The influence of endothelin-1 on human foeto-placental blood vessels: a comparison with 5-hydroxytryptamine

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1 The vasoconstrictor effect of endothelin-1 (3×10^{-11} M– 10^{-7} M) was studied in successive generations of blood vessels of the foeto-placental vascular tree. These were the human umbilical arteries and veins, primary surface chorionic plate arteries, secondary chorionic plate arteries, tertiary surface chorionic plate arteries and veins and the secondary stem villus arterioles. The responses to endothelin-1 were compared with those to 5-hydroxytryptamine (10^{-9} M– 10^{-5} M). Arterial preparations were gassed with 2.5% O₂, 8% CO₂ balance N₂ and venous preparations were gassed with 5% O₂, 6% CO₂ balance N₂ to simulate the conditions prevalent *in utero*. The influence of increasing the oxygen tension to 16% (that prevalent at birth) on the response to endothelin-1 on the umbilical arteries was also investigated.

2 All the arterial vessels tested were some ten times more sensitive to endothelin-1 than to 5-hydroxytryptamine and the venous preparations were ten times more sensitive to endothelin-1 than were their equivalent arteries. Increasing oxygen tension did not affect the responses to endothelin-1 in the umbilical artery.

3 Whilst the amplitude of the endothelin-1-induced response was uniform throughout the foeto-placental vascular tree, including the stem villus arterioles, the maximum response to 5-hydroxytryptamine decreased with successive generations and it had no significant effect on the stem villus arterioles. The ratios of the responses to 10^{-7} M endothelin-1: 10^{-7} M 5-hydroxytryptamine in human umbilical arteries, primary surface chorionic plate arteries, secondary chorionic plate arteries, tertiary surface chorionic plate arteries and the secondary stem villus arterioles in the vessels (listed in order of decreasing vessel size) were 1:1.2, 1:0.36, 1:0.33, 1:0.35 and 1:0.04 respectively.

4 In conclusion, endothelin-1 is a powerful vasoconstrictor at all levels of the foeto-placental vascular system including the stem villus resistance vessels. It may play an important role in maintaining foeto-placental vascular resistance at the low oxygen tension which exists in this vascular system *in utero*.

Keywords: Endothelin-1; human foeto-placental blood vessels; vasoconstriction

Introduction

Human umbilical and placental vessels are not innervated (Spivack, 1946). Regulation of vascular tone in the foeto-placental circulation must therefore depend on myogenic factors or on vasoactive factors which are produced locally or carried to their site of action by the blood. Endothelin-1 (ET-1) is a potent endothelium-derived vasoconstrictor (Yanagisawa, 1988) and is present in human umbilical vessels (Haegerstrand *et al.*, 1989). The preproendothelin-1 gene has been identified in human umbilical veins (Inoue *et al.*, 1989) and a cDNA encoding ET-1 has been isolated from a placental cDNA library (Itoh *et al.*, 1988). Umbilical vein endothelial cells can release ET-1 over time (Clozel & Frischli, 1989) or in response to thrombin (Kohno *et al.*, 1990). There is also evidence of increased maternal plasma levels of ET-1 during labour or on delivery and large amounts of ET-1 are present in the amniotic fluid (Usaki *et al.*, 1990). Binding sites for endothelin-1 exist in membranes from whole human placenta (Fischli *et al.*, 1989) and in purified membranes of placental sub-chorionic stem villus vessels (Mondon *et al.*, 1990).

Thus, evidence suggests that ET-1 is capable of being present and so, if it is active, it could be a locally produced modulator of foeto-placental vascular resistance. Whilst Haegerstrand *et al.* (1989) have shown that ET-1 is a potent vasoconstrictor of human umbilical vessels, no study has as yet been conducted to examine the effects of ET-1 on the placental surface chorionic plate arteries and the placental sub-chorionic stem villus arterioles. The sub-chorionic stem

villus arterioles are the major site of vascular resistance in the human placenta (Leiser *et al.*, 1991) and if ET-1 is capable of inducing substantial vasoconstriction of these vessels, this would suggest it is an important mediator of placental vascular resistance.

