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The effect of intra-arterial endothelin on resting blood flow and sympathetically mediated vasoconstriction in the forearm of man

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1 The hypothesis that endothelin (ET) influences sympathetically mediated vasoconstriction was investigated in 13 healthy, male subjects.

2 ET (1 pmol min^{-1}) was infused for 60 min into the left brachial arteries of seven healthy male subjects. Resting forearm blood flow, and sympathetic vasoconstriction produced by lower body negative pressure (LBNP; 15 mm Hg), was measured in both arms by strain gauge plethysmography. In a further six subjects, noradrenaline (NA) was infused intra-arterially at doses of 150–600 pmol min^{-1}, with and without co-infusion of ET (1 pmol min^{-1}), with blood flow measured in both forearms.

3 ET produced a small but significant reduction of blood flow in the infused forearm from 3.9 ± 0.6 ml 100 ml^{-1} min^{-1} during infusion of saline, to 3.3 ± 0.7 ml 100 ml^{-1} min^{-1} during infusion of ET at 60 min ($P < 0.05$). Blood flow in the non-infused forearm was not altered by ET infusion.

4 NA produced a significant and dose-dependent reduction of blood flow in the infused forearm, from 3.13 ± 0.5 ml 100 ml^{-1} min^{-1} during saline infusion, to 1.49 ± 0.2 ml 100 ml^{-1} min^{-1} with NA at 600 pmol min^{-1} ($P < 0.001$). During co-infusion of ET, blood flow was reduced similarly in the infused arm from 3.15 ± 0.7 ml 100 ml^{-1} min^{-1} during saline infusion to 1.55 ± 0.2 ml 100 ml^{-1} min^{-1} with NA at 600 pmol min^{-1}. Blood flow in the non-infused arm was not altered by ET and NA infusion.

5 During saline infusion, LBNP reduced blood flow in the infused and non-infused forearms from 3.9 ± 0.6 to 3.0 ± 0.4, and from 4.5 ± 0.6 to 3.2 ± 0.5 ml 100 ml^{-1} min^{-1} respectively. Following ET for 60 min, blood flow in the infused and non-infused forearms was similarly reduced by LBNP, from 3.3 ± 0.7 to 2.5 ± 0.4, and from 4.7 ± 1.2 to 3.6 ± 0.7 ml 100 ml^{-1} min^{-1} respectively. Infusion of ET did not affect responses to LBNP.

6 We conclude that ET is a potent vasoconstrictor of resistance vessels in the forearm circulation in man, producing a slowly progressive effect which is unique among the known constrictor agents and supports a role for ET in long-term maintenance of vascular tone. Intra-arterial ET does not, however, appear to affect NA or sympathetically (LBNP) mediated vasoconstriction in the human forearm.

Keywords endothelin sympathetic nervous system forearm blood flow lower body negative pressure

Introduction

Endothelin (ET) is a 21 amino acid peptide first isolated from the medium of porcine aortic endothelial cells in culture (Yanagisawa *et al.*, 1988). It is a potent vasoconstrictor *in vivo* both in animals (Yanagisawa *et al.*, 1988) and in man (Clarke *et al.*, 1989), with a slow onset, and prolonged duration of action.

Evidence from animal studies suggests that there may be an interaction between ET and the sympathetic nervous system. ET attenuates sympathetic activity in the perfused guinea pig femoral artery (Wiklund *et al.*, 1988), at least in part by reducing noradrenaline (NA) release presynaptically. ET increases catecholamine release from bovine adrenal cells in culture (Boarder & Marriott, 1989), and enhances the response to infused

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NA in the perfused rat mesenteric artery (Tabuchi *et al.*, 1989).

In the present experiments, we have investigated the effects of prolonged arterial infusion of ET (60 min) on resting blood flow of the upper limb in man, and on the vasoconstriction produced by lower body negative pressure (LBNP) and NA infusion. The dose of ET used (1 pmol min^{-1}) was selected to produce only a moderate reduction in resting forearm blood flow, based on earlier studies in man (Clarke *et al.*, 1989).

