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Endothelin receptor mediated constriction and dilatation in feline cerebral resistance arterioles in vivo

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Abstract

The receptors mediating the cerebrovascular actions of endothelins have been examined in feline cerebral resistance arterioles in vivo. The adventitial microapplication of the endothelin ET_A receptor antagonist BQ-123 (cyclo D-aspartate-D-tryptophan-L-leucine-D-valine-L-proline) (0.1–10 μM) per se had minimal effect on cerebral resistance arterioles examined. The adventitial microapplication of endothelin-1 (10 nM) elicited a marked vasoconstriction of cerebral resistance arterioles ($-29.1 \pm 1.9\%$ from pre-injection baseline). The endothelin-1 induced vasoconstriction was attenuated, in a dose dependent manner, by the adventitial co-application of BQ-123 and endothelin-1 (estimated IC_{50} 0.7 μM). The *adventitial* microapplication of the endothelin ET_B receptor agonist BQ-3020 *N*-acetyl[Ala^{11},Ala^{15}]ET-1 (6–21)) (0.001–1 μM) effected a dose dependent vasodilatation (EC_{50} 30 nM, maximum response $25 \pm 5\%$ from pre-injection baseline). The magnitude of the vasodilatation elicited by BQ-3020 (100 nM and 1 μM) was dependent on the pre-injection calibre of the arterioles examined. The *intracarotid* infusion (via the lingual artery) of BQ-3020 (0.5–500 pmol/min) had no significant effect on the calibre of cerebral resistance arterioles. These results suggest that the peptide endothelin ET_B receptor agonist fails to gain access to the cerebrovascular endothelin ET_B receptors following its intraluminal administration. These investigations indicate that endothelin ET_A receptors mediate vasoconstriction and endothelin ET_B receptors mediate vasodilatation in feline cerebral resistance arterioles in vivo.

Keywords: Endothelin-1; BQ-123; BQ-3020; Endothelin receptor; Cerebral circulation; Resistance arteriole, cerebral

1. Introduction

The cerebral circulation is a major therapeutic target area for the development of endothelin receptor antagonists. The endothelin peptides (endothelin-1, endothelin-2, endothelin-3) are potent constrictors of cerebrovascular smooth muscle in vivo and in vitro (Jansen et al., 1989; Robinson and McCulloch, 1990; Adner et al., 1993; Feger et al., 1994; Rubanyi and Polokoff, 1994; Schilling et al., 1995). The application of *exogenous* endothelin-1 on major cerebral arteries results in marked constriction of the vessels and reduces cerebral blood flow to levels that induce neuronal pathology (Macrae et al., 1993; Fuxe et al., 1992; Sharkey et al., 1993). Elevations in tissue, cerebrospinal fluid (CSF) and plasma endothelin levels have been reported following cerebral ischaemia and subarachnoid haemorrhage (Suzuki et al., 1992; Ziv et al., 1992; Barone et al., 1993; Bian et al., 1994; Spatz et al., 1995). Despite the potential significance of the endothelins in cerebrovascular disease, there has been limited pharmacological characterisation of the endothelin receptor subtypes present in cerebral blood vessels. The investigations conducted to date have examined the effects of endothelin receptor activation in major cerebral arteries, e.g. basilar artery, middle cerebral artery, spinal artery (Adner et al., 1993; Kitazono et al., 1993; Salom et al., 1993; Feger et al., 1994; Willette et al., 1994; Schilling et al., 1995). The cerebral resistance arterioles play the major role in the regulation of blood flow to the brain and there have been few investigations examining the effects of

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endothelin receptor activation in the cerebral resistance arterioles in vivo.

The effects of the endothelin peptides are mediated by at least two receptor subtypes, endothelin ET_A and endothelin ET_B (Arai et al., 1990; Sakurai et al., 1990; Masaki et al., 1994). The classification of endothelin receptors was initially carried out using molecular biology techniques and subsequently on the basis of agonist potencies of the endothelin isopeptides. Endothelin-1 and endothelin-2 displayed enhanced potency compared to endothelin-3 at the endothelin ET_A receptor while the three isopeptides were equipotent at the endothelin ET_B receptor (Arai et al., 1990; Sakurai et al., 1990; Masaki et al., 1994). More recently a putative endothelin ET_C receptor has been cloned and endothelin-3 displays a greater selectivity than the other isopeptides for this receptor (Karne et al., 1993). In the peripheral blood vessels, the vasoconstrictor response of the endothelins is mediated primarily by the stimulation of the endothelin ET_A receptor subtype, however, endothelin ET_B receptors mediating vasoconstriction and vasodilatation have been described (Shetty et al., 1993; Warner et al., 1993; Rubanyi and Polokoff, 1994). Although the endothelin receptors mediating the vasoconstrictor and vasodilator effects in peripheral arterioles and microvessels are similar to the large arteries, there are indications that the small calibre blood vessels may be more sensitive to the effects of the endothelin peptides (Homma et al., 1992; Rubanyi and Polokoff, 1994).

