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Endothelin-1-induced in vitro cerebral venoconstriction is mediated by endothelin ET_A receptors

Nuria Fernández, Luis Monge, Angel Luis García-Villalón, José Luis García, Bernardino Gómez, Godofredo Diéguez *

Departamento de Fisiología, Facultad de Medicina, Universidad Autónoma, 28029 Madrid, Spain

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Abstract

The in vitro effects of endothelin-1 on cerebral veins were studied using cylindrical segments, 5 mm long, from dog pial veins. Isometric responses to endothelin-1 (10^{-12}–10^{-7} M) and to the endothelin ET_B receptor agonist, IRL 1620 (Suc-[Glu^9,Ala^{11,15}]endothelin-1-(8–21), 10^{-12}–10^{-7} M), were recorded in veins under control conditions and pretreated with the endothelin ET_A receptor antagonist, BQ-123 (*cyclo*-(D-Asp-Pro-D-Val-Leu-D-Trp), 10^{-8}–10^{-5} M), and the endothelin ET_B receptor antagonist, BQ-788 (*N*-[*N*-[*N*-[(2,6-dimethyl-1-piperidinyl)carbonyl]-4-methyl-L-leucyl]-1-(methoxycarbonyl)-D-tryptophyl]-D-norleucine monosudium, 10^{-6} and 10^{-5} M). The response to endothelin-1 was also recorded in veins pretreated with the nitric oxide synthesis inhibitor, N^G-nitro-L-arginine methyl ester (L-NAME, 10^{-4} M), or the cyclooxygenase inhibitor, meclofenamate (10^{-5} M), and in veins without endothelium or placed in medium without Ca^{2+} but with EDTA (0.1 mM). In control veins, endothelin-1 produced a concentration-dependent contraction ($EC_{50} = 2.0 \times 10^{-10}$ M; maximal contraction = 113 ± 6 mg) and IRL 1620 induced no effects or a small contraction only with high concentrations (10^{-8}–10^{-6} M) ($EC_{50} = 1.5 \times 10^{-8}$ M; maximal contraction = 9 ± 3 mg). BQ-123 shifted the response to endothelin-1 to the right in a parallel, concentration-dependent way, whereas BQ-788, L-NAME or meclofenamate did not modify the response to endothelin-1. Compared with the control, veins in a medium without Ca^{2+} had similar EC_{50} values, but a lower maximal contraction induced by endothelin-1 (57 ± 10 mg, $P < 0.05$), and veins without endothelium exhibited similar EC_{50} values. Thus, endothelin-1 produces marked cerebral venoconstriction that could be mainly mediated by activation of endothelin ET_A receptors, may be dependent on extracellular Ca^{2+}, and may be independent of endothelium, nitric oxide and prostanoids.

Keywords: Pial vein; Endothelin ET_A receptor; Endothelin ET_B receptor; IRL 1620; (Dog)

1. Introduction

Endothelin-1 and its isopeptides, endothelin-2 and endothelin-3, are 21-amino acid residues that have a potent and prolonged vasoconstrictor effect. The effects of these endothelins seem to be mediated by at least two distinct types of receptors: the endothelin ET_A receptor, which has a higher affinity for endothelin-1 and endothelin-2 than for endothelin-3 (Arai et al., 1990), and the endothelin ET_B receptor, which has similar affinity for these three isopeptides (Sakurai et al., 1990). Various procedures have demonstrated that both endothelin ET_A and ET_B receptors are located in smooth muscle cells and can mediate the vascular contraction in response to endothelin-1, although the relative contribution of these receptors may vary between vascular beds, and between arteries and veins (Ihara et al., 1992; Moreland et al., 1992; Warner et al., 1993; Auguet et al., 1993; Gray et al., 1994). It has been also suggested that endothelin ET_B receptors, located in the endothelium, can also mediate vasodilatation in response to endothelin-1 (Sakurai et al., 1990). With regard to mechanisms involved in the vascular effects of endothelin-1, there are data suggesting that the vasoconstriction may be mediated by influx of extracellular Ca^{2+} (Yanagisawa et al., 1988) and that the vasodilatation may be mediated by release of nitric

* Corresponding author. Departamento de Fisiología, Facultad de Medicina, Universidad Autónoma, Arzobispo Morcillo 2, 28029 Madrid, Spain. Tel.: 34-1-3975424; fax: 34-1-3975317.

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oxide from the endothelium (Rigel and Lappe, 1993) or prostacyclin (De Nucci et al., 1988).