The foetus exists in an environment which is hypoxic by post-natal standards, reflected by the low oxygen tension of blood in the foeto-placental circulation. In the umbilical arteries, blood (returning from the foetus) has a PO_2 of approximately 16 mmHg and in the umbilical venous blood (going to the foetus) it is approximately 30 mmHg (Wulf, 1964). There is evidence which suggests that, in the pithed rat, ET-1 and its analogue [Ala^{1,3,11,15}]endothelin are more powerful pressor agents in hypoxic conditions *in vivo* (MacLean & Hiley, 1989; MacLean *et al.*, 1989) and that ET-1 is also a more powerful vasoconstrictor of the rat mesenteric bed in hypoxic conditions *in situ* (MacLean *et al.*, 1989) and *in vitro* (Douglas *et al.*, 1991). Hence, there is reason to suppose that ET-1 would be capable of playing a role in the maintenance of foeto-placental vascular resistance in the conditions of low PO_2 which prevail *in utero*.

The aim of this study is to assess ET-1-induced vasoconstriction in this unique circulatory system i.e. to assess ET-1-induced vasoconstriction in umbilical vessels and several placental surface chorionic plate arteries and to compare this with its effect in a placental subchorionic plate arteriole. Noradrenaline has no significant vasoconstrictor effect in human foeto-placental vessels and it has been established that 5-hydroxytryptamine (5-HT) is a potent vasoconstrictor of the umbilical artery (MacLennan & McGrath, 1986). Hence, the effects of ET-1 were compared with those of 5-HT. Increasing O₂ tension has been shown to stimulate

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thromboxane A₂ production which results in an increase in the sensitivity of umbilical arteries to 5-HT (Templeton *et al.*, 1991b). This can explain the role of 5-HT and thromboxane A₂ in closing down the umbilical circulation at birth (Tuveo, 1976). The possibility of a similar role for ET-1 was investigated by looking at the effect of a higher oxygen tension, equivalent to that expected at birth, on the activity of ET-1 on umbilical arteries.

A preliminary account of some of this work has been presented to the British Pharmacological Society (MacLean *et al.*, 1991).

Methods

Umbilical cords and placentae were obtained from recent full term normal pregnancies. They were collected from The Queen Mother's Maternity Hospital, Glasgow and used within 1 h of delivery. Tissue was obtained regardless of whether or not the delivery had been drug-free as it has been shown that variability in the responses to agonists in placentae and cords from different women is not related to the method of delivery (MacLennan, 1986). The following vessels were studied and compared: umbilical arteries and veins, primary surface chorionic plate arteries, secondary surface chorionic plate arteries, tertiary surface chorionic plate arteries and veins and the secondary stem villus arterioles (100–180 µm i.d.). All vessels were dissected free, cleaned and cut into rings (2–3 mm wide). All except the stem villus arterioles were suspended in 40 ml organ baths containing Krebs saline at 37°C. The *in vivo* distending pressures of the umbilical artery and chorionic plate arteries are unknown. The rings were stretched to the optimum point of their length active tension relationship as determined by their response to 75 mM KCl at progressive degrees of stretch. Thus, 1.5 g of tension was applied to all arteries, regardless of their situation in the vascular tree. Observation of these vessels show that the smooth muscle/lumen ratio appears to increase with each successive generation which probably accounts for this phenomenon. One gram tension was that which optimised the responses to the veins. Arteries were therefore set up under 1.5 g tension whilst veins were set up under 1 g tension. The composition of the Krebs-bicarbonate saline was as follows (in mM): NaCl 118.4, NaHCO₃ 25, KCl 4.7, KH₂PO₄ 1.2, MgSO₄ 1.2, CaCl₂ 2.5, glucose 11, Na₂EDTA 0.023. The arteries and arterioles were gassed with 2.5% O₂, 8% CO₂ balance N₂ and the veins were gassed with 5% O₂, 6% CO₂ balance N₂ to mimic the levels present *in utero*. In a further series of experiments, the responses to ET-1 in umbilical arteries gassed with 16% O₂, 8% CO₂ balance N₂ was studied (to mimic the levels present at birth). The gas tensions and pH were determined regularly by use of both an oxygen electrode inserted into one of the organ baths and a blood gas analyser (Instrumentation Laboratory system 1302).

