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Roles of endothelin receptors in the regional and systemic vascular responses to ET-1 in the anaesthetized ganglion-blocked rat: use of selective antagonists

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1 Endothelin-1 (ET-1) produces vasoconstriction, via activation of ET_A and ET_B receptors on vascular smooth muscle, and vasodilatation via ET_B receptors on endothelial cells. Here we have used the ET_A receptor-selective antagonist, BQ-123, the ET_B receptor-selective antagonist, BQ-788 and the ET_A/ET_B receptor non-selective antagonist, PD 145065, to study the role of these receptors in mediating the haemodynamic changes induced by an infusion of ET-1 to the anaesthetized ganglion-blocked rat.

2 Infusion of ET-1 (10 pmol kg^{-1} min^{-1}) increased the mean arterial pressure (MAP) by 57.5 ± 5.1 mmHg over 70 min. This pressor response was reduced by about 50% by coinfusion of BQ-123 (10 nmol kg^{-1} min^{-1}), but was unaffected by either BQ-788 (10 nmol kg^{-1} min^{-1}) or PD 145065 (10 nmol kg^{-1} min^{-1}).

3 After infusion of ET-1 for 70 min the cardiac output had fallen from 102.6 ± 11.3 to 55.7 ± 7.6 ml min^{-1} and the total peripheral resistance had increased from 3.24 ± 0.6 to 10.0 ± 0.8 mmHg ml^{-1} min^{-1} (per 100g body weight). BQ-123 decreased the magnitudes of these changes whereas BQ-788 potentiated them. PD 145065 was without effect.

4 ET-1 increased the vascular resistances of all the organs studied except the brain and stomach. These changes were attenuated by BQ-123 in the kidneys, skin, adrenal glands and caecum and potentiated by BQ-788 in the kidneys, small intestine, large intestine and mesentery. PD 145065 had little effect on the individual tissues.

5 Thus, BQ-123, a selective ET_A receptor antagonist, inhibits the pressor and vascular constrictor effects of ET-1 more actively than PD 145065. As BQ-788 potentiates some of the vasoconstrictor effects of ET-1 and increases the effects of ET-1 on total peripheral resistance, the predominant role of ET_B receptors in the rat circulation is to limit the pressor effects of ET-1.

Keywords: Endothelin-1; endothelin receptors; endothelin antagonists

Introduction

The vasoconstrictor and pressor effects of endothelin-1 (ET-1) are mediated by ET_A and/or ET_B receptors depending on the species and particular vascular bed (Clozel *et al.*, 1992; McMurdo *et al.*, 1993; Shetty *et al.*, 1993; Warner *et al.*, 1993a,b). It has therefore been assumed that non-selective antagonists would be of greatest therapeutic benefit in those cardiovascular disease states where ET-1 has been implicated (Clozel *et al.*, 1993; Ohlstein *et al.*, 1994). However, this assumption is made less certain by the observation that stimulation of ET_B receptors on the vascular endothelium elevates the release of the vasodilator autacoids, nitric oxide and prostacyclin (De Nucci *et al.*, 1988; see Rubanyi & Polokoff, 1994). Thus, the net effect of endothelin receptor blockade will be determined by the balance between the inhibition of ET_A and ET_B receptors mediating vasoconstriction and ET_B receptors mediating vasodilatation. To investigate this further we have examined the effects of the endothelin receptor antagonists, BQ-123 (ET_A receptor-selective; Ihara *et al.*, 1992), BQ-788 (ET_B receptor-selective; Ishikawa *et al.*, 1994) and PD 145065 (ET_A/ET_B receptor non-selective; Cody *et al.*, 1993) on the systemic and regional vascular changes induced by an infusion of ET-1.

Some of this work has been presented to the British Pharmacological Society (Allcock *et al.*, 1995).

Methods

Surgical procedure

Male Wistar rats 330-430 g were anaesthetized with sodium thiopentone (120 mg kg^{-1}, i.p.) and a cannula introduced into the trachea to facilitate spontaneous breathing. The right femoral artery was cannulated and connected to a pressure transducer (Elcomatic type 750) for the measurement of systemic blood pressure while the left femoral artery was cannulated and connected to a syringe pump (Perfusor VI, Braun) for the later withdrawal of the reference blood sample. Whilst monitoring pressure, a cannula was introduced into the left ventricle via the right carotid artery. The left jugular vein was cannulated for the administration of drugs. Body temperature was maintained at 37°C via a homeothermic blanket regulated by a rectal thermometer (Biosciences, Sheerness, Kent).