Recently peptide and non-peptide antagonists and agonists selective for the endothelin receptors have been developed (Ihara et al., 1991; Sogabe et al., 1992; Clozel et al., 1993, 1994; Doherty et al., 1995). BQ-123 (cyclo D-aspartate-D-tryptophan-L-leucine-D-valine-L-proline) is a pentapeptide endothelin receptor antagonist that displays a 4000 fold selectivity for the endothelin ET_A receptor subtype (Ihara et al., 1991; Rubanyi and Polokoff, 1994). The selectivity of BQ-123 for the endothelin ET_A receptor has been utilised to examine the vasoconstrictor actions of endothelin-1 in a variety of vascular and non vascular tissues (Ihara et al., 1991). BQ-3020 (*N*-acetyl[Ala^{11},Ala^{15}]ET-1-(6–21)) is a linear analogue of the endothelin-1 molecule and has demonstrated a ~4000 fold selectivity for the endothelin ET_B receptor subtype compared to the endothelin ET_A receptor in in vitro and in vivo investigations (Ihara et al., 1992). The present investigations were designed to characterise the effects of endothelin receptor activation in feline small cerebral resistance arterioles in vivo.

2. Materials and methods

2.1. Surgical preparation

The experiments were performed on 8 adult female cats weighing between 2–3 kg. Anaesthesia was induced using alphaxolone/alphadolone (Saffan, Glaxo) administered into the radial vein. The animals were intubated and positive pressure ventilation with N_2O-O_2 (70%-30%) initiated. The femoral arteries and veins were cannulated for the monitoring of arterial blood pressure and arterial blood gas status and also for the administration of fluids and drugs. Anaesthesia was maintained using α-chloralose (60 mg/kg) and supplemented as required to prevent the return of the corneal reflex during the course of the experiment. The inspired gas mixture was altered to O_2 supplemented air (25% O_2) and arterial blood samples were taken at regular intervals to monitor blood gas status and the animals maintained normocapnic by adjusting the stroke volume. Metabolic acidosis was controlled by the administration of sodium bicarbonate (8.4% solution) where necessary. At the outset of the study, mean arterial blood pressure (MABP) was 97 ± 3 mm Hg, arterial pH was 7.42 ± 0.01, arterial carbon dioxide tension was 30 ± 1 mm Hg and arterial oxygen tension was 201 ± 5 mm Hg. During the course of the study, the physiological variables did not vary significantly from the levels at the outset.

The animals were placed in a stereotactic frame and after a midline incision the scalps were retracted and sutured onto a metal ring to form a well over the calvaria. The temporalis muscle was retracted and a rectangular craniectomy (2.5 cm × 1.5 cm) was made over the parietal cortex using a saline cooled dental drill. The exposed dura was bathed continuously with mineral oil at 38°C. With the aid of a stereomicroscope (Bausch and Lomb) the dura was excised and then reflected laterally. Bipolar diathermy was used to eliminate bleeding from the dural vessels. The preparation was allowed to equilibrate for 30 min before any adventitial microapplications were performed. In a separate group of animals the lingual artery was exposed and catheterised. The catheter was advanced into the carotid artery and subsequently used for the intravascular infusions of the endothelin ET_B receptor agonist BQ-3020.

2.2. Measurement of cerebral resistance arteriolar calibre

Cerebral resistance arteriolar calibre was measured by the method of Baez (1966) using an image splitter linked through a closed circuit video display system. A detailed description of the surgical preparation and measurement techniques has appeared previously (Robinson and McCulloch, 1990). Briefly, the individual cerebral resistance arterioles were viewed through a stereomicroscope and the arteriolar diameter was measured from the degree of shear applied to the image splitter in order to tangentially appose the two images. The system was calibrated at ×40 and ×70 against threads of known diameter and this allows for the direct measurement of vascular diameter in absolute units (μm). The vessel diameter was measured pre-insertion of micropipettes, pre- and post-injection of substances and the vessel diameter was monitored over a period of 1–2 min at each instance. In the intracarotid infusion studies, the calibres of selected cerebral resistance arteri-

oles were examined at 10 min and 20 min following the start of the infusion of BQ-3020 at each concentration. The infusion of BQ-3020 was carried out for 30 min at each concentration.

2.3. Administration of drugs

Artificial cerebrospinal fluid (CSF) was prepared with the following composition: Na^+ 156 mM, K^+ 3 mM, Ca^{2+} 2.5 mM, HCO_3^- 12 mM, Cl^- 152 mM. The pH of the CSF was adjusted to 7.2 by aeration with 95% O_2-5% CO_2. The agents to be administered by adventitial microapplication were dissolved in CSF. All solutions were prepared freshly on the day of the study. Glass micropipettes (tip diameter 10–12 μm) were filled under vacuum with the solution to be studied and stored under mineral oil in CSF until required. BQ-3020 for intravascular infusions was dissolved in 0.9% saline and infused at a rate of 0.15 ml/min.

The substances were applied by adventitial microapplication using a micromanipulator to position the pipettes in the adventitial space around individual cerebral resistance arterioles (pre-injection calibre range 42–334 μm). Small volumes (approximately 5 μl) were delivered by a pressure ejection system.

2.4. Statistical analysis

All results were analysed using a one way analysis of variance followed by two-tailed Student's unpaired *t*-test using a Bonferroni correction factor for multiple group comparisons. All results are expressed as means ± S.E.M. of the percentage change in arteriolar calibre from baseline immediately prior to microapplication or infusion of substances.

3. Results

3.1. Perivascular microapplication of BQ-123 per se

The adventitial microapplications of CSF (pH 7.2) had no significant effect on cerebral arteriolar calibre (−0.93 ± 1.6% from pre-injection baseline; $n = 14$). The adventitial microapplications of BQ-123 per se (0.1–10 μM) had no significant effect on cerebral arteriolar calibre (Fig. 1).