The cerebral vasculature also appears to be sensitive to endothelin-1 as most of data about this issue show that this peptide produces marked cerebral vasoconstriction in vitro (Yanagisawa et al., 1988; García et al., 1991b) and in vivo (Armstead et al., 1989; Diéguez et al., 1992; García et al., 1991a). These observations, together with data showing that the concentrations of endothelin-1 are about seven times higher in cerebrospinal fluid than in plasma of humans (Hoffman et al., 1989), and plasma concentrations of this peptide are elevated in patients with cerebral vasospasm after subarachnoid haemorrhage (Masaoka et al., 1989), suggest that this peptide could be involved in the regulation of the cerebral circulation and in the pathophysiology of some cerebrovascular abnormalities. It has been reported that cerebral vasoconstriction caused by endothelin-1 is mediated by endothelin ET_A receptors (Salom et al., 1993), is endothelium-independent (García et al., 1991b) and is dependent on extracellular Ca^{2+} (García et al., 1991b).

Experiments for studying the effects of endothelin-1 on the cerebral circulation have been done mainly with the arterial bed, and there are very few studies analysing its effects on cerebral veins (Hardebo et al., 1989; Robinson and McCulloch, 1990). One of these studies (Robinson and McCulloch, 1990) showed that the sensitivity of cerebral veins to endothelin-1 is slightly higher, whereas the other (Hardebo et al., 1989) indicates that it is lower than that of cerebral arteries. The endothelin receptors and mechanisms of action involved in the cerebral venoconstriction caused by endothelin-1 have not yet been explored to our knowledge. In the present study and using pharmacological procedures we investigated endothelin receptors (ET_A and ET_B), as well as the role of some possible mediators (nitric oxide, prostanoids, extracellular Ca^{2+}), involved in the cerebral venoconstriction induced by endothelin-1. We have used endothelin-1 and IRL 1620, a specific endothelin ET_B receptor agonist (Takai et al., 1992), as agonists for activating endothelin receptors. BQ-123, a specific endothelin ET_A receptor antagonist (Ihara et al., 1992), and BQ-788, a specific endothelin ET_B receptor antagonist (Ishikawa et al., 1994), were used for testing the antagonism of the agonist-induced response of veins.

2. Materials and methods

2.1. Tissue preparation

36 mongrel dogs of either sex, weighing 15–23 kg, provided by the Centro de Protección Animal (Ayuntamiento de Madrid, Spain), were anaesthetised by i.v. injection of sodium pentobarbital (50 mg/kg body weight) and killed by intracardiac injection of suxamethonium chloride (10 mg/kg). The brain was then removed and placed into isotonic saline (NaCl 0.9%) on ice, and both brain basalis veins (pial veins), carefully dissected, were cut into cylindrical segments 5 mm in length and about 1 mm in external diameter. Each vein segment was prepared for isometric tension recording in a 6-ml organ bath containing modified Krebs-Henseleit solution with the following composition (millimolar): NaCl, 115; KCl, 4.6; KH_2PO_4, 1.2; $MgSO_4$, 1.2; $CaCl_2$, 2.5; $NaHCO_3$, 25; glucose, 11.1. The solution was equilibrated with 95% oxygen and 5% carbon dioxide to give a pH of 7.3–7.4, and the temperature was held at 37°C. Briefly, the method consists of passing two fine, stainless steel pins, 150 μm, through the lumen of the vein segment. One pin was fixed to the organ bath wall while the other was connected to a strain gauge, thus permitting the application of passive tension in a plane perpendicular to the long axis of the vascular cylinder. The recording system included a Universal Transducing Cell UC3 (Statham Instruments), a Statham Microscale Accessory UL5 (Statham Instruments) and a Beckman Type RS Recorder (model R-411, Beckman Instruments). A resting tension of 150 mg was applied and the venous segments were allowed to equilibrate for 40–60 min before any drugs were added. We selected this resting tension because it represents an effective transmural pressure of 3–4 mm Hg, which is in the physiological range for transmural pressure in cerebral veins (Auer and MacKenzie, 1984). The experimental arrangement enabled the wall tension and the internal circumference length of the vessel to be controlled, and assuming that the venous wall was sufficiently thin for Laplace's equation to apply, the effective transmural pressure (P) under applied resting tension was determined. The Laplace equation is,

$$P = 2\pi T/L$$

where T, venous wall tension is the circumferential wall force per unit length given by $F/2g$. F is the force applied to the tissue under resting conditions, and g is the vascular segment length. L, the internal circumference length corresponding to a wall tension T, was calculated by using the formula

$$L = d(2 + \pi) = 1l$$

where d is the diameter of the pins and l is distance between the inner edges of the pins measured by displacement of the micrometer head. The wall tension, T, was calculated in mg/mm and transmural pressures, P, in mg/mm^2. To calculate P in mm Hg the values in mg/mm^2 were divided by 13.6.

