinical Application*. ed. Soma, H. Basel: Karger.

LERMAN, A., EDWARDS, B.S., HALLETT, J.W., HEUBLEIN, D.M., SANDBERG, S.M. & BURNETT, Jr, J.C. (1991). Circulating and tissue endothelin immunoreactivity in advanced atherosclerosis. *N. Engl. J. Med.*, **325**, 997–1001.

LIPPTON, H.L., COHEN, G.A., MCMURTRY, I.F. & HYMAN, A.L. (1991). Pulmonary vasodilatation to endothelin isopeptides *in vivo* is mediated by potassium channel activation. *J. Appl. Physiol.*, **70**, 947–952.

LIPPTON, H.L., HAUTH, T.A., SUMMER, W.R. & HYMAN, A.L. (1989). Endothelin produces pulmonary vasoconstriction and systemic vasodilation. *J. Appl. Physiol.*, **66**, 1008–1012.

MCLEAN, M.R., TEMPLETON, A.G.B. & MCGRATH, J.C. (1992). The influence of endothelin-1 on human foeto-placental blood vessels: a comparison with 5-hydroxytryptamine. *Br. J. Pharmacol.*, **106**, 937–941.

MAGGI, C.A., GIULIANI, S., PATACCHINI, R., ROVERO, P., GIACHETTI, A. & MELI, A. (1989). The activity of peptides of the endothelin family in various mammalian smooth muscle preparations. *Eur. J. Pharmacol.*, **174**, 23–31.

MASTROGIANNIS, D.S., O'BRIEN, W.F., KRAMMER, J. & BENOIT, R. (1991). Potential role of endothelin-1 in normal and hypertensive pregnancies. *Am. J. Obstet. Gynecol.*, **165**, 1711–1716.

MCQUEEN, J., KINGDOM, J.C.P., CONNELL, J.M.C. & WHITTLE, M.J. (1993). Fetal endothelin levels and placental vascular endothelin receptors in intrauterine growth retardation. *Obstet. Gynecol.*, **82**, 992–998.

MONDON, F., MALASSINÉ, A., ROBAUT, C., VIAL, M., BANDET, J., TANGUY, G., ROSTÈNE, W., CAVERO, I. & FERRÉ, F. (1993). Biochemical characterization and autoradiographic localization of [^{125}I]endothelin-1 binding sites on trophoblast and blood vessels of human placenta. *J. Clin. Endocrinol. Metab.*, **76**, 237–244.

MOORE, P.K., AL-SWAYEH, O.A., CHONG, N.W.S., EVANS, R.A. & GIBSON, A. (1990). L-N^G-nitro-arginine, (L-NOARG), a novel L-arginine-reversible inhibitor of endothelium-dependent vasodilatation *in vitro*. *Br. J. Pharmacol.*, **99,** 408–412.

MULVANY, M.J. & AALKJAER, C. (1990). Structure and function of small arteries. *Physiol. Rev.*, **70,** 921–961.

MULVANY, M.J. & HALPERN, W. (1977). Contractile properties of small arterial resistance vessels in spontaneously hypertensive and normotensive rats. *Circ. Res.*, **41,** 19–26.

MYATT, L., BREWER, A.S. & BROCKMAN, D.E. (1992). The comparative effects of big endothelin-1, endothelin-1, and endothelin-3 in the human fetal-placental circulation. *Am. J. Obstet. Gynecol.*, **167,** 1651–1656.

NAKASHIMA, M. & VANHOUTTE, P.M. (1993). Endothelin-1 and -3 cause endothelium-dependent hyperpolarization in the rat mesenteric artery. *Am. J. Physiol.*, **265,** H2137–H2141.

NOVA, A., SIBAI, B.M., BARTON, J.R., MERCER, B.M. & MITCHELL, M.D. (1991). Maternal plasma level of endothelin is increased in preeclampsia. *Am. J. Obstet. Gynecol.*, **165,** 724–727.

OSOL, G., CIPOLLA, M. & KNUTSON, S. (1989). A new method for mechanically denuding the endothelium of small (50–150 μm) arteries with a human hair. *Blood Vessels*, **26,** 320–324.

PEETERS, L.L., GRUTTERS, G. & MARTIN, C.B. (1980). Distribution of cardiac output in the unstressed pregnant guinea-pig. *Am. J. Obstet. Gynecol.*, **138,** 1177–1184.