Protocol of drug administration

After surgery, animals were allowed to stabilize for 30 min before receiving a 5 min infusion of hexamethonium (10 mg kg^{-1}, i.v.) to remove reflexes which could confuse the analysis of the vascular responses. ET-1 (10 pmol kg^{-1} min^{-1}) or vehicle plus antagonist (10 nmol kg^{-1} min^{-1}; a dose as great as the infusion rate, 600 $\mu l\,h^{-1}$, and availability would allow) or vehicle was infused 20 min later. After a further 70 min the microspheres were injected.

Microsphere injection

^{57}Co-labelled microspheres (15 ± 3 μm diameter), 60,000-80,000, were suspended in 0.3 ml 0.9% w/v saline containing 0.01% w/v polyoxyethylene 80 sorbitan mono-oleate (Tween 80), a detergent added to prevent microsphere aggregation. The microsphere suspension was drawn into a syringe and injected into the left ventricle over a 20 s period. The cannula was then flushed through with a further 0.3 ml of vehicle. A reference arterial blood sample was concurrently withdrawn at a rate of 0.5 ml min^{-1} during and for 70 s following the injection period.

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Quantitation of blood flow

After the reference blood sample had been removed the animals were immediately killed with an air embolism and the tissues dissected out, weighed and placed in vials. Organs with a low blood flow per unit mass (e.g. skin and skeletal muscle) had multiple samples taken to ensure that a minimum of 400 microspheres were counted for each tissue, thus compensating for any random variability in microsphere distribution. The reference blood sample and the injection syringe and cannula were also placed in vials. All the vials were then placed in a gamma counter (Nuclear Enterprises, NE 1600) and counted for radioactivity for 5 min on the same day to avoid count variations due to radioactive decay. The amount of radioactivity, and thus the number of microspheres, injected into the rat were calculated by subtracting the counts for the waste (i.e. microspheres caught in the injection syringe and cannula etc) from the total starting radioactivity, as measured in an aliquot taken just prior to the experiment.

Cardiac output (CO) was calculated by the reference blood sample method described by McDevitt & Nies (1976). The fraction of cardiac output received by an organ and organ vascular resistance were calculated as described by Thomas *et al.* (1988).

Materials

The microspheres were obtained from Du Pont, NEN research products, (Boston, U.S.A.). ET-1 was purchased from the Peptide Institute (Osaka, Japan) and dissolved in 0.9% w/v saline containing 1% bovine serum albumin. Sodium thiopentone (Intraval) was obtained from May and Baker Ltd. (Dagenham, Essex). Hexamethonium and bovine serum albumin were obtained from Sigma Chemical Co. (Poole, Dorset) and were dissolved in saline. BQ-123 (cyclo-(D-Trp-D-Asp-Pro-D-Val-Leu-)) and PD 145065 (Ac-D-Bhg-L-Leu-L-Asp-L-Ile-L-Ile-L-Trp; Bhg = 5H-dibenzyl[a,d]cycloheptene-10,11-dihydroglycine) were supplied by Parke-Davis Pharmaceutical Research, Division of Warner-Lambert Company. BQ-788(N-*cis*-2, 6-dimethylpiperidinocarbonyl-L-γMeLeu-D-Trp (COOMe)-D-Nle-ONa) was from Banyu Pharmaceutical Co., Japan. All antagonists were dissolved in saline.

Statistics

Groups were compared by a Mann Whitney U test and $P < 0.05$ was taken as significant. A one sample test was used to compare normalised data to control. $P < 0.05$ was taken as significant.

Results

Systemic effects

Hexamethonium induced a fall in the mean arterial blood pressure (MAP) from 96.7±2.1 mmHg to 78.5±2 mmHg ($n = 23$) after 20 min. Infusion of ET-1 (10 pmol kg^{-1} min^{-1}) caused an immediate increase in MAP ($P < 0.05$ at 1 min), which after 70 min had risen by 57.5±5.1 mmHg ($n = 5$) (Figure 1). Coadministration of BQ-123 (10 nmol kg^{-1} min^{-1}) delayed the onset of the pressor response to ET-1 such that there was no significant increase in MAP until 15 min ($P > 0.05$, 1 to 15 min). Additionally, coadministration of BQ-123 reduced by about 50% the increase in MAP after 70 min compared to ET-1 alone. Conversely, when BQ-788 (10 nmol kg^{-1} min^{-1}) was co-infused with ET-1, the MAP increased much more rapidly, although after 70 min the change in MAP was not different from that induced by ET-1 alone. PD 145065 produced a similar effect to BQ-788, although the early potentiation of the pressor effect of ET-1 was not so great.

After 70 min, the infusion of ET-1 increased total peripheral resistance (TPR) by >200% and decreased the cardiac output (CO) by approximately 45%. The increase in TPR induced by ET-1 was reduced by BQ-123, potentiated by BQ-788 and unaffected by PD 145065. Similarly the fall in CO induced by ET-1 was attenuated by BQ-123 and potentiated by BQ-788, but unaffected by PD 145065 (see Table 1).