2.2. Experimental protocol

The responses of venous segments to endothelin-1 (10^{-12}–10^{-7} M) and to IRL 1620 (specific endothelin ET_B receptor agonist, 10^{-12}–10^{-7} M) were determined in a cumulative manner in the veins immersed in the Krebs-Henseleit solution under control conditions and in the presence of the specific endothelin ET_A receptor antagonist, BQ-123 (10^{-8}–10^{-5} M), or of the specific endothelin ET_B receptor antagonist, BQ-788 (10^{-6} and 10^{-5} M). As the vein response to IRL 1620 was very small, the response to only endothelin-1 was also obtained in the presence of the inhibitor of nitric oxide synthase, N^G-nitro-L-arginine methyl ester (L-NAME, 10^{-4} M), and of the inhibitor of cyclooxygenase, meclofenamate (10^{-5} M). The response to endothelin-1 was also recorded in veins placed in the organ bath containing Krebs-Henseleit solution without Ca^{2+}. This solution was as indicated above, except that it did not contain $CaCl_2$ and did contain Na_2K_2EDTA (0.1 mM). The veins remained immersed in this solution without Ca^{2+} during the equilibration period (40–60 min) and during testing. BQ-123, BQ-788, L-NAME, or meclofenamate was added to the organ bath 20–30 min before endothelin-1 or IRL 1620 was applied to the tissues.

In another group of experiments the response to endothelin-1 was recorded in veins where the endothelium had been removed by gently rubbing their luminal surface. Morphological examination of these rubbed veins at the end of the experiments by direct observation after en face silver staining of their luminal surface, revealed that these veins had less than 5% of their intima covered with endothelium. Using this morphological technique we also found that, at the end of the experiments, the control veins showed more than 50% of their intima covered with endothelium.

In vein segments precontracted with endothelin-1 (10^{-8} M) ($n = 5$), or with noradrenaline (10^{-5} M) ($n = 4$), acethylcholine (10^{-7}–10^{-4} M) did not produce any relaxant effect but produced a concentration-dependent contraction ($EC_{50} = 4.4 \times 10^{-6}$ M, maximal effect = 26 ± 3 mg). This contractile effect was observed both in veins considered as controls (> 50% of endothelium preserved) and in veins pretreated with L-NAME (10^{-4} M) or with meclofenamate (10^{-5} M). As previous studies suggested that the venous endothelium does not appear to mediate the venous response to acethylcholine (García-Villalón et al., 1993; Rubanyi and Vanhoutte, 1988; Seidel and LaRochelle, 1987), it is probable that the venous response to acetylcholine is not a suitable functional test for evaluating the activity of venous endothelium. For this reason, only the morphological test was used to evaluate the presence or the absence of endothelium in the vein segments studied.

To have a reference for the vein contraction in response to stimuli different from endothelin-1 in our system, we also recorded the effects of noradrenaline (10^{-8}–10^{-3} M) on a group of vein segments; the veins were pretreated with propranolol (10^{-6} M) to block β-adrenoceptors. In some vein preparations, the ability of BQ-123 (10^{-5} M) to block the response to noradrenaline was also tested.

All drugs used in this study were prepared in physiological saline

2.3. Data analysis

The concentration of endothelin-1 causing 50% of the maximal response (EC_{50}) was calculated by nonlinear regression analysis for each vein segment, and the geometrical mean and 95% confidence interval of the EC_{50} were calculated for each group of experiments. pA_2 values for BQ-123 were determined by Schild analysis (Arunlakshana and Schild, 1959). The data, expressed as means ± S.E.M., were evaluated by analysis of variance followed by Dunnett's test to compare each experimental condition with its control. In each case, $P < 0.05$ was considered statistically significant.

2.4. Chemicals

Drugs used were: endothelin-1 (human, porcine) and IRL 1620 (Suc-[Glu^9,$Ala^{11,15}$]endothelin-1-(8–21)) from Peninsula Laboratories Europe; BQ-123 (*cyclo*-(D-Asp-Pro-D-Val-Leu-D-Trp)) from Nova-Biochem; BQ-788 (*N*-[*N*-[*N*-[(2,6-dimethyl-1-piperidinyl)-carbonyl]-4-methyl-L-leucyl]-1-(methoxycarbonyl)-D-tryptophyl]-D-norleucine monosudium) from Research Biochemicals International; N^G-nitro-L-arginine methyl ester (L-NAME), noradrenaline ((±)-norepinephrine hydrochloride) and propranolol hydrochloride from Sigma, and sodium meclofenamate from Parke Davis.

