Br. J. Pharmacol. (1988), **95**, 1005–1007

Endothelin induces potent microvascular constriction

[1]S.D. Brain, *J.R. Tippins & **T.J. Williams

Section of Vascular Biology, MRC Clinical Research Centre, Watford Road, Harrow, Middx. HA1 3UJ, *Cardiovascular Research Unit, Royal Postgraduate Medical School, Hammersmith Hospital, Du Cane Road, London W12 0NN and **Department of Applied Pharmacology, Cardiothoracic Institute, Dovehouse Street, London SW3 6LY

Endothelin is a recently discovered peptide produced by endothelial cells. It has been shown to have potent constrictor effects on major arteries *in vitro* and to raise rat blood pressure *in vivo*. The present experiments show that endothelin has a potent constrictor action on the microvasculature. Blood flow changes were measured by a xenon clearance technique in rabbit skin. Endothelin, when injected intradermally into rabbit skin, decreased local blood flow in a dose-dependent manner. Endothelin reduced basal skin blood flow and reversed the increased blood flow induced by a vasodilator. These results show that endothelin, administered extravascularly, has potent vasoconstrictor activity. This adds further support to the suggestion that endothelin may have an important role in the physiological control of blood flow and pressure.

Introduction The 21 amino acid peptide endothelial product, endothelin, has recently been isolated and sequenced (Yanagisawa *et al.*, 1988). The expression of the endothelin gene has been shown to be regulated by vasoactive agents, suggesting that endothelin could have a critical role in the maintenance of blood flow and pressure (Yanagisawa *et al.*, 1988).

Endothelin has potent constrictor effects on major arteries *in vitro* and produces a long lasting pressor response when injected intravenously in the rat (Yanagisawa *et al.*, 1988). If endothelin has an important role in the control of local blood flow, it must be able to exert its effects on resistance vessels at the microvascular level. Previously, we have demonstrated that the 37 amino acid neuropeptide, calcitonin gene-related peptide (CGRP) is a potent and long lasting vasodilator which acts via arterioles to induce increased blood flow in the microvasculature (Brain *et al.*, 1985; 1986). In this study we have examined the ability of endothelin when administered extravascularly to affect basal blood flow and vasodilatation induced by CGRP in the rabbit skin microvasculature, using a multi-site ^{133}xenon clearance technique.

Methods Local blood flow was measured in the dorsal skin of New Zealand White rabbits (2.5–3.5 kg) by a ^{133}xenon clearance method (Williams, 1976; 1979). Rabbits were anaesthetized with sodium pentobarbitone (30 mg kg^{-1}, May and Baker Ltd, Dagenham, England) and the dorsal skin was shaved. The agents under test were mixed with a solution of ^{133}xenon in saline (Amersham International, Amersham, England) and then rapidly injected (0.1 ml volumes) intradermally according to a balanced site pattern. The injections were given in random order with six replicates per sample in each animal. After a 15 min clearance period the rabbits were killed by barbiturate overdose. The dorsal skin was removed and injected sites were punched out and counted for radioactivity. Changes in blood flow were calculated as % change in blood flow in test sites compared with that at control, phosphate buffered saline (PBS)-injected sites (Williams, 1979). Endothelin was purchased from Peninsula Laboratories, St Helens, England. Aliquots of 1 nmol 0.1 ml^{-1} were stored at −20°C in PBS until just before use. Other vasoconstrictor agents were tested for comparison. Vasopressin, angiotensin II, noradrenaline and neuropeptide Y were purchased from Sigma Chemical Company Ltd, Poole, England.

Results Endothelin acted in a potent and dose-dependent manner to reduce basal blood flow in the rabbit skin microvasculature as shown in Figure 1 and pallor was seen with the highest dose of endothelin. A significant vasoconstrictor effect was observed with 1 pmol endothelin ($P < 0.05$, paired t test). In an experiment where the effects of higher doses of endothelin were investigated a maximal decrease in blood flow was obtained with 10 pmol per site endothelin (endothelin: 1 pmol per site 44.1 ± 1.0% decrease; 10 pmol per site 68.1 ± 2.1% decrease; 100 pmol per site 73.5 ± 1.9% decrease; 1 nmol per site 69.7 ± 3.4% decrease. The results are expressed as the mean ± s.e.mean for $n = 6$ replicate injections and as the percentage decrease in blood flow when compared with PBS-injected sites. CGRP (10 pmol per site) increased skin blood flow as previously reported (Brain *et al.*, 1985). The co-injection of endothelin with CGRP caused an inhibition of the dilator response of CGRP as shown in Figure 1. When a 10 pmol dose of CGRP was injected with a

[1] Author for correspondence.