Methods

Thirteen healthy male volunteers, aged between 21 and 24 years, participated in these studies which were conducted with the approval of the St George's Hospital Ethics Committee and with the informed consent of each volunteer. Studies were performed after subjects had rested supine in a quiet clinical laboratory for a minimum of 30 min. Room temperature (between 25 and 27° C) was maintained constant ± 1° C for each study.

Blood flow was measured in both forearms using venous occlusion plethysmography with temperature compensated mercury-in-silastic strain gauges (Whitney, 1953). Collecting cuff pressure was 40 mm Hg and wrist cuff occlusion pressure was 200 mm Hg. Flows were recorded for 10 s in every 15 s, and the mean of the final five measurements of each recording period was used for analysis. A 27 gauge (SWG) steel cannula was inserted into the left brachial artery using lignocaine hydrochloride (1%; Antigen Ltd, Ireland) to provide local anaesthesia. Saline (0.9%; Travenol, UK), ET (1 pmol min^{-1} in 0.9% saline; Penninsula Labs, Europe), and NA (150–600 pmol min^{-1} in 0.9% saline (with ascorbic acid as antioxidant); Winthrop, UK) were given at a rate of 0.5 ml infusate min^{-1} throughout the experiments by means of a constant-rate infusion pump (Harvard 944A).

Lower body negative pressure (LBNP) was applied using the method described by Brown and colleagues (Brown *et al.*, 1966). Subjects rested supine in a plastic-covered steel cage enclosing the lower limbs and hips and sealed above the level of the anterior superior iliac spines. Suction was applied using an industrial vacuum cleaner to produce a constant negative pressure of 15 mm Hg below atmospheric pressure. The alteration from atmospheric pressure was both applied and relieved rapidly.

In the first series of experiments six subjects received saline for 12 min, followed by three incremental doses of NA (150, 300, then 600 pmol min^{-1}), with each dose given for 12 min. After a further 12 min period of saline infusion, subjects received ET (1 pmol min^{-1}) for 12 min before, and then throughout a second period of incremental infusion of NA (150, 300, then 600 pmol min^{-1}), with each dose again given for 12 min. Forearm blood flow was measured for the second 6 min of each 12 min infusion period throughout the study.

In the second series of experiments, seven subjects received saline for 12 min, followed by ET (1 pmol min^{-1}), given for 60 min. Forearm blood flow was measured for the second 6 min of each 12 min period throughout the study. LBNP (15 mm Hg) was applied for the second 3 min of each measurement period.

The effect of ET on responses to LBNP and NA, was assessed by repeated measures analysis of variance followed by application of the Wilcoxon signed rank test where appropriate. Results are presented as mean ± s.e. mean.

Results

NA alone produced a significant and dose related reduction in blood flow in the infused forearm from 3.1 ± 0.5 ml 100 ml^{-1} min^{-1} during saline infusion, to 1.5 ± 0.2 ml 100 ml^{-1} min^{-1} with NA at 600 pmol min^{-1} ($P < 0.001$). During co-infusion of ET (1 pmol min^{-1}), NA reduced blood flow in the infused forearm from 3.2 ± 0.7 ml 100 ml^{-1} min^{-1} during ET alone to 1.6 ± 0.2 ml 100 ml^{-1} min^{-1} with NA at 600 pmol min^{-1} ($P < 0.001$). The absolute and percentage reductions in forearm blood flow (Figure 1) produced by NA were, however, unaffected by co-infusion of ET. Blood flow in the non-infused forearm was not affected by infusion of either NA or ET.

Infusion of ET for 60 min produced a 15% reduction in blood flow in the infused forearm, from 3.9 ± 0.6 ml 100 ml^{-1} min^{-1} during saline infusion to 3.3 ± 0.7 ml 100 ml^{-1} min^{-1} after 60 min ET ($P < 0.05$). Blood flow in the non-infused forearm, during the same period, increased from 4.5 ± 0.6 to 4.7 ± 1.2 ml 100 ml^{-1} min^{-1} (NS) (Figure 2, Table 1).