3.2. Perivascular co-application of endothelin-1 (10 nM) and BQ-123

The adventitial microapplication of endothelin-1 (10 nM) effected a marked constriction of the cerebral arterioles examined (−29.1 ± 1.9% from pre-injection baseline; $n = 12$). The concentration of endothelin-1 (10 nM) used in the present investigations is the EC_{50} concentration for the endothelin-1 induced constrictions of cerebral resistance arterioles determined in previous investigations (Robinson and McCulloch, 1990). The adventitial co-application of endothelin-1 (10 nM) and BQ-123 demonstrated a dose dependent attenuation of the endothelin-1 mediated vasoconstrictive response (Fig. 1). The concentration of BQ-123 that produced a half maximal attenuation (IC_{50}) of endothelin-1 induced vasoconstriction was estimated to be approximately 0.7 μM.

3.3. Perivascular and intravascular administration of BQ-3020

The adventitial microapplication of CSF had minimal effect on arteriolar calibre, whereas, the adventitial microapplication of BQ-3020 (0.001–1 μM) effected dose dependent dilatations of cerebral arterioles examined (EC_{50}

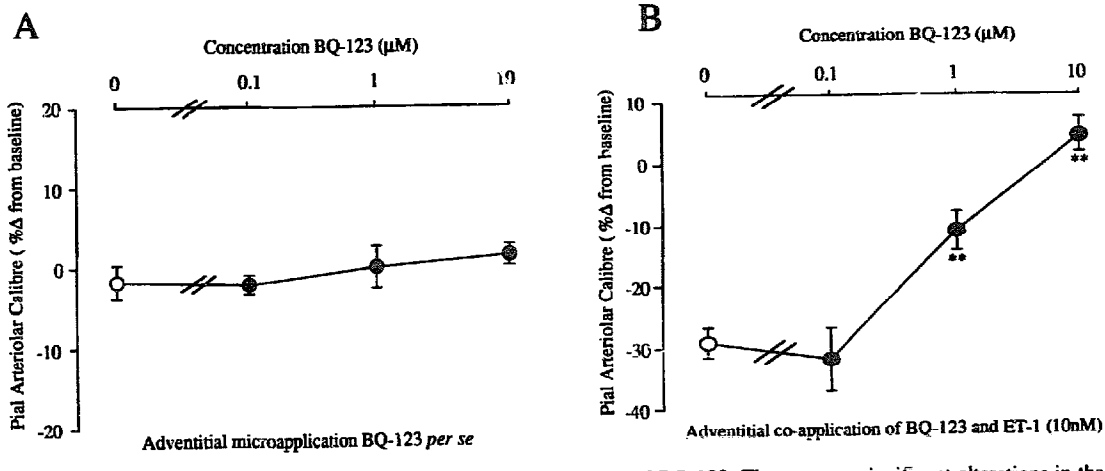

Fig. 1. (A) Vasomotor responses of pial arterioles to the adventitial microapplication of BQ-123. There are no significant alterations in the pial arteriolar calibre at any concentration of BQ-123. Data are expressed as percent alteration from baseline of pial arteriolar calibre. Data are presented as mean ± S.E.M. (n, number of arterioles examined = 6 for each concentration). (B) Vasomotor responses of pial arterioles to adventitial co-application of endothelin-1 (10 nM) and BQ-123. BQ-123 significantly attenuated the endothelin-1 induced vasoconstrictions (** $P < 0.01$ for the comparison with endothelin-1 alone). Data are expressed as percent alteration from baseline of pial arteriolar calibre. Data are presented as means ± S.E.M (n, number of arterioles examined = 5–12 for each concentration).

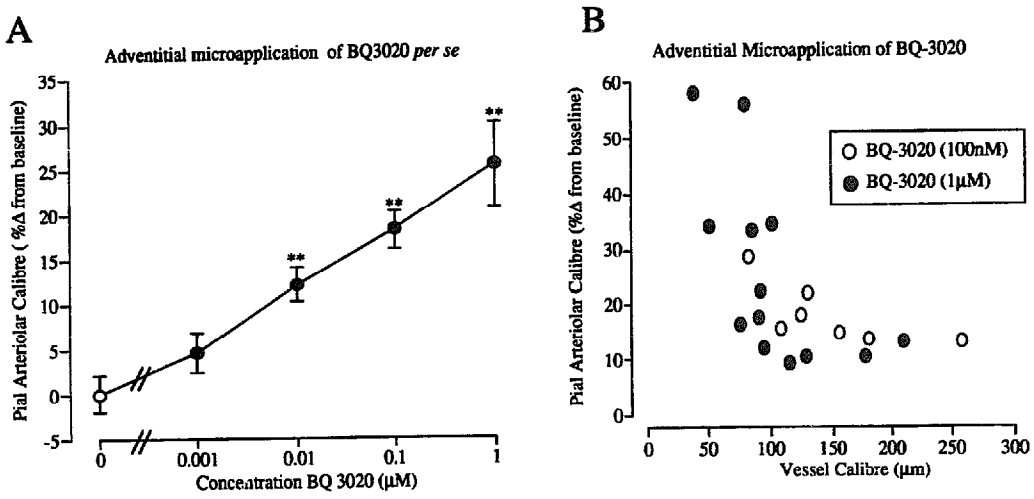

Fig. 2. (A) Vasomotor responses of pial arterioles to the adventitial microapplication of BQ-3020. BQ-3020 effected a dose dependent vasodilatation of the pial arterioles examined (** $P < 0.01$ for the comparison with CSF). Data are expressed as percent alteration from baseline of pial arteriolar calibre. Data are presented as means ± S.E.M. (n, number of arterioles examined = 7–13 for each concentration). (B) The dependence of the pial arteriolar dilatation elicited by BQ-3020 on the calibre of arteriole examined. Pial arteriolar diameter was determined prior to the adventitial microapplication of BQ-3020. The arteriolar responses are expressed as the percent change from pre-injection baseline. ($r^2 = 0.36$; $P < 0.01$).