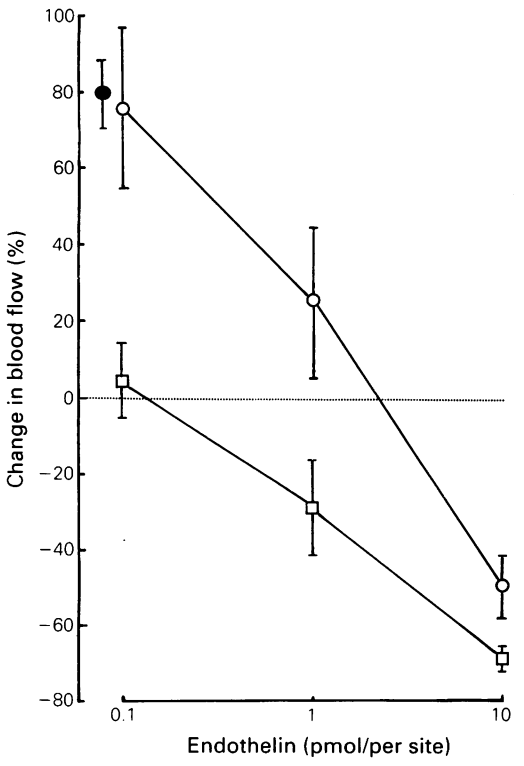

Figure 1 Effect of endothelin on blood flow in rabbit skin. Changes in local blood flow were measured by ^{133}xenon clearance over a period of 15 min after intradermal injections. Endothelin (0.1–10 pmol per site) reduced basal blood flow (□). The vasodilator calcitonin gene-related peptide (CGRP, 10 pmol per site) increased local blood flow (●) which was reversed when CGRP was injected together with endothelin (○). Results are expressed as mean for $n = 5$ rabbits (endothelin alone) and $n = 4$ rabbits (CGRP alone and CGRP + endothelin); vertical bars show s.e.mean. The dotted line represents the control phosphate buffered saline-injected sites.

10 pmol dose of endothelin the net effect was vasoconstriction.

An approximate comparison of potency was obtained by testing 3 doses of endothelin against 3 doses of other vasoconstrictors. Endothelin had a similar potency to that of vasopressin and angiotensin II i.e. a 1 pmol dose gave reduced blood flow of $37.4 \pm 3.8\%$ (endothelin, $n = 9$ rabbits), $51.4 \pm 7.8\%$ (vasopressin, $n = 7$ rabbits), $32.6 \pm 8.5\%$ (angiotensin II, $n = 4$ rabbits). Noradrenaline was approximately 100 times less potent i.e. 100 pmol noradrenaline reduced blood flow by $48.2 \pm 8.3\%$ ($n = 5$ rabbits). Noradrenaline was injected into skin in the presence of the antioxidant ascorbic acid (5×10^{-6} M) which had no effect on basal blood flow (9.8 ± 10.4, mean ± s.e.mean % change, $n = 4$ rabbits, when compared with a site which received PBS). Endothelin was at least 1000 times more active than either human or porcine neuropeptide Y which produced no detectable change in flow at doses up to 1 nmol per site.

Discussion Our results show that endothelin is a potent vasoconstrictor in the microvascular bed, acting to inhibit basal and stimulated blood flow in rabbit skin. Thus, endothelin could have an important role in the control of blood flow and pressure at the microvascular level. Our experiments show that it is of comparable potency as a microvascular vasoconstrictor with vasopressin and angiotensin II and more potent than noradrenaline and neuropeptide Y.

It has been suggested that the production of endothelin by endothelial cells is regulated by haemodynamic conditions, as endogenous mediators such as adrenaline and thrombin regulate gene expression (Yanagisawa *et al.*, 1988). Our experiments show that endothelin is active when injected at extravascular sites. As endothelin has been shown to be released from vascular endothelial cells *in vitro*, we suggest that endothelin, released in low amounts from the abluminal surface of endothelial cells, could exert its vasoconstrictor effect on adjacent smooth muscle cells, thus acting locally to modulate microvascular blood flow. Endothelium-derived relaxant factor (EDRF) released from the endothelium may have a counteracting relaxant effect on smooth muscle cells (Furchgott & Zawadzki, 1980). Yanagisawa and coworkers have shown that the intravenous administration of endothelin causes a long lasting pressor response in the rat. Thus, in situations where excessive stimulation of endothelin production occurs, the role of endothelin could change from that of a locally acting vasoconstrictor to that of a circulating pressor factor. It is therefore essential to determine factors that influence the production of endothelin, which will in turn lead to a greater insight into the importance of this peptide in the control of blood flow and pressure in both physiological and pathological conditions.

References

BRAIN, S.D., TIPPINS, J.R., MORRIS, H.R., MACINTYRE, I. & WILLIAMS, T.J. (1986). Potent vasocilator activity of calcitonin gene-related peptide in human skin. *J. Invest. Dermatol.*, **87**, 533–536.

BRAIN, S.D., WILLIAMS, T.J., TIPPINS, J.R., MORRIS, H.R. & MACINTYRE, I. (1985). Calcitonin gene-related peptide is a potent vasodilator. *Nature*, **313**, 54–56.

FURCHGOTT, R.F. & ZAWADZKI, J.V. (1980). The obli-

gatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. *Nature*, **288**, 373–376.

WILLIAMS, T.J. (1976). Simultaneous measurement of local plasma exudation and blood flow changes induced by intradermal injection of vasoactive substances using ^{131}I-albumin and ^{133}Xe. *J. Physiol.*, **254**, 4–5P.

WILLIAMS, T.J. (1979). Prostaglandin E2, prostaglandin I2 and the vascular changes in inflammation. *Br. J. Pharmacol.*, **65**, 517–524.

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.

(Received July 20, 1988
Accepted August 22, 1988)