3. Results

3.1. Control conditions

Endothelin-1 (10^{-12}–10^{-7} M) produced a slowly developing and then sustained contraction, which was concentration-dependent in every vein segment tested (Figs. 1 and 2A,B). For 31 vein segments, the EC_{50} values were 2.0×10^{-10} M (95% confidence interval: 1.2×10^{-10}–3.3×10^{-10} M) and the maximal contraction was 113 ± 6 mg.

IRL 1620 (10^{-12}–10^{-6} M), a specific agonist for endothelin ET_B receptors, produced no effects, or a small contraction only when higher concentrations were

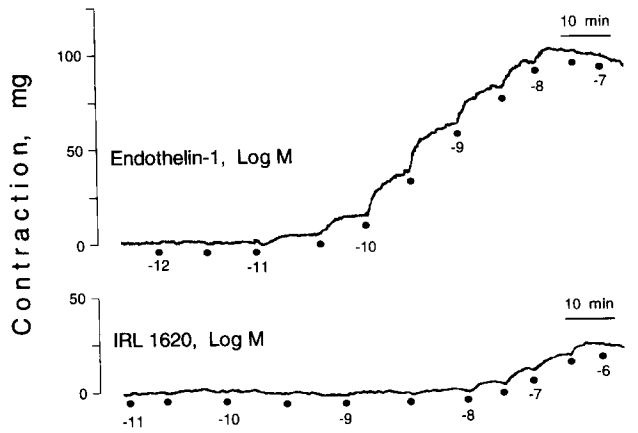

Fig. 1. Representative recordings showing the effects of endothelin-1 and IRL 1620 on segments of canine cerebral veins under control conditions.

applied (10^{-8}–10^{-6} M) (Figs. 1 and 2C,D). For 14 vein segments, the EC_{50} values were 1.5×10^{-8} M (95% confidence interval: 7.2×10^{-9}–3.1×10^{-8} M) and the maximal contraction was 9 ± 3 mg.

Noradrenaline (10^{-8}–10^{-3} M), tested in nine vein segments pretreated with propranolol (10^{-6} M), produced a contraction that was concentration-dependent from 10^{-8} to 10^{-5} M, and was not further increased, or even was decreased when higher concentrations were applied (3×10^{-5}–10^{-3} M). In the concentration-response curve for 10^{-8}–10^{-5} M noradrenaline, the maximal contraction was 55 ± 7 mg and the EC_{50} values were 3.1×10^{-7} M (95% confidence interval: 2.4×10^{-7}–4.1×10^{-7} M).

3.2. Effects of endothelin ET_A and ET_B receptor antagonists

The specific antagonist for endothelin ET_A receptors, BQ-123 (10^{-8}–10^{-5} M), and the specific antagonist for endothelin ET_B receptors, BQ-788 (10^{-6} and 10^{-5} M), themselves did not induce changes in resting tension of the veins.

BQ − 123 produced concentration-dependent, rightward parallel displacement of the concentration-response curve to endothelin-1 (Fig. 2A). Schild analysis of BQ-123 antagonism yielded a pA_2 value of 7.64 (slope of 0.84) against endothelin-1. Analysis of the regression line showed that the slope value was not significantly different from unity. This antagonist, however, did not significantly modify the concentration-response curve for noradrenaline in 11 vein segments (not shown).

BQ-788 did not significantly modify the EC_{50} values or the maximal response of the concentration-response curve for endothelin-1 (Fig. 2B).

BQ-123 (seven vein segments) and BQ-788 (8 vein segments) did not affect either the EC_{50} values or the maximal response to IRL 1620 (Fig. 2C,D).