Regional effects

Infusion of ET-1 increased the vascular resistances in almost all organs (Figure 2a–l). ET-1 did not affect the vascular resistance of the brain, and reduced the vascular resistance of the stomach by 50%. The increases in vascular resistance induced by ET-1 in the skin, kidneys, adrenal glands and caecum were reduced by coadministration of BQ-123, whereas BQ-788 potentiated the increases in vascular resistance induced by ET-1 in the small intestine, large intestine, mesentery and pancreas and the kidneys. PD 145065 attenuated only the increase in vascular resistance induced by ET-1 in the caecum and was without effect in the other tissues examined (Figure 2a–l). In the stomach, in contrast to the other tissues, ET-1 infusion caused a fall in vascular resistance, an effect which was reversed by all the antagonists. Finally, it was

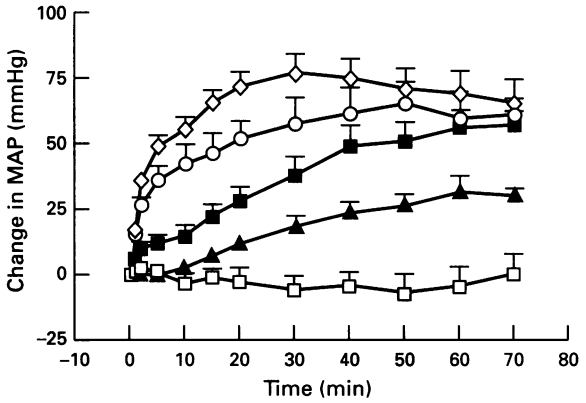

Figure 1 Changes in mean arterial pressure induced by an infusion of vehicle (□; $n = 5$) or ET-1 (10 pmol kg^{-1} min^{-1}) in the presence of vehicle (■; $n = 5$), BQ-123 (10 nmol kg^{-1} min^{-1}) (▲; $n = 4$), BQ-788 (10 nmol kg^{-1} min^{-1}) (◇; $n = 5$) or PD 145065 (10 nmol kg^{-1} min^{-1}) (○; $n = 4$).

Table 1 Systemic parameters after a 70 min infusion of endothelin-1 (10 pmol kg^{-1} min^{-1}) or vehicle in the presence of an endothelin antagonist (10 nmol kg^{-1} min^{-1}) or vehicle

	Control	*ET-1*	*ET-1 + BQ-123*	*ET-1 + BQ-788*	*ET-1 + PD 145065*
MAP (mmHg) before ET-1 infusion	83.6±4.0	82.2±5.9	81.3±3.1	70.9±4.6	75.0±3.1
Increase in MAP (mmHg)	0.7±7.2	57.5±5.1†	30.5±2.5*†	65.3±9.6†	61.4±6.3†
CO (ml min^{-1})	102.6±11.3	55.7±7.6†	88.1±11.3*	33.5±4.7†*	64.3±9.6†
TPR (mmHg ml^{-1} min^{-1}) per 100 g body weight	3.2±0.6	10.1±0.8†	5.2±0.8*	15.6±1.8†*	8.7±1.5†

CO is the cardiac output as calculated by the microsphere method. TPR is the total peripheral resistance calculated from the cardiac output and the mean arterial pressure (MAP), assuming central venous pressure to be zero. Values are given as the mean±s.e.mean of 4–5 determinations. †$P < 0.05$ compared to control values, *$P < 0.05$ compared to ET-1.

noticeable that the lungs received significantly more microspheres in rats treated with ET-1 than in control animals, an effect which was little altered by the endothelin antagonists ($n=4-5$, data not shown).

Treatment with the antagonists alone did not affect TPR, CO or MAP ($n=4$ for each, Table 2). Similarly, vascular resistances of the individual tissues were not significantly different from those in control rats ($n=4$, data not shown), except for the skin, where the vascular resistance was doubled by infusion of BQ-788 (10 nmol kg^{-1} min^{-1}), and the spleen, where BQ-123 induced a small fall in the organ vascular resistance.

Discussion

We have compared the ability of selective and non-selective endothelin antagonists to effect the systemic and regional haemodynamic changes induced by an infusion of ET-1 in the anaesthetized, ganglion-blocked rat. ET_A receptor antagonism, i.e. treatment with BQ-123, was more effective in attenuating the regional and systemic haemodynamic changes induced by ET-1 than non-selective antagonism of both ET_A and ET_B receptors by PD 145065. This is most probably because blockade of ET_B receptors, as shown by treatment with BQ-788, potentiates the regional and systemic vasoconstrictions caused by ET-1.