approximately 30 nM) (Fig. 2). The magnitude of the response of cerebral arterioles to BQ-3020 (100 nM and 1 μM) was dependent on the pre-injection calibre of the arteriole examined. Cerebral arterioles less than 100 μm demonstrated an increased reactivity to BQ-3020 (Fig. 2). The reactivity of cerebral arterioles to CSF was not dependent on the pre-injection arteriolar calibre (data not shown). The intracarotid infusion of 0.9% saline had minimal effect on pial arteriolar calibre or arterial blood pressure. The intracarotid infusion of BQ-3020 (0.5–500 pmol/min) had minimal effect on pial arteriolar calibre (Fig. 3). The intracarotid infusion of BQ-3020 at a the rate of 500 pmol/min elicited a transient decrease in mean arterial blood pressure (16.3 ± 8.6 mm Hg from pre-infusion baseline) and the blood pressure returned to baseline within 5 min.

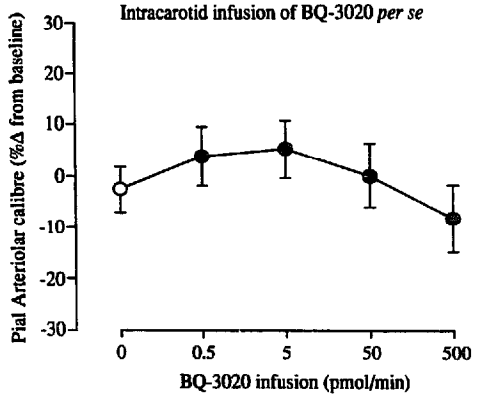

Fig. 3. Vasomotor responses of pial arterioles to the intracarotid infusion of BQ-3020. BQ-3020 or saline had minimal effect on pial arterioles examined. Data are expressed as percentage alteration from baseline of pial arteriolar calibre. Data are presented as means ± S.E.M. (n, number of arterioles examined = 11 for each concentration).

4. Discussion

In contrast to the vast literature on the characterisation of endothelin receptors in the peripheral vasculature, there is less information concerning the endothelin receptors in the cerebral resistance arterioles. A number of investigators have characterised the endothelin receptors in the major cerebral arteries (spinal, basilar, middle cerebral) in different species (Adner et al., 1993; Kitazono et al., 1993; Salom et al., 1993; Feger et al., 1994; Willette et al., 1994; Schilling et al., 1995). Resistance arterioles play an important role in the regulation of cerebral blood flow and in this investigation we have attempted to characterise the endothelin receptors in cerebral resistance arterioles. Investigations in the peripheral vasculature indicate that the endothelin ET_A receptor is the predominant receptor mediating endothelin-1 induced vasoconstrictions (Warner et al., 1993; Masaki et al., 1994; Rubanyi and Polokoff, 1994). The role of endothelin ET_B receptors is more controversial with endothelin ET_B receptor mediated vasodilatation and vasoconstriction reported in different vascular beds (Clozel et al., 1992; Sumner et al., 1992; Shetty et al., 1993). In the feline skeletal muscle, the constriction of resistance arterioles is mediated by the endothelin ET_A receptor (Ekelund et al., 1993; Ekelund, 1994) while endothelin-1 mediated vasodilatation is mediated by the endothelin ET_B receptor (Ekelund et al., 1995). The present investigations have demonstrated that endothelin ET_A receptors mediate vasoconstriction while endothelin ET_B receptors mediate vasodilatation in feline small cerebral resistance arterioles in vivo. The failure of the intracarotid infusion of BQ-3020 to alter cerebral arteriolar calibre indicates that the endothelin ET_B receptors may not be readily accessible by intraluminal endothelin receptor agonists which are peptide analogues.

The role of endogenous endothelin in the regulation of vascular tone in peripheral and cerebral blood vessels has been suggested (Yoshimoto et al., 1990; Haynes and Webb, 1994). In feline peripheral blood vessels, an absence of basal endothelin mediated tone has been demonstrated (Ekelund et al., 1993, 1995; Ekelund, 1994). In the current investigations the adventitial microapplication of the ET_A receptor antagonist BQ-123 had minimal effect on the calibre of the pial arterioles. The perivascular microapplication of other endothelin receptor antagonists, e.g. Bosentan, PD145065, PD155080, have demonstrated similar effects indicating that under the present experimental conditions there is minimal endothelin mediated tone in the cerebral resistance arterioles (Patel et al., 1994, 1995b). These observations contrast with the reports of Yoshimoto et al. (1990) which suggest that there is a basal production of endothelin by cerebrovascular endothelial cells in culture, and those of Haynes and Webb (1994) which indicate the presence of basal endothelin mediated tone in human forearm vessels. The adventitial co-application of endothelin-1 with increasing concentrations of BQ-123 demonstrates a dose dependent attenuation of the endothelin-1 induced vasoconstriction. The estimated IC_{50} for BQ-123 determined from these experiments is 0.7 μM and this is comparable to the values obtained for this antagonist in other tissues (Ihara et al., 1991). In cerebral resistance arterioles it appears that the vasoconstrictor effect of endothelin-1 is mediated primarily via endothelin ET_A receptors. The ability of BQ-123 to attenuate the effects of exogenous endothelin-1 in cerebral resistance arterioles is similar to the responses obtained with the combined endothelin ET_A/ET_B receptor antagonists Bosentan and PD145065 and endothelin ET_A receptor antagonist PD155080 (Patel et al., 1994, 1995b).