QUAST, U. & COOK, N.S. (1989). Moving together: K^+ channel openers and ATP-sensitive K^+ channels. *Trends Pharmacol. Sci.*, **10,** 431–435.

ROBAUT, C., MONDON, F., BANDET, J., FERRÉ, F. & CAVERO, I. (1991). Regional distribution and pharmacological characterization of [^{125}I] endothelin-1 binding sites in human fetal placental vessels. *Placenta*, **12,** 55–67.

SABRY, S., MONDON, F., FERRÉ, F. & DINH-XUAN, A.T. (1995). *In vitro* contractile and relaxant responses of human resistance placental stem villi arteries of healthy parturients: role of endothelium. *Fund. Clin. Pharmacol.*, **9,** 46–51.

SAKATA, K., OZAKI, H., KWON, S.C. & KARAKI, H. (1989). Effects of endothelin on the mechanical activity and cytosolic calcium levels of various types of smooth muscle. *Br. J. Pharmacol.*, **98,** 483–492.

SIMONSON, M.S., WANN, S., MENÉ, P., DUBYAK, G.R., KESTER, M., NAKAZATO, Y., SEDOR, J.R. & DUNN, M.J. (1989). Endothelin stimulates phospholipase C, Na^+/H^+ exchange, c-fos expression, and mitogenesis in rat mesangial cells. *J. Clin. Invest.*, **83,** 708–712.

STANDEN, N.B., QUAYLE, J.M., DAVIES, N.W., BRAYDEN, J.E., HUANG, Y. & NELSON, M.T. (1989). Hyperpolarizing vasodilators activate ATP-sensitive K^+ channels in arterial smooth muscle. *Science*, **245,** 177–180.

SUZUKI, H. & CHEN, G. (1990). Endothelium-derived hyperpolarizing factor (EDHF): an endogenous potassium-channel activator. *News Physiol. Sci.*, **5,** 212–215.

TARE, M., PARKINGTON, H.C., COLEMAN, H.A., NEILD, T.O. & DUSTING, G.J. (1990). Hyperpolarization and relaxation of arterial smooth muscle caused by nitric oxide derived from the endothelium. *Nature*, **346,** 69–71.

TAYLOR, S.G. & WESTON, A.H. (1988). Endothelium-derived hyperpolarizing factor: a new endogenous inhibitor from the vascular endothelium. *Trends Pharmacol. Sci.*, **9,** 272–274.

TOD, M.L. & CASSIN, S. (1992). Endothelin-1-induced pulmonary arterial dilatation is reduced by N^{ω}-nitro-L-arginine in fetal lambs. *J. Appl. Physiol.*, **72,** 1730–1734.

VAN RENTERGHEM, C., VIGNE, P., BARHANIN, J., SCHMID-ALLIANA, A., FRELIN, C. & LAZDUNSKI, M. (1988). Molecular mechanism of action of the vasoconstrictor peptide endothelin. *Biochem. Biophys. Res. Commun.*, **157,** 977–985.

WARNER, T.D., DE NUCCI, G. & VANE, J.R. (1989). Rat endothelin is a vasodilator in the isolated perfused mesentery of the rat. *Eur. J. Pharmacol.*, **159,** 325–326.

WILKES, B.M., MENTO, P.F., HOLLANDER, A.M., MAITA, M.E., SUNG, S. & GIRARDI, E.P. (1990). Endothelin receptors in human placenta: relationship to vascular resistance and thromboxane release. *Am. J. Physiol.*, **258,** E864–E870.

WILKIN, P. (1958). Morphogénèse. In *Le Placenta Humain: Aspects Morphologiques et Fonctionnels*. ed. Snoeck, J. pp. 23–70. Paris: Masson.

YANAGISAWA, M., KURIHARA, H., KIMURA, S., TOMOBE, Y., KOBAYASHI, M., MITSUI, Y., YAZAKI, Y., GOTO, K. & MASAKI, T. (1988). A novel potent vasoconstrictor peptide produced by vascular endothelial cells. *Nature*, **332,** 411–415.

YANAGISAWA, M. & MASAKI, T. (1989). Endothelin, a novel endothelium-derived peptide: pharmacological activities, regulation and possible roles in cardiovascular control. *Biochem. Pharmacol.*, **38,** 1877–1883.

(Received January 3, 1995
Revised March 22, 1995
Accepted March 30, 1995)