3.3. Effects of L-name, meclofenamate and endothelium removal

Addition of L-NAME (10^{-4} M) (eight venous segments) or meclofenamate (10^{-5} M) (nine venous seg-

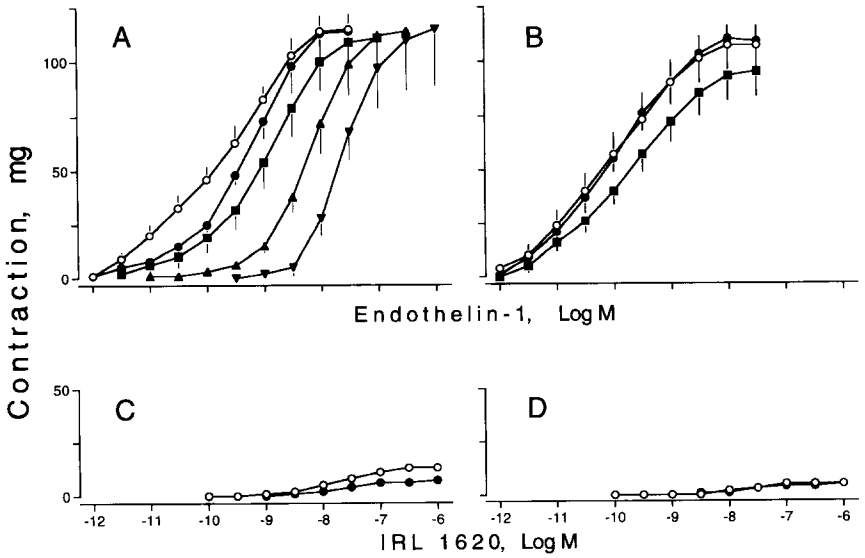

Fig. 2. Concentration-response curves for endothelin-1 (top) and for IRL 1620 (bottom) obtained in canine cerebral veins. Top, for endothelin-1 in veins (A) under control conditions (○) and treated with BQ-123 10^{-8} M (●), 10^{-7} M (□), 10^{-6} M (▲) and 10^{-5} M (▼), and (B) under control conditions (○) and treated with BQ-788 10^{-6} M (●) and 10^{-5} M (□). Bottom, for IRL 1620 in veins (C) under control conditions (○) and treated with BQ-123 10^{-6} M (●), and (D) under control conditions (○) and treated with BQ-788 10^{-5} M (●). Values are means ± S.E.M. for six to nine vein segments in each case.

ments) neither altered the resting tension of the vein segments, nor affected the maximal response or EC_{50} values for endothelin-1 in the vein segments tested (Fig. 3A,B).

In seven vein segments after endothelium removal the EC_{50} values were not significantly altered ($P > 0.05$), but the maximal contraction with endothelin-1 was reduced by 35% (70 ± 9 vs. 108 ± 14 mg, $P < 0.05$), in comparison to that obtained in the veins considered as controls (Fig. 3C).

3.4. Effects of Ca^{2+} free solution

In the presence of Krebs-Henseleit solution without Ca^{2+} but with Na_2K_2EDTA (0.1 mM), endothelin-1 also produced a slowly developing then sustained contraction that was concentration-dependent in the eight vein segments tested. In this case, however, the maximal contraction was reduced about 40% ($P < 0.01$), whereas the EC_{50} values were not significantly modified with regard to the control conditions (Fig. 4A,B)).

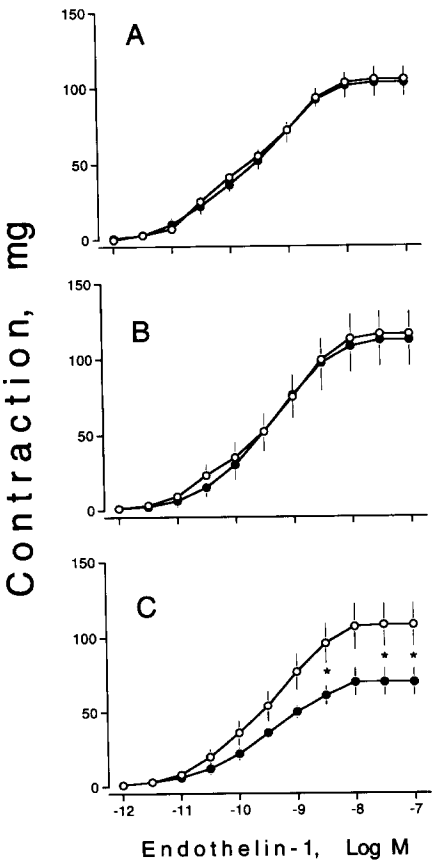

Fig. 3. Concentration-response curves for endothelin-1 obtained in canine cerebral veins: (A) under control conditions (○) and treated with 10^{-4} M L-NAME (●), (B) under control conditions (○) and treated with 10^{-5} M meclofenamate (●) and (C) under control conditions (○) and after endothelium removal (●). Values are means ± S.E.M. for seven to nine vein segments in each case. * $P < 0.05$ compared with its control.