Previous investigations of Armstead et al. (1989) in the piglet and Faraci (1989) in the rat indicated the existence of endothelin mediated vasodilatation in cerebral resistance arterioles. However, the absence of receptor selective agonists and antagonists prevented the characterisation of the endothelin receptor subtypes. In the present investigations, the adventitial microapplication of the endothelin ET_B receptor agonist BQ-3020 effected a dose dependent vasodilatation of cerebral resistance arterioles in vivo. These observations are in agreement with reports indicating that endothelin ET_B receptors mediate vasodilatation in the rat basilar artery in vivo and in vitro (Kitazono et al., 1993; Feger et al., 1994; Schilling et al., 1995). Preliminary investigations have demonstrated that the BQ-3020 mediated vasodilatations can be attenuated by the combined endothelin ET_A/ET_B receptor antagonist bosentan (T.R. Patel, unpublished observations) suggesting that the endothelin ET_{B1} receptor subtype may mediate vasodilatation in feline cerebral arterioles (Masaki et al., 1994). The cranial window technique used in the present investigations allows us to examine cerebral resistance arterioles (40–350 μm) under normal physiologic and neurogenic

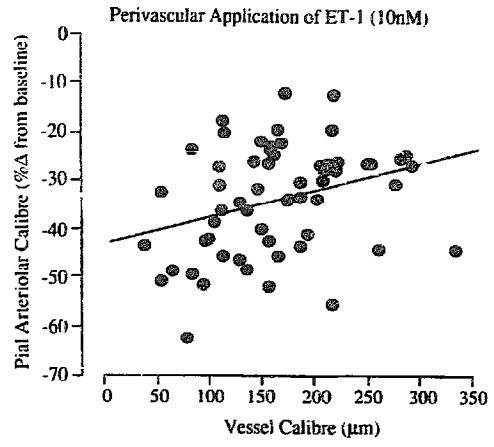

Fig. 4. The dependence of the pial arteriolar constriction elicited by endothelin-1 (10 nM) on the calibre of arteriole examined. Pial arteriolar diameter was determined prior to the adventitial microapplication of endothelin-1. The arteriolar responses are expressed as the percent change from pre-injection baseline. Post hoc analyses of these responses have demonstrated significant association between the arteriolar response and pre-injection calibre ($r^2 = 0.11$; $P < 0.01$) (see text for references).

control. This in situ preparation facilitates the observation of vasodilator responses of cerebral arterioles without the pre-constriction. Previous investigations have demonstrated that the responses of cerebral resistance arterioles in vivo, to vasodilator or vasoconstrictor agents, may be dependent on the pre-injection calibre of arterioles in vivo (Kuschinsky and Wahl, 1975; Harper and MacKenzie, 1977; McCulloch and Edvinsson, 1980). The present investigations have demonstrated that cerebral arterioles less than 100 μm elicited a greater vasodilator response following the adventitial application of BQ-3020. In the present study we have been unable to demonstrate a similar correlation following the adventitial application of endothelin-1 (10 nM). The absence of a correlation with endothelin-1 may be due to the small number of arterioles examined. We have re-examined the responses of cerebral arterioles to endothelin-1 from the present study and those conducted previously and can demonstrate a significant correlation between the pre-injection calibre of arterioles and response to adventitial endothelin-1 (Fig. 4) (Patel et al., 1994, 1995b, 1996). The responses of arterioles to the microapplication of CSF was not dependent on the calibre of the arterioles.

Intracarotid infusion of BQ-3020 failed to demonstrate a significant alteration in arteriolar calibre of cerebral resistance vessels. A distinguishing feature of the cerebral circulation is the blood-brain barrier. The blood-brain barrier can prevent the access of molecules, e.g. peptides, to the adventitial surface of cerebral arterioles (Ermisch et al., 1993). Endothelin receptor agonists or antagonists targeted at the cerebrovasculature would have to gain access to the abluminal surface of cerebral arterioles in order to exert their effects. BQ-3020 with its peptide structure would not be expected to penetrate the blood-brain barrier and gain