The increase in MAP induced by ET-1 was due to a strong increase in TPR, despite a fall in CO related most probably to a reduction in stroke volume (Beyer *et al.*, 1994). BQ-123 reduced the speed of the onset of the pressor response to ET-1, such that after 70 min, the increase in MAP was only half that seen when ET-1 was applied alone. Similarly, most of the regional increases in vascular resistance caused by ET-1 were largely suppressed by BQ-123, as has been reported before in individual tissues such as the skin (Lawrence & Brain, 1994) and kidneys (Cristol *et al.*, 1993). Thus, it is clear that in the rat, activation of ET_A receptors primarily drives vasoconstriction. Indeed, in this species the abilities of antagonists to reverse the pressor effects of ET-1 are directly related to their binding affinity to ET_A, but not ET_B, receptors (Sargent *et al.*, 1995).

In the presence of BQ-123 there was still a residual pressor response to ET-1, which may have been mediated by unblocked ET_A receptors, or more likely by ET_B receptors as has been suggested previously (Clozel *et al.*, 1992; McMurdo *et al.*, 1993). However, selective blockade of these ET_B receptors with BQ-788 did not reduce the increase in MAP induced by ET-1 after 70 min, but rather accelerated its onset, as has been shown following a bolus injection of ET-1 (Ishikawa *et al.*, 1994). This was associated with a potentiation of both the fall in CO and the increase in TPR caused by ET-1. Thus, despite

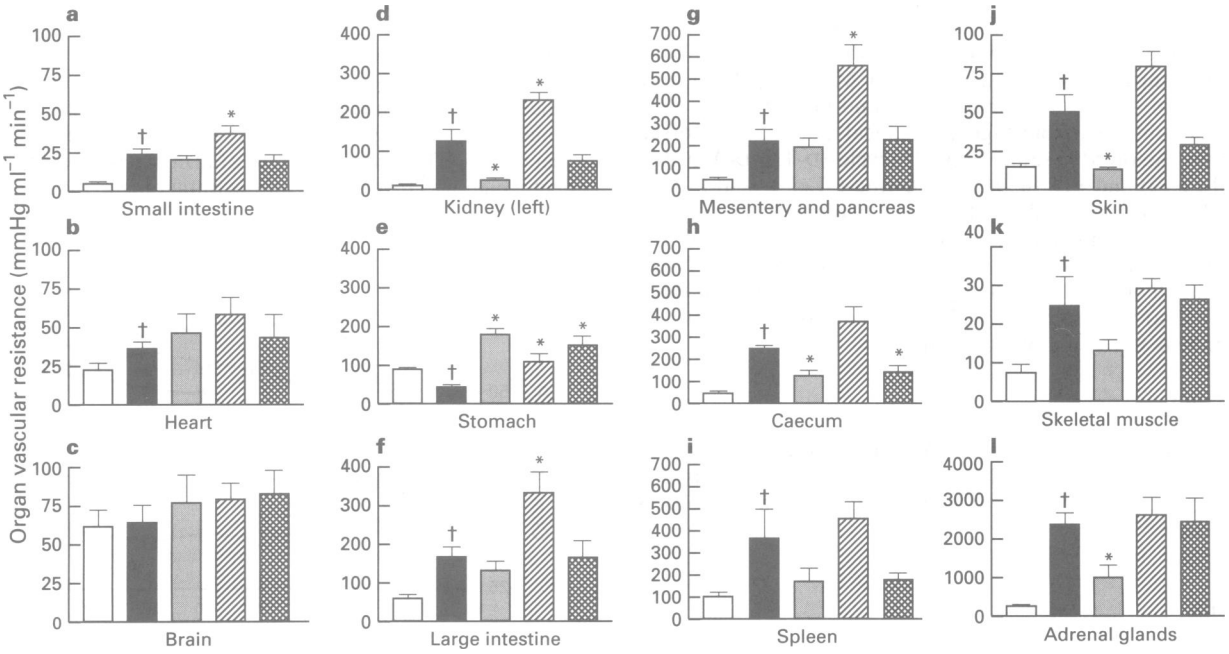

Figure 2 Tissue vascular resistances in rats treated with vehicle (open columns) or ET-1 in the presence of vehicle (solid columns), BQ-123 (stippled columns), BQ-788 (hatched columns) or PD 145065 (cross-hatched columns). Tissue vascular resistances were determined by the microsphere method of determining regional blood flows. For the skin and skeletal muscle, of which the whole tissue masses were not collected, the vascular resistances were calculated by assuming that they comprised, respectively, 32% and 45% of the body mass. Data for the right kidney (not shown) and the left kidney were not different. †$P<0.05$, between control and ET-1 treated animals; *$P<0.05$, between ET-1 and ET-1 plus antagonist-treated rats.