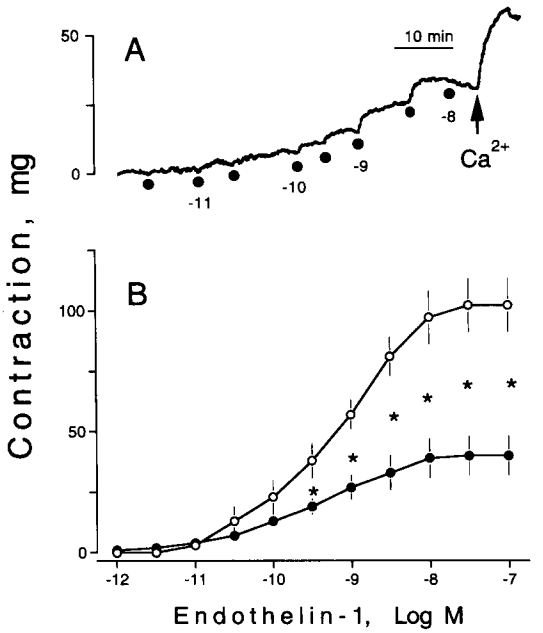

Fig. 4. (A) Representative recording showing the effects of endothelin-1 on one segment of canine cerebral veins placed in a Ca^{2+}-free solution with EDTA 0.1 mM. At right (↑Ca^{2+}) the effect produced by the addition of Ca^{2+} (2.5 mM) to the bath containing Ca^{2+}-free solution and endothelin-1 is shown. (B) Concentration-response curves for endothelin-1 in the veins under control conditions (○) and placed in a Ca^{2+}-free solution with EDTA 0.1 mM (●). Values are means ± S.E.M. for seven to eight vein segments. * $P < 0.01$.

4. Discussion

The results of our study showed that endothelin-1 produces marked cerebral venoconstriction, and suggest that this endothelin-1-induced venoconstriction is mainly mediated by activation of endothelin ET_A receptors.

When we compare the present data for dog cerebral veins with results from our laboratory for cerebral arteries of the same species (García et al., 1991b), it is apparent that cerebral veins exhibit about 34 times more sensitivity, considering the EC_{50} values, and a much lower maximal response (about 25 times), than cerebral arteries. The lower maximal contraction of cerebral veins is probably because their wall has a lower content of smooth muscle than that of cerebral arteries. We also found that cerebral veins showed a higher sensitivity (about 1100 times) and maximal contraction (about 2 times) in response to endothelin-1 than to noradrenaline, suggesting that they have a relatively high reactivity to this peptide. Studies by others also show that cerebral veins constrict in situ

(Robinson and McCulloch, 1990) and in vitro (Hardebo et al., 1989) and in one of these studies (Robinson and McCulloch, 1990) it was shown that cat cerebral veins are slightly more sensitive than cerebral arteries to endothelin-1. Therefore, the effects of endothelin-1 on cerebral veins should be considered for an overall understanding of the regulation of cerebral circulation by this peptide. As cerebral veins are the main determinants of cerebrovascular capacitance and intracranial blood volume, the effects of endothelin-1 on cerebral veins may be of significance in controlling intracranial blood volume and pressure, specially in those situations such as subarachnoid haemorrhage (Masaoka et al., 1989), where plasma concentrations of this peptide are elevated.

Other venous beds such as the jugular, saphenous and omental of different species also contract with endothelin-1. In terms of the EC_{50} values the results suggest that cerebral veins of the dog (present study) are more sensitive to endothelin-1 than the saphenous vein from the rabbit, dog and monkey (Moreland et al., 1992) and the human omental veins (Riezebos et al., 1994), and have a sensitivity similar to that of the rabbit jugular vein (Sumner et al., 1992).