access to the abluminal surface of cerebral vessels. Previous investigations using intraluminal administration of endothelin-1 have demonstrated alterations in cerebral blood flow and cerebral blood volume (Kobari et al., 1994a; Willette et al., 1990). The intraluminal infusion of endothelin-1 induces systemic hypertension, and hypertension per se produces alterations in cerebral arteriolar calibre as part of the cerebral autoregulatory response. Endothelin-1 can also activate endothelin ET_A and endothelin ET_B receptors and for these reasons it was not examined in the present investigations. The failure of intracarotid infusions, in contrast to the adventitial application, of BQ-3020 to alter arteriolar calibre indicates that the endothelin ET_B receptors may be located on the abluminal surface of cerebral resistance arterioles. In the present investigations, assuming a carotid blood flow of 5 ml/min, we estimate that the intravascular concentration of BQ-3020 (with these infusion rates) would be in the range of 0.1–100 nM. The adventitial microapplication of these concentrations of BQ-3020 elicit a dilatation of cerebral resistance arterioles. The failure of the intracarotid infusion of BQ-3020 to alter pial arteriolar calibre is at variance with the observations of Kobari et al. (1994b). The differences in the observations could be the result of the different methodological approaches employed in the two investigations. Kobari et al. (1994b) have used alterations in cerebral blood volume as an indicator of cerebral vasodilatation. Cerebral blood volume is a measure of cerebral capacitance (a combination of arterial, arteriolar, capillary and venous diameters) and alterations in cerebral capacitance will not necessarily reflect increases in cerebral blood flow. In the present investigations, BQ-3020 was administered as a continuous infusion while Kobari and colleagues administered the agents as a bolus (Kobari et al., 1994b). The alterations in cerebral blood volume reported may be the result of an injection artefact (Kobari et al., 1994b). Evidence from in vitro investigations have indicated the susceptibility of endothelin receptors to desensitisation (Hollenberg et al., 1993). The initial intracarotid infusions of low doses of BQ-3020 could have resulted in the desensitisation of endothelin ET_B receptors in the cerebral arterioles. The infusion of 500 pmol/min of BQ-3020 resulted in a transient reduction in mean arterial blood pressure indicating that the endothelin ET_B receptors in the peripheral vasculature were activated.

In the rat basilar artery, endothelium dependent vasodilatation has been demonstrated following activation of endothelin ET_B receptors (Feger et al., 1994; Schilling et al., 1995). The vasodilatation in the rat basilar artery is mediated by nitric oxide (Feger et al., 1994; Schilling et al., 1995). The mediation of the dilator response following the activation of endothelin ET_B receptors in feline cerebral resistance arterioles is unknown but a functional link to either nitric oxide or prostacyclin are possibilities. The present investigations do not discount the existence of endothelin ET_B receptors on the luminal surface of endothelial cells or on cerebrovascular smooth muscle since northern blot analysis and functional studies on human blood vessels have indicated the existence of receptors without a functional link to either nitric oxide or prostacyclin (Lüscher, 1993; Seo et al., 1994).

The feline species has been used for investigating the role of the endothelins in the pathophysiology of focal cerebral ischaemia (Patel et al., 1995a). The present investigations indicate that the location of the endothelin ET_B receptors is on the abluminal surface of cerebral resistance arterioles and that the endothelin ET_B receptors mediate vasodilatation. The dilatation of cerebral resistance arterioles would result in an increase in cerebral blood flow. The ability of pharmacologic agents to increase cerebral blood flow would have beneficial effects in conditions of impaired cerebral blood flow e.g. stroke. In contrast to the description of endothelin ET_B receptor mediated vasoconstriction in peripheral tissues, similar responses have not been observed in cerebral arteries or arterioles in vivo or in vitro. The present investigations indicate a vasoconstrictor action for endothelin ET_A receptors in feline cerebral resistance arterioles. The present investigations suggest that antagonists selective for the endothelin ET_A receptors may be of greater utility than the combined endothelin ET_A/ ET_B receptor antagonists in cerebrovascular investigations.

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References

Adner, M., J. You and L. Edvinsson, 1993, Characterisation of endothelin-A receptors in the cerebral circulation, Neuroreport 4, 441.

Arai, H., S. Hori, I. Aramori, H. Ohkubo and S. Nakanishi, 1990, Cloning and expression of a cDNA encoding an endothelin receptor, Nature 348, 730.

Armstead, W. M., R. Mirro, C. W. Leffler and D. W. Busija, 1989, Influence of endothelin on piglet cerebral microcirculation, Am. J. Physiol. 257, H707.

Baez, S., 1966, Recording of microvascular dimensions with an image splitter television microscope, J. Appl. Physiol. 21, 299.

Barone, F.C., M.Y.-T. Globus, W.J. Price, R.F. White, B.L. Storer, G.Z. Feuerstein, R. Busto and E.H. Ohlstein, 1993, Endothelin levels increase in rat focal and global ischaemia, J. Cereb. Blood Flow Metab. 14, 337.

Bian, L.G., T.X. Zhang, W.G. Zhao, J.K. Shen and G.Y. Yang, 1994, Increased endothelin-1 in the rabbit model of middle cerebral artery occlusion, Neurosci. Lett. 174, 47.

Clozel, M., G.A. Gray, V. Breu, B.-M. Loffler and R. Osterwalder, 1992, The endothelin ET_B receptor mediates vasodilatation and vasoconstriction in vivo, Biochem. Biophys. Res. Commun. 186, 867.

Clozel, M., V. Breu, K. Burri, J.-M. Cassal, W. Fischli, G.A. Gray, G. Hirth, B-M. Loffler, M. Muller, W. Neidhart and H. Ramuz, 1993,

Pathophysiological role of endothelin revealed by the first orally active endothelin receptor antagonist, Nature 365, 759.

Clozel, M., V. Breu, G.A. Gray, B. Kalina, B-M. Loffler, K. Burri, J-M. Cassal, G. Hirth, M. Muller, W. Neidhart and H. Ramuz, 1994, Pharmacological characterisation of Bosentan, a new potent orally active non-peptide endothelin receptor antagonist, J. Pharmacol. Exp. Ther. 270, 228.