Table 2 Systemic parameters after a 70 min infusion of vehicle in the presence of an endothelin antagonist (10 nmol kg^{-1} min^{-1}) or vehicle

	Control	*+BQ-123*	*+BQ-788*	*+PD 145065*
MAP (mmHg)	83.6±2.7	80.2±2.5	87.5±9.2	85.4±3.5
CO (ml min^{-1})	94.8±5.9	93.3±4.6	87.2±8.6	94.5±11.4
TPR (mmHg ml^{-1} min^{-1}) per 100 g body weight	2.7±0.2	2.7±0.1	3.4±0.1	3.1±0.4

CO is the cardiac output as calculated by the microsphere method. TPR is the total peripheral resistance calculated from the cardiac output and the mean arterial pressure (MAP), assuming central venous pressure to be zero. Values are given as the mean±s.e.mean of 4 determinations. †$P<0.05$ compared to control values.

their presence on the vascular smooth muscle, the predominant role of ET_B receptors in the normotensive, anaesthetized rat is to limit the vasoconstrictor and pressor effects of ET-1. This may be associated with the release of vasodilators such as prostacyclin and nitric oxide from the endothelium (Walder *et al.*, 1989; Warner *et al.*, 1989; Clozel *et al.*, 1992) and possibly other vasodilators (Ohlstein *et al.*, 1990), and/or the clearance of ET-1 from the circulation (Fukuroda *et al.*, 1994a). Interestingly, this limiting effect of ET_B receptors lasted far beyond the period of transient vasodilatation seen following a bolus intravenous injection of ET-1 (Yanigasawa *et al.*, 1988; De Nucci *et al.*, 1988). Thus, prolonged activation of endothelial ET_B receptors may well limit the vasoconstrictor and pressor effects of ET-1. This long term regulation may be at the level of clearance as it has been suggested that the dilator response may become quickly desensitized (Le Monnier de Gouville *et al.*, 1990).

PD 145065, an antagonist of ET_A and ET_B receptors, reduces the constrictor effects of ET-1 in isolated organs (Warner *et al.*, 1993b) and blood vessels with a pA_2 of 6.8 (Cody *et al.*, 1993) at the ET_A receptor. This compares favourably with BQ-123, which has a pA_2 of 6.9 against responses to ET-1 mediated by ET_A receptors in the rat thoracic aorta (Sumner *et al.*, 1992). Additionally, in the isolated perfused kidney 1000 fold excess of PD 145065 (as used in this study) is effective in reversing established vasoconstrictions induced by ET-1 (Warner *et al.*, 1994). However, we have previously found that PD 145065 is less effective in limiting the *in vivo* pressor effects of ET-1 than these *in vitro* assays would suggest (Warner *et al.*, 1994). Our data with BQ-788 suggest very strongly that this is due to the accompanying loss of ET_B receptor-mediated vasodilator and/or ET clearance pathways. This conclusion is also supported by the finding that PD 145065, at the same dose as used here, though unable to reverse an established pressor response to ET-1, reduces the degree of reversal elicited by BQ-123, and readily blocks the depressor response following bolus injection of ET-1 (Warner *et al.*, 1994). Thus, we found that in general terms, PD 145065 produced very little effect against either the pressor or regional vasoconstrictor effects of ET-1. This lack of effect cannot be explained as PD 145065 being inactive at the dose used since PD 145065 attenuated vasoconstrictions induced by ET-1 in the caecum, reduced the ET-1-induced increase in blood flow in the stomach, and accelerated the onset of the pressor response to ET-1.

The vascular changes that we recorded in the kidney agree well with the above hypothesis. Thus, although both ET_A and ET_B receptors mediate vasoconstriction in the rat kidney (Cristol *et al.*, 1993; Gardiner *et al.*, 1994b; Warner *et al.*, 1994; Wellings *et al.*, 1994), the net effect of administration of BQ-788 was to potentiate the renal vasoconstriction induced by ET-1. This suggests that, as in the general circulation, although ET_B receptors can directly mediate renal vasoconstriction, their more important role is to limit the vasoconstrictor effects of ET-1. This also explains why high concentrations of non-selective endothelin receptor antagonists are required to attenuate increases in renal vascular resistance induced by ET-1 (Gardiner *et al.*, 1994a; Wellings *et al.*, 1994).