The stem villus arterioles were too small to be set up in standard organ baths. Under a microscope, they were dissected out and mounted as ring preparations (approximately 2 mm long) in a microvascular myograph (Mulvany & Halpern, 1976) by threading the vessels onto two 40 µm stainless steel wires and subsequently securing the wires to supports. They were equilibrated for 1 h at 37°C and set, using a computer programme, to a circumference L₁, estimated to be 0.9 times the circumference they would maintain if relaxed and exposed to a transmural pressure of 100 mmHg, the normalised circumference (Mulvany & Halpern, 1977). This resulted in a force of 305 ± 16 mg wt being applied to the vessels (*n* = 7). The actual *in vivo* distending pressures of human stem villus arterioles is unknown. As this normalisation procedure resulted in optimal response of these vessels to both ET-1 and 5-HT, we considered it acceptable to study differences in sensitivity of these vessels to these two agonists under these conditions.

Cumulative concentration-response curves were constructed

to 5-HT (Sigma, Poole) and compared with those elicited by ET-1 (Bio Mac, Glasgow) in the same ring preparations (in random sequence). Results are shown as the absolute response to the vasoconstrictor agent as measured in mg wt force. Stock solutions of ET-1 were 10⁻⁴ M and due to the financial cost of taking the concentration further, the maximum bath concentration used was 10⁻⁷ M. Hence, a true maximum response was only consistently attained in the umbilical arteries and tertiary surface chorionic plate arteries, preventing calculation of accurate EC₅₀ value for ET-1 in all the vessels. To enable a comparison of the responses to ET-1 with those to 5-HT, concentration-response curves to 5-HT and ET-1 were constructed for every ring preparation. The concentration of 5-HT and ET-1 required to induce a response of 200 mg wt (EC_{200mg wt}) was calculated for every ring preparation and mean values calculated. The ratio of ET-1: 5-HT sensitivity was obtained by calculating the antilog [(EC_{200(ET-1)} - EC_{200(5-HT)})]. To compare the magnitude of responses, the responses to 10⁻⁷ M ET-1 and 5-HT were determined.

A paired Student's *t* test was used to assess the significance of differences between the responses to ET-1 and 5-HT and an unpaired Student's *t* test used to assess the significance of differences between the veins and their corresponding arteries.

Results

Blood gas and pH analysis showed that the *in vitro* conditions for the arterial and arteriolar preparations were as follows: PO₂, 14.1 ± 3.1 mmHg; PCO₂, 45 ± 1.5 mmHg; pH 7.27 ± 0.04 and for the venous preparations: PO₂, 30.5 ± 0.6 mmHg; PCO₂, 37.3 ± 1.4 mmHg; pH 7.39 ± 0.01 (all *n* = 6). In those arterial preparations gassed with 16% oxygen, the values were as follows (*n* = 6): PO₂, 122.6 ± 2.8 mmHg; PCO₂, 43.3 ± 1 mmHg; pH 7.3 ± 0.02.