Blood flow was consistently reduced in both forearms in all subjects by LBNP. During saline infusion, LBNP reduced blood flow from 3.9 ± 0.6 to 3.0 ± 0.4 ml 100 ml^{-1} min^{-1}, and from 4.5 ± 0.6 to 3.2 ± 0.5 ml 100 ml^{-1} min^{-1} in the infused and non-infused forearms respectively. After 60 min ET infusion, LBNP reduced blood flow from 3.3 ± 0.7 to 2.5 ± 0.4 ml 100 ml^{-1} min^{-1}, and from 4.7 ± 1.2 to 3.6 ± 0.7 ml 100 ml^{-1} min^{-1} in the infused and non-infused forearms respectively. The reduction in forearm blood flow produced by LBNP was unaffected by infusion of ET, either in terms of absolute or percentage change in flow (Figure 2, Table 1).

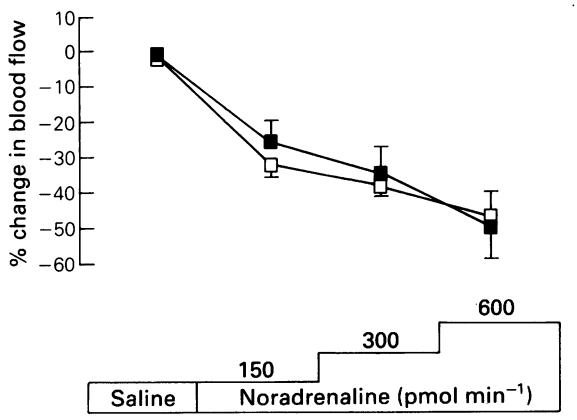

Figure 1 Effect on forearm blood flow of infusion of incremental doses of noradrenaline (150–600 pmol min^{-1}) with (■) and without (□) co-infusion of endothelin (1 pmol min^{-1}).

Table 1 Effect of endothelin (1 pmol min^{-1}) on resting forearm blood flow (mean ± s.e. mean; ml 100 ml^{-1} min^{-1}) and on the response to lower body negative pressure (15 mm Hg; LBNP)

	Time (min)					
	0	12	24	36	48	60
Blood flow: infused arm						
Resting	3.9 ± 0.6	3.8 ± 0.6	3.5 ± 0.7	3.4 ± 0.6	3.3 ± 0.6	3.3 ± 0.7
During LBNP	3.0 ± 0.4	2.8 ± 0.5	2.7 ± 0.5	2.7 ± 0.5	2.6 ± 0.5	2.5 ± 0.4
Blood flow: non-infused arm						
Resting	4.5 ± 0.6	4.5 ± 0.7	4.3 ± 0.9	4.6 ± 1.0	4.7 ± 1.1	4.7 ± 1.2
During LBNP	3.2 ± 0.5	3.2 ± 0.6	3.4 ± 0.6	3.8 ± 0.8	3.6 ± 0.8	3.6 ± 0.7

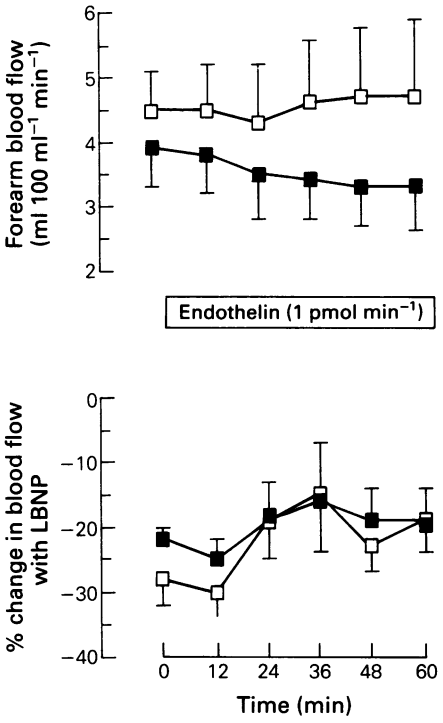

Figure 2 Effect of endothelin (1 pmol min^{-1}), given for 60 min, on resting forearm blood flow (upper panel), and on percentage change in blood flow during lower body negative pressure (lower panel), in the infused (■) and non-infused (□) forearms.