To our knowledge there are no previous studies determining the type of endothelin receptor, ET_A and ET_B, involved in the contraction of cerebral veins in response to endothelin-1. The data herein indicate that endothelin-1 produces marked cerebral venoconstriction, whereas the endothelin ET_B receptor agonist, IRL 1620, induced a very small contraction of cerebral veins. The results also showed that the sensitivity of dog cerebral veins, in terms of the EC_{50} values, is about 75 times higher for endothelin-1 than for IRL 1620. We observed that the endothelin ET_A receptor antagonist, BQ-123, induced a parallel and concentration-dependent rightward shift of the concentration-response curve, without depressing the maximal contraction in response to endothelin-1, and that BQ-123 failed to block the venoconstriction induced by noradrenaline. The concentration-response curve for endothelin-1, however, was not modified by the endothelin ET_B receptor antagonist, BQ-788. Together, these data suggest that the endothelin ET_A, rather than the endothelin ET_B, receptor subtype mediates the cerebral venoconstriction in response to endothelin-1, and that BQ-123 is a competitive antagonist for endothelin-1-induced cerebral venoconstriction as occurs in other vessels (Ihara et al., 1992; Salom et al., 1993). Further evidence for competitive antagonism by BQ-123 was provided by analysis of the slope of the Schild plot which was not significantly different from unity (slope = 0.84). In our study we found that the pA_2 value for BQ-123 in dog cerebral veins was 7.64, which is comparable to that found in goat cerebral arteries (Salom et al., 1993) as well as other arterial (Riezebos et al., 1994) and venous (Riezebos et al., 1994; Lodge and Halaka, 1993) beds. Under our experimental conditions, we observed that the cerebral venoconstriction in response to IRL 1620 was present only when relatively high concentrations of this endothelin ET_B receptor agonist were applied, and that it was not affected by BQ-123 or BQ-788. This suggests that high concentrations of IRL 1620 may induce cerebral venoconstriction which is not probably mediated by endothelin ET_A and ET_B receptors.

Most available studies about venous reactivity to endothelin-1 have been performed with saphenous and jugular vein preparations, and most of the results reported show that the endothelin ET_B receptor subtype is the main mediator for constriction of these veins in response to this peptide (Moreland et al., 1992; Sumner et al., 1992; Lodge and Halaka, 1993; Auguet et al., 1993; Gray et al., 1994). Some of these studies suggest, however, that the endothelin ET_A receptor subtype is also involved, although to a minor degree, in the venoconstriction induced by endothelin-1 (Moreland et al., 1992; Auguet et al., 1993; Gray et al., 1994). On the other hand, results of studies with human omental veins (Riezebos et al., 1994) and rat mesenteric veins (D'Orléans-Juste et al., 1993) suggest that the venoconstriction caused by endothelin-1 is mainly mediated by endothelin ET_A receptors. All these findings suggest that the relative contribution of endothelin receptors, ET_A and ET_B, to the venoconstriction with endothelin-1 may differ between vascular beds and perhaps species, a phenomenon that, according to the literature, also seems to occur between arterial beds.

With regard to mechanisms of action, results of studies performed with cerebral (Jansen et al., 1989; Saito et al., 1989) and non-cerebral (Yanagisawa et al., 1988; Eglen et al., 1989) arteries suggest that endothelin-1 induces contraction by influx of extracellular Ca^{2+}. The present data suggest that, in cerebral veins, the contraction induced by endothelin-1 depends, at least in part, on the concentration of extracellular Ca^{2+} as the veins placed in a Ca^{2+}-free medium exhibited a lower contraction with each concentration of endothelin-1 than those placed in normal Ca^{2+} medium. Thus, endothelin-1 may induce cerebral venoconstriction in part by activating the influx of extracellular Ca^{2+} into the smooth muscle cells of veins, as may occur in cerebral arteries from the same species (García et al., 1991b). The present experimental design did not allow exploration of the way of entrance of extracellular Ca^{2+} into the vein musculature after its activation with endothelin-1, as the aim was only to test if the cerebral venoconstriction with this peptide was or was not dependent on the concentration of extracellular Ca^{2+}.

The role of endothelium in the vascular response to endothelin-1 is not clear as there are contradictory results reported about this issue for cerebral (Hardebo

et al., 1989; Vila et al., 1990; Jansen et al., 1989) and non-cerebral (Marsden et al., 1989; Warner et al., 1989) arteries. In dog cerebral arteries we have previously found that the contraction in response to endothelin-1 is not affected by removal of the endothelium (García et al., 1991b). In the present study we observed that endothelium removal did not affect sensitivity, but decreased by 35% the maximal contraction of cerebral veins with endothelin-1. In this case, the decrease in the capacity of cerebral veins to contract could be related to damage to the smooth musculature of veins after rubbing of their luminal surface. If sensitivity rather than maximal contraction is considered, we suggest that the reactivity of dog cerebral veins to endothelin-1 may be endothelium-independent as occurs in cerebral arteries from this same species (García et al., 1991b).