At first sight, data derived from the intestinal circulation may not appear to agree with the hypothesis put forward above. Thus, in agreement with data derived by Gardiner *et al.* (1994b) using a low dose of ET-1, we found that in tissues receiving blood from the mesentery, such as the small intestine, large intestine and the mesentery and pancreas, vasoconstrictions induced by ET-1 were unaffected by ET_A receptor antagonism. This suggests that in these tissues ET_B receptors play the predominant role in mediating ET-1 induced vasoconstrictions. It may appear contradictory, therefore, that BQ-788 also potentiated vasoconstrictions induced by ET-1 in these organs. However, it should be remembered that BQ-788 will block ET_B receptors on the endothelium in addition to those present on the vascular smooth muscle. Thus, the net effect of administration of BQ-788 may be to reveal a subpopulation of ET_A receptors present on the vascular smooth muscle, as has been shown, for instance, in the rabbit pulmonary artery (LaDouceur *et al.*, 1993). This conclusion would also explain why PD 145065 did not behave like BQ-788 in these tissues, as it will additionally block these ET_A constrictor receptors. Interestingly, as these tissues receive a sizeable fraction of the cardiac output, it may be this that drives the remaining pressor response to ET-1 seen in the presence of BQ-123.

Of all the tissues examined, ET-1 caused a fall in vascular resistance only in the stomach. This at first appears to contradict earlier studies (Wallace *et al.*, 1989) but may be explained by the ability of ET-1 to increase the local production of vasodilator prostanoids (Lopez-Belmonte & Whittle, 1993; 1994). This is likely to be mediated by both ET_A and ET_B receptors (Warner *et al.*, 1993b) explaining why all three antagonists tended to decrease the gastric vasodilatation.

ET-1 infusion caused an increase in the accumulation of microspheres in the lungs which could indicate vasodilatation of the bronchial artery. However, it is more likely that ET-1 causes an increased passage of microspheres through anastomoses into the venous circulation and hence back into the lungs.

The endothelin antagonists alone had no direct effects on any of the systemic parameters measured, which is in agreement with the findings of other groups (Nishikibe *et al.*, 1993; Ishikawa *et al.*, 1994). This clearly suggests that endothelin is not important in the regulation of blood pressure or regional blood flow in normotensive rats. Interestingly, this may contrast with what is found in human subjects, for one group has reported that an endothelin receptor antagonist directly increases forearm blood flow (Haynes & Webb, 1994), implicating endothelin in the maintenance of vascular tone. Thus, species differences mean that care must be taken when using findings from normotensive anaesthetized animals to make estimates of what would occur in humans. In addition, it should be remembered that the expression of endothelin receptors may vary in different pathological states (see, Rubanyi & Polokoff, 1994).

Thus, our data suggest that although both ET_A and ET_B receptors mediate vasoconstriction (Warner *et al.*, 1993a; Fukuroda *et al.*, 1994b), the ET_A receptor is more important for producing the profound systemic haemodynamic changes induced by ET-1 *in vivo*, and the ET_B receptor for limiting these ET_A-mediated effects. These counteractive roles of ET_A and ET_B receptors may explain why non-selective compounds appear less effective anti-pressor agents *in vivo* than would be predicted from their activity *in vitro* (Warner *et al.*, 1994), though clearly this problem may be surmounted by the application of higher doses of antagonist (Gardiner *et al.*, 1994a).

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References

ALLCOCK, G.H., WARNER, T.D. & VANE, J.R. (1995). Antagonism of endothelin-A receptors, but not endothelin-B receptors, attenuates the regional and systemic haemodynamic changes to endothelin-1 in the anaesthetised rat. *Br. J. Pharmacol.*, **114**, 80P.

BEYER, M.E., NERZ, S., KRAMER, B.K. & HOFFMEISTER, H.M. (1994). Hemodynamic and inotropic effects of endothelin-1 in vivo. *Basic. Res. Cardiol.*, **89**, 39–49.

CLOZEL, M., BREU, V., BURRI, K., CASSAL, J.M., FISCHLI, W., GRAY, G.A., HIRTH, G., LÖFFLER, B.-M., MULLER, M., NEIDHART, W. & RAMUZ, H. (1993). Pathophysiological role of endothelin revealed by the first orally active endothelin receptor antagonist. *Nature*, **365**, 759–761.

CLOZEL, M., GRAY, G.A., BREU, V., LÖFFLER, B.-M., & OSTERWALDER, R. (1992). The endothelin ET_B receptor mediates both vasodilation and vasoconstriction *in vivo*. *Biochem Biophys. Res. Commun.*, **186**, 867–873.

CODY, W.L., DOHERTY, A.M., HE, J.X., DEPUE, P.L., WAITE, L.A., TOPLISS, J.G., HALEEN, S.J., LADOUCEUR, D., FLYNN, M.A., HILL, K.E. & REYNOLDS, E.E. (1993). The rational design of a highly potent combined ET_A and ET_B receptor antagonist (PD 145065) and related analogues. *Med. Chem. Res.*, **3**, 154–162.

CRISTOL, J.-P., WARNER, T.D., THIEMERMANN, C. & VANE, J.R. (1993). Mediation via different receptors of the vasoconstrictor effects of endothelins and sarafotoxins in the systemic circulation and renal vasculature of the anaesthetized rat. *Br. J. Pharmacol.*, **108**, 776–779.