Doherty, A.M., W.C. Patt, J.J. Edmunds, K.A. Berryman, B.R. Reisdorph, M.S. Plummer, A. Shahripour, C. Lee, X.-M. Cheng, D.M. Walker, S.J. Haleen, J.A. Keiser, M.A. Flynn, K.M. Welch, H. Hallak, D.G. Taylor and E.E. Reynolds, 1995, Discovery of a novel series of orally active non peptide endothelin-A (ET_A) receptor selective antagonists, J. Med. Chem. 38, 1259.

Ekelund, U., 1994, In vivo effects of endothelin-2, endothelin-3 and ET(A) receptor blockade on arterial, venous and capillary functions in the cat skeletal muscle, Acta Physiol. Scand. 150, 147.

Ekelund, U., U. Albert, L. Edvinsson and S. Mellander, 1993, In vivo effects of endothelin-1 and ET(A) receptor blockade on arterial, venous and capillary functions in skeletal muscle, Acta Physiol. Scand. 148, 273.

Ekelund, U., M. Adner, L. Edvinsson and S. Mellander, 1995, Effects of the combined ET(A) and ET(B) receptor antagonist PD145065 on arteries, arterioles and veins in the cat hindlimb, J. Cardiovasc. Pharmacol. 26 (Suppl. 3), S211.

Ermisch, A., P. Brust, R. Kretzschmar and H.-J. Ruhle, 1993, Peptides and blood-brain barrier transport, Physiol. Revs. 73, 489.

Faraci, F. M., 1989, Effects of endothelin and vasopressin on cerebral blood vessels, Am. J. Physiol. 257, H799.

Feger, G.I., L. Schilling, H. Ehrenreich and M. Wahl, 1994, Endothelin-induced contraction and relaxation of rat isolated basilar artery: Effect of BQ-123, J. Cereb. Blood Flow Metab. 14, 845.

Fuxe, K., N. Kurosawa, A. Cintra, A. Hallstrom, M. Goiny, L. Rosen, L.F. Agnati and U. Ungerstedt, 1992, Involvement of local ischaemia in endothelin-1 induced lesions of the neostriatum of the anaesthetised rat, Exp. Brain Res. 88, 131.

Harper, A.M. and E.T. MacKenzie, 1977, Effects of 5-hydroxytryptamine on pial arteriolar calibre in anaesthetised cats, J. Physiol. 271, 735.

Haynes, W.G. and D.J. Webb, 1994, Contribution of endogenous generation of endothelin-1 to basal vascular tone, Lancet 344, 852.

Hollenberg, S.M., J.H. Shelhamer and R.E. Cunnion, 1993, Tachyphylaxis to the vasopressor effects of endothelin in rat aortic rings, Am. J. Physiol. 264, H352.

Homma, S., T. Miyauchi, Y. Sugishita, K. Goto, M. Sato and N. Ohshima, 1992, Vasoconstrictor effects of endothelin-1 on myocardium microcirculation studied by the Langendorff perfusion method: differential sensitivities among microvessels, Microvasc. Res. 43, 205.

Ihara, M., K. Noguchi, T. Saeki, T. Fukuroda, S. Tsuchida, S. Kimura, T. Fukani, K. Ishikawa, M. Nishikibe and M. Yano, 1991, Biological profiles of highly potent novel endothelin antagonists for the ET_A receptor, Life Sci. 50, 247.

Ihara, M., T. Saeki, T. Fukuroda, S. Kimura, S. Ozaki, A.C. Patel and M. Yano, 1992, A novel radioligand [^{125}I] BQ-3020 selective for endothelin ETB receptors, Life Sci. 51, PL47.

Jansen, I., B. Fallgren and L. Edvinsson, 1989, Mechanisms of actions of endothelin on isolated feline cerebral arteries: in vitro pharmacology and electrophysiology, J. Cereb. Blood Flow Metab. 9, 743.

Karne, S., C.K. Jayawickreme and M.R. Lerner, 1993, Cloning and characterisation of an endothelin-3 specific receptor (ET_C receptor) from Xenopus Laevis dermal melanocytes, J. Biol. Chem. 268, 29126.

Kitazono, T., D. Heistad and F.M. Faraci, 1993, Activation of endothelin B receptors produces dilatation of the basilar artery in vivo, Circulation 88, I-170.

Kobari, M., Y. Fukuuchi, M. Tomita, N. Tanahashi, S. Konno and H. Takeda, 1994a, Constriction/dilatation of the cerebral microvessels by intravascular endothelin-1 in cats, J. Cereb. Blood Flow Metab. 14, 64.

Kobari, M., Y. Fukuuchi, M. Tomita, N. Tanahashi, S. Konno and H. Takeda, 1994b, Dilatation of cerebral microvessels by endothelin ET_B receptor and nitric oxide in cats, Neurosci. Lett. 176, 157.

Kuschinsky, W. and M. Wahl, 1975, Alpha-receptor stimulation by endogenous and exogenous norepinephrine and blockade by phentolamine in pial arteries of cats, Circ. Res. 37, 168.

Lüscher, T.F., 1993, Do we need endothelin antagonists?, Cardiovasc. Res. 27, 2089.

Macrae, I.M., M.J. Robinson, D.I. Graham, J.L. Reid and J. McCulloch, 1993, Endothelin-1-induced reductions in cerebral blood flow: dose dependency, time course and neuropathological consequences, J. Cereb. Blood Flow Metab. 13, 276.

Masaki, T., J.R. Vane and P.M. Vanhoutte, 1994, International Union of Pharmacology Nomenclature of Endothelin Receptors, Pharamcol. Rev. 46, 137.