Discussion

Endothelin (ET) is a recently discovered peptide vasoconstrictor (Yanagisawa *et al.*, 1988). Although the potency of ET in the forearm of man is similar to that of angiotensin II (Clarke *et al.*, 1989), the slowly progressive onset and offset of its action (Clarke *et al.*, 1989; Hughes *et al.*, 1989) is unique, differing from the actions of other constrictor agents in the forearm. In the present study in man, as in previous experiments employing a higher dose of ET (5 pmol min^{-1}), there was a slowly progressive reduction in forearm blood flow during ET infusion, maximal at the end of the 60 min infusion period. The maximum 15% reduction of blood flow associated with infusion of ET at 1 pmol min^{-1} compares with a 39% reduction at 5 pmol min^{-1} given for 60 min (Clarke *et al.*, 1989), showing this response to be dose dependent. Experiments with shorter infusions of ET at 5 pmol min^{-1} suggest that the maximum effect does not develop when ET infusion is stopped before 60 min (unpublished observations). The characteristics of the response to ET are compatible with progressive and tight binding of the peptide to its receptor in vascular smooth muscle. These experiments provide no evidence for the tachyphylaxis to ET which has been demonstrated *in vitro* (Hughes *et al.*, 1989) and the characteristics of the response to ET would provide support for its role in long term, rather than short term regulation of vascular tone (Editorial, 1988).

Immunoreactive ET has recently been detected in the plasma of uraemic patients undergoing haemodialysis, with the majority of patients having circulating plasma levels greater than 4×10^{-12} mol l^{-1}, and some as high as 20×10^{-12} mol l^{-1} (Koyama *et al.*, 1989). Infusion of ET (1×10^{-12} mol min^{-1}) in the present study, into a forearm blood flow of approximately 50 ml min^{-1}, would be predicted to produce local concentrations of ET in blood in the order of 20×10^{-12} mol l^{-1}, similar to dialysed patients with the highest plasma levels of ET. If, as our experiments suggest, the response to circulating ET in man is dose dependent, and if the effect on forearm resistance vessels is common to other resistance vessels in man, similar circulating concentrations of ET might be expected to exert a direct effect on arteriolar tone. This view is supported by the results of a recent report of responses to systemic infusion of ET in man (Vierhapper *et al.*, 1990). Infusion of ET at a dose sufficient to provide a circulating plasma ET concentration of 50×10^{-12} mol min^{-1} produced a significant increase in arterial pressure. Assuming that measurement of plasma immunoreactive ET provides a true reflection of its biological activity, our findings are consistent with the hypothesis that ET could act as a circulating pressor agent in patients with renal failure.

In the present experiments in man, ET had no effect on vasoconstriction in forearm resistance vessels produced either by NA or LBNP. The latter has been shown to be a reliable stimulus for reflex sympathetic vasoconstriction in the upper limb (Ardill *et al.*, 1967) and a moderate degree of LBNP, as with the 15 mm Hg used in our experiments, produces constriction of the resistance vessels of forearm muscle through activation of low pressure cardiopulmonary baroreceptors (Abboud *et al.*, 1979; Johnson *et al.*, 1974), with no change in heart rate or arterial pressure (Johnson *et al.*, 1974). The

absence of an effect of ET on responses to exogenous NA in human resistance vessels *in vivo* indicates that ET does not affect postsynaptic responses to NA in man, contrasting with its enhancement of the response to NA in the rat mesenteric artery (Tabuchi *et al.*, 1989). In the absence of a postsynaptic action, the lack of an effect of ET on the response to LBNP suggests that ET does not affect presynaptic neurotransmitter release in human resistance vessels *in vivo*. This contrasts with its effect on guinea pig femoral artery (Wiklund *et al.*, 1988), and with the enhancement of responses to LBNP produced by the pressor peptide angiotensin II via a presynaptic action (Webb *et al.*, 1988). It is possible, however, that concentrations of ET close to sympathetic nerves in vascular smooth muscle, and generated locally by vascular endothelial cells, may be much higher than those in plasma. If ET acts predominantly as a local rather than circulating mediator, it is not possible to entirely exclude an effect of ET on peripheral sympathetic function in man.