DE NUCCI, G., THOMAS, G., D'ORLEANS-JUSTE, P., ANTUNES, E., WALDER, C., WARNER, T.D. & VANE, J.R. (1988). Pressor effects of 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.

FUKURODA, T., FUJIKAWA, T., OZAKI, S., ISHIKAWA, K., YANO, M. & NISHIKIBE, M. (1994a). Clearance of circulating endothelin-1 by ET_B receptors in rats. *Biochem. Biophys. Res. Commun.*, **199**, 1461–1465.

FUKURODA, T., OZAKI, S., IHARA, K., ISHIKAWA, K., YANO, M. & NISHIKIBE, M. (1994b). Synergistic inhibition by BQ-123 and BQ-788 of endothelin-1-induced contractions of the rabbit pulmonary artery. *Br. J. Pharmacol.*, **113**, 336–338.

GARDINER, S.M., KEMP, P.A., MARCH, J.E. & BENNETT, T. (1994a). Effects of bosentan (Ro 47-0203), an ET_A-, ET_B-receptor antagonist, on regional haemodynamic responses to endothelins in conscious rats. *Br. J. Pharmacol.*, **112**, 823–830.

GARDINER, S.M., KEMP, P.A., MARCH, J.E., BENNETT, T., DAVENPORT, A.P. & EDVINSSON, L. (1994b). Effects of an ET_1-receptor antagonist, FR139317, on regional haemodynamic responses to endothelin-1 and [Ala11,15]Ac-endothelin-1 (6-21) in conscious rats. *Br. J. Pharmacol.*, **112**, 477–486.

HAYNES, W.G. & WEBB, D.J. (1994). Contribution of endogenous generation of endothelin-1 to basal vascular tone. *Lancet*, **344**, 852–854.

IHARA, M., NOGUCHI, K., SAEKI, T., FUKURODA, T., TSUCHIDA, S., KIMURA, S., FUKAMI, T., ISHIKAWA, K., NISHIKIBE, M. & YANO, M. (1992). Biological profiles of highly potent endothelin antagonists selective for the ET_A receptor. *Life Sci.*, **50**, 247–255.

ISHIKAWA, K., IHARA, M., NOGUCHI, K., MASE, T., MINO, N., SAEKI, T., FUKURODA, T., FUKAMI, T., OZAKI, S., NAGASE, T., NISHIKIBE, M. & YANO, M. (1994). Biochemical and pharmacological profile of a potent and selective endothelin B-receptor antagonist, BQ-788. *Proc. Natl. Acad. Sci. U.S.A.*, **91**, 4892–4896.

LADOUCEUR, D.M., FLYNN, M.A., KEISER, J.A., REYNOLDS, E. & HALEEN, S.J. (1993). ET_A and ET_B receptors coexist on rabbit pulmonary artery vascular smooth muscle mediating contraction. *Biochem. Biophys. Res. Commun.*, **196**, 209–215.

LAWRENCE, E. & BRAIN, S.D. (1994). Effect of BQ-123 and Ro 47-0203 (bosentan) on endothelin-induced vasoconstriction in the rat skin. *Eur. J. Pharmacol.* **260**, 103–106.

LE MONNIER DE GOUVILLE, A.C., MONDOT, S., LIPPTON, H., HYMAN, A. & CAVERO, I. (1990). Hemodynamic and pharmacological evaluation of the vasodilator effects of endothelin-1 in rats. *J. Pharmacol. Exp. Ther.*, **252**, 300–311.

LOPEZ-BELMONTE, J. & WHITTLE, B.J.R. (1993). The paradoxical vascular interactions between endothelin-1 and calcitonin gene-related peptide in the rat gastric mucosal microcirculation. *Br. J. Pharmacol.*, **110**, 496–500.

LOPEZ-BELMONTE, J. & WHITTLE, B.J.R. (1994). The involvement of endothelial dysfunction, nitric oxide and prostanoids in the rat gastric microcirculatory responses to endothelin-1. *Br. J. Pharmacol.*, **112**, 267–271.

MCDEVITT, D.G. & NIES, A.S. (1976). Simultaneous measurement of cardiac output and its distribution with microspheres in the rat. *Cardiovasc. Res.*, **10**, 494–498.

MCMURDO, L., CORDER, R., THIEMERMANN, C. & VANE, J.R. (1993). Incomplete inhibition of the pressor effects of endothelin-1 and related peptides in the anaesthetised rat with BQ-123 provides evidence for more than one vasoconstrictor receptor. *Br. J. Pharmacol.*, **108**, 557–561.