McCulloch, J. and L. Edvinsson, 1980, Cerebral circulatory and metabolic effects of vasoactive intestinal polypeptide, Am. J. Physiol. 238, H449.

Patel, T.R., M.A. McAuley and J. McCulloch, 1994, Effects on feline pial arterioles in situ of bosentan, a non-peptide, endothelin receptor antagonist, Eur. J. Pharmacol. 260, 65.

Patel, T.R., S. Galbraith, M.A. McAuley and J. McCulloch, 1995a, Cerebrovascular significance of endothelin receptor antagonism in focal ischaemia, J. Cereb. Blood Flow Metab. 15 [Suppl. 1], S140.

Patel, T.R., M.A. McAuley, A.M. Doherty and J. McCulloch, 1995b, Pharmacological characterisation of peptide and non-peptide endothelin antagonists in cerebral arterioles in situ, J. Cereb. Blood Flow Metab. 15 [Suppl. 1], S537.

Patel, T.R., S. Galbraith, D.I. Graham, H. Hallak, A.M. Doherty and J. McCulloch, 1996, Endothelin (ET_A) receptor antagonist increases cerebral perfusion and reduces ischaemic damage in feline focal cerebral ischaemia, J. Cereb. Blood Flow Metab. (in press).

Robinson, M.J. and J. McCulloch, 1990, Contractile responses to endothelin in feline cortical vessels in situ, J. Cereb. Blood Flow Metab. 10, 285.

Rubanyi, G.M. and M.A. Polokoff, 1994, Endothelins: molecular biology, biochemistry, pharmacology, physiology and pathophysiology, Pharmacol. Rev. 46, 325.

Sakurai, T., M. Yanagisawa, Y. Takuwa, H. Miyazaki, S. Kimura, K. Goto and T. Masaki, 1990, Cloning of cDNA encoding a non isopeptide selective subtype of the endothelin receptor, Nature 348, 732.

Salom, J., G. Torregrossa, M. Barbera, T. Jover and E. Alborch, 1993, Endothelin receptors mediating contraction in goat cerebral arteries, Br. J. Pharmacol. 109, 826.

Schilling, L., G.I. Feger, H. Ehrenreich and M. Wahl, 1995, Endothelin-3 induced relaxation of isolated rat basilar artery is mediated by an endothelial ET_B type endothelin receptor, J. Cereb. Blood Flow Metab. 15, 699.

Seo, B.G., B.S. Oemar, R. Siebenmann, L. von Segesser and T.F. Luscher, 1994, Both ET_A and endothelin ETB receptors mediate contraction to endothelin-1 in human blood vessels, Circulation 89, 1203.

Sharkey, J., I.M. Ritchie and P.A.T. Kelly, 1993, Perivascular microapplication of endothelin-1: a new model of focal cerebral ischaemia in the rat, J. Cereb. Blood Flow Metab. 13, 865.

Shetty, S.S., T. Okada, R.L. Webb, D. Del Grande and R.W. Lappe, 1993, Functionally distinct endothelin ET_B receptor in vascular endothelium and smooth muscle, Biochem. Biophys. Res. Commun. 191, 459.

Sogabe, K., H. Nirei, M. Shoubo, A. Nomoto, S. Ao, S. Notsu and T. Ono, 1992, Pharmacological profile of FR139317, a novel, potent endothelin ET_A receptor antagonist, J. Pharmacol. Exp. Ther. 264, 1040.

Spatz, M., D. Stanimirovic, A. Strasser and R. M. McCarron, 1995, Nitro-L-arginine augments endothelin-1 content of cerebrospinal fluid induced by cerebral ischaemia, Brain Res. 684, 99.

Sumner, M., T. Cannon, J. Mundi, D. White and I. Watts, 1992, Endothelin ET_A and ET_B receptor mediate vascular smooth muscle contraction, Br. J. Pharmacol. 107, 858.

Suzuki, R., H. Masaoka, Y. Hirata, F. Marumo, F. Isotani and K. Hirakawa, 1992, The role of endothelin-1 in the origin of cerebral vasospasm in patients with aneurysmal subarachnoid hemorrhage, J. Neurosurg. 77, 96.

Warner, T.D., G.H. Allcock, R. Corder and J.R. Vane, 1993, Use of the endothelin antagonists BQ-123 and PD-142893 to reveal 3 endothelin receptors mediating smooth-muscle contraction and the release of EDRF, Br. J. Pharmacol. 110, 777.

Willette, R.N., C. Sauermelch, M. Ezekiel, G. Feuerstein and E.H. Ohlstein, 1990, Effect of endothelin on cortical microvascular perfusion in rats, Stroke 21, 451.

Willette, R.N., H. Zhang, M. P. Mitchell, C. F. Sauermelch, E.H. Ohlstein and A.C. Sulpizio, 1994, Nonpeptide endothelin antagonist: cerebrovascular characterisation and effects on delayed cerebral vasospasm, Stroke 25, 2450.

Yoshimoto, S., Y. Ishizaki, M. Kurihara, T. Sasaki, M. Yoshizumi, M. Yanagisawa, Y. Tazaki, T. Masaki, K. Takahura and S. Murota, 1990, Cerebral microvessel endothelium is producing endothelin, Brain Res. 580, 283.

Ziv, I., G. Heminger, R. Djaldetti, A. Achiron, E. Melamed and M. Sokolovsky, 1992, Increased plasma endothelin-1 in acute ischaemic stroke, Stroke 23, 1014.