Figure 1 illustrates concentration-response curves for ET-1 and 5-HT in the arterial vessels studied and Table 1 gives a comparison of their EC_{200mg wt} values and their responses at a concentration of 10⁻⁷ M. Only two of the seven stem villus arterioles tested responded to 5-HT. Hence no EC_{200mg wt} value was determined for these vessels. It can be seen that the potency of ET-1 was uniform throughout the arterial tree as was the amplitude of its response at 10⁻⁷ M. ET-1 was some ten times more potent than 5-HT in the vessels tested. The potency of 5-HT did not vary between the arterial preparations but, as mentioned above, it induced a significant contraction in only two out of the seven stem villus arterioles tested. Table 1 shows that the ratio of ET-1: 5-HT sensitivity tended to increase with successive generations of the foeto-placental vasculature and that the increased potency of ET-1 compared with that to 5-HT was most marked in the umbilical vein. Table 1 shows that, unlike with ET-1, the amplitude of response to 5-HT declined with successive chorionic plate arterial generations and was negligible in the subchorionic stem villus arterioles. It also shows that, in all but the umbilical artery, the amplitude of the response to 10⁻⁷ M 5-HT was significantly smaller than that elicited by 10⁻⁷ M ET-1. It can also be seen from Figure 1 that the responses to 5-HT in the primary surface chorionic plate arteries, secondary surface chorionic plate arteries and tertiary surface chorionic plate arteries fall off at concentrations exceeding 3 × 10⁻⁷–10⁻⁶ M.

Figure 2 illustrates concentration-response curves for ET-1 and 5-HT in the umbilical vein and the tertiary surface chorionic plate vein. It compares these responses with the concentration-response curves for the relevant corresponding arteries. Table 1 also gives a comparison of their EC_{200mg wt} values and their responses to 10⁻⁷ M ET-1 and 5-HT. Whilst the potency of 5-HT was the same in the veins and arteries, it can be seen that ET-1 is some ten times more potent in the venous preparations. From Figure 2 and Table 1 it can be

Figure 1 (a). Concentration-response curves for endothelin-1 (ET-1) in umbilical arteries (●) $n = 6$, primary surface chorionic plate arteries (■) $n = 6$, secondary chorionic plate arteries (▲) $n = 6$, tertiary surface chorionic plate arteries (□) $n = 6$ and secondary stem villus arterioles (○) $n = 7$. (b) Concentration-response curves for 5-hydroxytryptamine (5-HT) in umbilical arteries (●) $n = 6$, primary surface chorionic plate arteries (■) $n = 6$, secondary chorionic plate arteries (▲) $n = 6$, tertiary surface chorionic plate arteries (□) $n = 6$ and secondary stem villus arterioles (○) $n = 7$. Responses are expressed as absolute changes in tension (mg wt.). Preparations were gassed with 2.5% O_2 , 8% CO_2 balance N_2 . All values are given as means with the vertical lines representing s.e.mean.

Figure 2 (a). Concentration-response curves for endothelin-1 (ET-1) in umbilical veins (●) $n = 6$, tertiary surface chorionic plate veins (■) $n = 5$, umbilical arteries (○) $n = 6$ and tertiary surface chorionic plate arteries (□) $n = 6$. (b) Concentration-response curves for 5-hydroxytryptamine (5-HT) in umbilical veins (●) $n = 6$, tertiary surface chorionic plate veins (■) $n = 5$, umbilical arteries (○) $n = 6$ and tertiary surface chorionic plate arteries (□) $n = 6$. Responses are expressed as absolute changes in tension (mg wt.). The arteries and arterioles were gassed with 2.5% O_2 , 8% CO_2 balance N_2 and the veins were gassed with 5% O_2 , 6% CO_2 balance N_2 . The values are given as means with the vertical lines representing s.e.mean.