In conclusion, the infusion of ET causes constriction of the resistance vessels in the forearm circulation in man, producing a slowly progressive effect which is unique among the known constrictor agents. The concentrations of ET producing vasoconstriction intra-arterially are similar to those detected in the plasma of patients with renal failure, suggesting that circulating ET may contribute to elevated peripheral vascular tone in this circumstance. Intra-arterial ET does not, however, appear to affect NA or sympathetically (LBNP) mediated vasoconstriction in the human forearm.

References

Abboud, F. M., Eckberg, D. L., Johannsen, U. J. & Mark, A. L. (1979). Carotid and cardiopulmonary baroreceptor control of splanchnic and forearm vascular resistance during venous pooling in man. *J. Physiol.*, **286**, 173–184.

Ardill, B. L., Bannister, R. G., Fentem, P. H., Greenfield, A. D. M. (1967). Circulatory responses of supine subjects to the exposure of parts of the body below the xiphisternum to subatmospheric pressure. *J. Physiol.*, **193**, 57–72.

Boarder, M. R. & Marriott, D. B. (1989). Characterization of endothelin-1 stimulation of catecholamine release from adrenal chromaffin cells. *J. cardiovasc. Pharmac.*, **13** (suppl. 5), S223–S224.

Brown, E., Goei, J. S., Greenfield, A. D. M. & Plassaras, G. C. (1966). Circulatory responses to simulated gravitational shifts of blood in man induced by exposure of the body below the iliac crests to sub-atmospheric pressure. *J. Physiol.*, **183**, 607–627.

Clarke, J. G., Benjamin, N., Larkin, S. W., Webb, D. J., Davies, G. J. & Maseri, A. (1989). Endothelin is a potent long-lasting vasoconstrictor in men. *Am. J. Physiol.*, **257**, H2033–H2035.

Editorial (1988). Yin and yang in vasomotor control. *Lancet*, **ii**, 19–20.

Hughes, A. D., Thom, S. A. McG., Woodall, N., Schachter, M., Hair, W. M., Martin, G. N. & Sever, P. S. (1989). Human vascular responses to endothelin-1: observations *in vivo* and *in vitro*. *J. cardiovasc. Pharmac.*, **13** (suppl. 5), S225–S228.

Johnson, J. M., Rowell, L. B., Niederberger, M. & Eisman, M. M. (1974). Human splanchnic and forearm vasoconstrictor responses to reductions of right atrial and aortic pressures. *Circulation Res.*, **34**, 515–524.

Koyama, H., Tabata, T., Nishzawa, Y., Inoue, T., Morii, H. & Yamaji, T. (1989). Plasma endothelin levels in patients with uraemia. *Lancet*, **i**, 991–992.

Tabuchi, Y., Nakamaru, M., Rakugi, H., Nagano, M. & Ogihara, T. (1989). Endothelin enhances adrenergic vasoconstriction in perfused rat mesenteric arteries. *Biochem. Biophys. Res. Comm.*, **159**, 1304–1308.

Vierhapper, H., Wagner, O., Nowotny, P. & Waldhäusl, W. (1990). Effect of endothelin-1 in man. *Circulation*, **81**, 1415–1418.

Webb, D. J., Seidelin, P. H., Benjamin, N., Collier, J. G., Struthers, A. D. (1988). Sympathetically mediated vasoconstriction is augmented by angiotensin II in man. *J. Hypertension*, **6** (suppl. 4), S542–S543.

Whitney, R. J. (1953). The measurement of volume changes in human limbs. *J. Physiol.*, **121**, 1–27.

Wiklund, N. P., Öhlen, A. & Cederqvist, B. (1988). Inhibition of adrenergic neuroeffector transmission by endothelin in the guinea-pig femoral artery. *Acta physiol. Scand.*, **134**, 311–312.

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.

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