NISHIKIBE, M., TSUCHIDA, S., OKADA., FUKURODA, T., SHIMAMOTO, K., YANO, M., ISHIKAWA, K. & IKEMOTO, F. (1993). Antihypertensive effect of a newly synthesized endothelin antagonist, BQ-123, in a genetic hypertensive model. *Life Sci.*, **52**, 717–724.

OHLSTEIN, E.H., NAMBI, P., DOUGLAS, S.A., EDWARDS, R.M., GELLAI, M., LAGO, A., LEBER, J.D., COUSINS, R.D., GAO, A., FRAZEE, J.S., PEISHOFF, C.E., BEAN, J.W., EGGLESTON, D.S., ELSHOURBAGY, N.A., KUMAR, G., LEE, J.A., YUE, T.-L., LOUDEN, C., BROOKS, D.P., WEINSTOCK, J., FEUERSTEIN, G., POSTE, G., RUFFOLO, R.R. JR., GLEASON, J.G. & ELLIOTT, J.D. (1994). SB 209670, a rationally designed potent nonpeptide endothelin receptor antagonist. *Proc. Natl. Acad. Sci. U.S.A.*, **91**, 8052–8056.

OHLSTEIN, E.H., VICKERY, L., SAUERMELCH, C. & WILLETTE, R.N. (1990). Vasodilation induced by endothelin: role of EDRF and prostanoids in rat hindquarters. *Am. J. Physiol.*, **259**, H1835–H1841.

RUBANYI, G.M. & POLOKOFF, M.A. (1994). Endothelins: molecular biology, biochemistry, pharmacology, physiology and pathophysiology. *Pharmacol. Rev.*, **46**, 328–415.

SARGENT, C.A., BRAZDIL, R., BROWN, T.J. & ROACH, A.G. (1995). Ability of non-peptide endothelin antagonists to reverse an established endothelin-1 pressor response in the pithed rat. *Br. J. Pharmacol.*, **114**, 198P.

SHETTY, S.S., OKADA, T., WEBB, R.L., DELGRANDE, D. & LAPPE, R.L. (1993). Functionally distinct endothelin B recptors in vascular endothelium and smooth muscle. *Biochem. Biophys. Res. Commun.*, **191**, 459–464.

SUMNER, M.J., CANNON, T.R., MUNDIN, J.W., WHITE, D.G. & WATTS, I.S. (1992). Endothelin ET_A and ET_B receptors mediate vascular smooth muscle contraction. *Br. J. Pharmacol.*, **107**, 858–860.

THOMAS, G.R., THIEMERMANN, C., WALDER, C. & VANE, J.R. (1988). The effects of endothelium-dependent vasodilators on cardiac output and their distribution in the anaesthetized rat: a comparison with sodium nitroprusside. *Br. J. Pharmacol.*, **95**, 986–992.

WALDER, C.E., THOMAS, G.R., THIEMEREMANN, C. & VANE, J.R. (1989). The hemodynamic effects of endothelin-1 in the pithed rat. *J. Cardiovasc. Pharmacol.*, **13**, (Suppl 5), S93–S97.

WALLACE, J.L., KEENEAN, C.M., MACNAUGHTON, W.K. & MCKNIGHT, G.W. (1989). Comparison of the effects of endothelin-1 and endothelin-3 on the rat stomach. *Eur. J. Pharmacol.*, **167**, 41–47.

WARNER, T.D., ALLCOCK, G.H., CORDER, R. & VANE, J.R. (1993a). Use of the endothelin antagonists BQ-123 and PD 142893 to reveal three endothelin receptors mediating smooth muscle contraction and the release of EDRF. *Br. J. Pharmacol.*, **110**, 777–782.

WARNER, T.D., ALLCOCK, G.H. & VANE, J.R. (1994). Reversal of established responses to endothelin-1 in vivo and in vitro by the endothelin receptor antagonists, BQ-123 and PD 145065. *Br. J. Pharmacol.*, **112**, 207–213.

WARNER, T.D., BATTISTINI, B., ALLCOCK, G.H. & VANE, J.R. (1993b). Endothelin ET_A and ET_B receptors mediate vasoconstriction and prostanoid release in the isolated kidney of the rat. *Eur. J. Pharmacol.*, **250**, 447–453.

WARNER, T.D., MITCHELL, J.A., DE NUCCI, G. & VANE, J.R. (1989). Endothelin-1 and endothelin-3 release EDRF from isolated perfused arterial vessels of rat and rabbit. *J. Cardiovasc. Pharmacol.*, **17**, (suppl.5), S85.

WELLINGS, R.P., CORDER, R., WARNER, T.D., CRISTOL, J.-P., THIEMERMANN, C. & VANE, J.R. (1994). Evidence from receptor antagonists of an important role for ET_B receptor mediated vasoconstrictor effects of endothelin-1 in the rat kidney. *Br. J. Pharmacol.*, **111**, 515–520.

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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