Table 1 $EC_{200 \text{ mg wt}}$ values and maximum responses to 10^{-7} M endothelin-1 (ET-1) and 10^{-7} M 5-hydroxytryptamine (5-HT) in placental and umbilical blood vessels

	$-\log \text{ concentration of the } EC_{200 \text{ mg wt}}$			Response to 10^{-7} M (tension [mg wt.])		
	ET-1	5-HT	Ratio ET-1:5-HT	ET-1	5-HT	n
Umbilical artery	8.3 ± 0.2	$7.6 \pm 0.1^*$	5.0	562 ± 200	690 ± 150	6
1° Surface chorionic plate artery	8.1 ± 0.2	$6.7 \pm 0.1^{**}$	31.6	623 ± 100	$224 \pm 79^*$	6
2° Surface chorionic plate artery	7.8 ± 0.2	$6.6 \pm 0.2^{**}$	15.8	458 ± 63	$153 \pm 60^*$	6
3° Surface chorionic plate artery	8.0 ± 0.4	$6.4 \pm 0.3^*$	39.8	450 ± 110	$156 \pm 50^*$	6
2° Stem villus arteriole	7.9 ± 0.2	—	—	460 ± 65	$20 \pm 16^{***}$	7
Umbilical vein	$10.0 \pm 0.8^\dagger$	$7.5 \pm 0.4^*$	316.2	856 ± 265	757 ± 250	6
3° Surface chorionic plate vein	$8.6 \pm 0.2^\dagger$	$7.0 \pm 0.5^{**}$	39.8	580 ± 150	$70 \pm 5^*$	5

All arterial preparations were gassed with 2.5% oxygen, 8% carbon dioxide, balance nitrogen whilst the venous preparations were gassed with 5% oxygen, 6% carbon dioxide, balance nitrogen. Statistical differences as assessed by the unpaired Student's t test for veins vs. their relevant arteries

$^\dagger P < 0.05$; $^\ddagger P < 0.01$; statistical differences as assessed by the paired Student's t test for 5-HT vs. ET-1

$^* P < 0.05$; $^{**} P < 0.01$; $^{***} P < 0.001$.

seen that ET-1 has a particularly profound effect on the umbilical vein with 10^{-10} M ET-1 being the threshold effective concentration. Figure 2 also demonstrates that concentration-response curves to 5-HT in foeto-placental vessels are often bell-shaped, concentrations over 3×10^{-6} M tending to fall off. This phenomenon is currently under investigation.

Figure 3 shows the responses to ET-1 in the umbilical artery gassed with either 14 mmHg oxygen or 123 mmHg oxygen. Increasing the oxygen tension had no significant effect on the sensitivity to ET-1 in the umbilical artery. In fact the trend, though not significant, was for the increased oxygen tension to decrease absolute responses to ET-1. The $-\log$ concentrations of the $EC_{200 \text{ mg wt}}$ for ET-1 in the umbilical artery in 16% O_2 and 0% O_2 were 7.9 ± 0.2 and 8.3 ± 0.2 respectively. Alternative statistical tests such as analysis of variance also failed to pick up any significant difference in this data.

Discussion

The aim of this study was to investigate the potential of ET-1 to be a locally produced modulator of foeto-placental vascular resistance by studying the ability of ET-1 to induce vasoconstriction in successive generations of the human foeto-placental vasculature. This is a unique system existing in a low oxygen environment *in utero*, conditions that are known to enhance the effects of ET-1 in other circulatory systems. The results show that ET-1 is a potent agonist at all levels of the foeto-placental circulation. The responses elicited by ET-1 were uniform throughout the foeto-placental vascular tree unlike those to 5-HT which declined with successive generations. Increasing O_2 tension has been shown to stimulate thromboxane A_2 production which results in an increase in the sensitivity of umbilical arteries to 5-HT (Templeton *et al.*, 1991b). It has previously been shown that the maximum responses to thromboxane A_2 diminish with foeto-placental vessel size and that the maximum response achieved in the stem villus arterioles was only 20% of the response seen in the primary surface chorionic plate arteries (Templeton *et al.*, 1991a). The stem villus arterioles are the

Figure 3 The effect of increasing oxygen tension from 2.5% (+ 8% CO_2 balance N_2) to 16% (+ 8% CO_2 balance N_2) on the responses to endothelin-1 (ET-1) in the umbilical artery. (●) ET-1 at 16% oxygen, (○) ET-1 at 2.5% oxygen. $n = 6$ for both groups. Responses are expressed as absolute changes in tension (mg wt.). The values are given as means with the vertical lines representing s.e.mean.

main site of resistance in the foeto-placental vasculature (Leiser *et al.*, 1991), they are not innervated and as other vasoactive agents have little effect on these vessels, locally produced endogenous ET-1 is the best candidate that we have found, so far, to play a significant role in the maintenance of foeto-placental vascular resistance. Placental vascular ET-1 synthesis may therefore be of great physiological importance.

The results of this study show that ET-1 is an extremely effective vasoconstrictor in the umbilical vein compared with 5-HT with 10^{-10} M being the threshold concentration for a marked vasoconstriction. It may, therefore, play an important role in maintaining umbilical venous pressure and foetal blood supply. Systemic plasma levels of ET-1 have been measured and are in the pM range (Tomita *et al.*, 1989; Saito *et al.*, 1990; Usaki *et al.*, 1990). However, it has been shown by Ånggård *et al.* (1989) that following left ventricular injection of ^{125}I -labelled ET-1, 60% of the ET-1 was removed from the circulation in the first minute after injection. The highest uptake being in the lung, followed by the kidney and liver. Hence, it will be local concentrations of ET-1 which determine the response to this peptide and these are likely to be higher than those detected in plasma. During labour, maternal ET-1 plasma levels can increase though the concentration in the foeto-placental circulation could well be higher still. Indeed, the level of ET-1 detected in the amniotic fluid on delivery exceeds 10 pM (Usaki *et al.*, 1990). The threshold of sensitivity to ET-1 in the vessels studied here were therefore just at physiological levels as measured from the systemic circulation though local levels in the foeto-placental circulation are probably higher than these.

The increase in O_2 tension that occurs at birth is believed to assist in closing down the umbilical circulation by inducing a profound vasoconstriction of the umbilical arteries. This is due to stimulation of thromboxane A_2 production which increases the sensitivity of the umbilical arteries to 5-HT (Templeton *et al.*, 1991b). This study shows that the potency of ET-1 was not increased by increasing oxygen tension in the umbilical artery. These results suggest that the intracellular mechanisms behind the ET-1-induced vasoconstriction are different from those involved in the thromboxane A_2 -sensitive 5-HT response. However, as there is an increase in maternal plasma levels of ET-1 on delivery (Usaki *et al.*, 1990), ET-1 may still play a role in closing down the umbilical circulation at birth i.e. because its concentration changes in contrast to the sensitivity of the vessel to it. In particular, increased levels of ET-1 may play an essential role in closing down the umbilical venous circulation at birth.

In the systemic system ET-1 is a relatively better agonist on veins than on arteries. This has previously been demonstrated in various isolated vessel preparations from the rabbit and dog *in vitro* (D'Orléans-Juste *et al.*, 1988). The results of the present study show that it is also the case in human umbilical blood vessels that ET-1 is more potent on the umbilical vein and the tertiary surface chorionic plate vein than in the corresponding arteries, i.e. the umbilical artery and the tertiary surface chorionic plate artery. However an analogy with the systemic system is inappropriate with respect to the metabolic environment since the umbilical 'vein' contains the oxygenated blood. A closer analogy is with the pulmonary circulation where the veins also carry the oxygenated blood although the absolute PO_2 levels are much higher. There is, however, evidence that the primary site of action of ET-1 in the lung is in the pulmonary veins (e.g. in the guinea pig: Horgan *et al.*, 1991) suggesting that there is a greater 'sensitivity' of venous smooth muscle cells to ET-1 (e.g. greater receptor number or affinity).

In conclusion, ET-1 is a potent agonist at all levels of the foeto-placental circulation and hence ET-1 has a unique capacity to maintain foeto-placental vascular resistance in the hypoxic conditions that prevail *in utero*. The increase in ET-1 levels observed during labour and at birth may assist in closing down the umbilical circulation at birth.

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