In a number of studies it has been found that the endothelin ET_B receptor subtype, located in the endothelium, could mediate vasodilatation by releasing nitric oxide (Rigel and Lappe, 1993; Warner et al., 1993) or prostacyclin (De Nucci et al., 1988). Riezebos et al. (1994) have reported that nitric oxide modulates the contraction of human omental arteries, but not of veins, in response to endothelin-1. In our study we observed that L-NAME, an inhibitor of nitric oxide synthesis, and meclofenamate, an inhibitor of prostanoid metabolism, did not affect the venoconstriction induced by endothelin-1, suggesting that nitric oxide and prostanoids are not involved in the effects of this peptide on cerebral veins. A lack of effect of indomethacin on the contraction of human omental arteries and veins (Riezebos et al., 1994) and on the contraction of porcine coronary arteries (Yanagisawa et al., 1988) induced by endothelin-1 has also been reported.

In conclusion, the present results show that endothelin-1 produces marked cerebral venoconstriction, which may be mainly mediated by activation of endothelin ET_A receptors. These results also suggest that cerebral venoconstriction in response to endothelin-1 may be independent of endothelium and nitric oxide or prostanoid release, but may depend on entry of extracellular Ca^{2+} into cells of the vein smooth musculature.

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References

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

Arunlakshana, O. and H.O. Schild, 1959, Some quantitative uses of drug antagonists, Br. J. Pharmacol. 14, 48.

Auer, L.M. and E.T. MacKenzie, 1984, Physiology of the cerebral venous system, in: The Cerebral Vonous System and Its Disorders, ed. J. P. Kapp and H. M. Schmideck (Grune and Stratton, London) p. 169.

Auguet, M., S. Delaflotte, P.E. Chabrier and P. Braquet, 1993, Characterization of endothelin receptors mediating contraction and relaxation in rabbit saphenous artery and vein, Can. J. Physiol. Pharmacol. 71, 818.

De Nucci, G., R. Thomas, P. D'Orléans-Juste, E. Antunes, C. Walder, T.D. Warner and J.R. Vane, 1988, Pressor effects of circulating 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. USA 85, 9797.

Diéguez, G., J.L. García, N. Fernández, A.L. García-Villalón, L. Monge and B. Gómez, 1992, Cerebrovascular and coronary effects of endothelin in the goat, Am. J. Physiol. 263, R834.

D'Orléans-Juste, P., A. Claing, T.D. Warner, M. Yano and S. Télémaque, 1993, Characterization of receptors for endothelins in the perfused arterial and venous mesenteric vasculatures of the rat, Br. J. Pharmacol. 110, 687.

Eglen, R.M., A.D. Michel, N.A. Sharif, S.R. Swank and R.L. Whiting, 1989, The pharmacological properties of the peptide endothelin, Br. J. Pharmacol. 97, 1297.

García, J.L., B. Gómez, L. Monge, A.L. García-Villalón and G. Diéguez, 1991a, Endothelin action on cerebral circulation in unanesthetized goats, Am. J. Physiol. 261, R581.

García, J.L., L. Monge, B. Gómez and G. Diéguez, 1991b, Response of canine cerebral arteries to endothelin-1, J. Pharm. Pharmacol. 43, 281.

García-Villalón, A.L., N. Fernández, J.L. García, L. Monge, B. Gómez and G. Diéguez, 1993, Reactivity of the dog cavernous carotid artery. The role of the arterial and venous endothelium, Pflügers Arch. 425, 256.

Gray, G.A., B.M. Löffler and M. Clozel, 1994, Characterization of endothelin receptors mediating contraction of rabbit saphenous vein, Am. J. Physiol. 266, H959.

Hardebo, J.E., J. Kåhrström, C. Owman and L.G. Salford, 1989, Endothelin is a potent constrictor of human intracranial arteries and veins, Blood Vessels 26, 249.

Hoffman, A., H.R. Keiser, E. Grossman, D.S. Goldstein, P.W. Gold and M. Kling, 1989, Endothelin concentrations in cerebrospinal fluid in depressive patients, Lancet ii, 1519.

Ihara, M., K. Noguchi, T. Saeki, T. Fukuroda, S. Tsuchida, S. Kimura, T. Fukami, K. Ishikawa, M. Nishikibe and M. Yano, 1992, Biological profiles of highly potent novel endothelin antagonists selective for the ETA receptor, Life Sci. 50, 247.

Ishikawa, K., M. Ihara, K. Noguchi, T. Mase, N. Mino, T. Saeki, T. Fukuroda, T. Fukami, S. Ozaki, T. Nagase, M. Nishikibe and M. Yano, 1994, Biochemical and pharmacological profile of a potent and selective endothelin B-receptor antagonist, BQ-788, Proc. Natl. Acad. Sci. USA 91, 4892.

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

Lodge, N.J. and N.N. Halaka, 1993, Endothelin receptor subtype(s) in rabbit jugular vein smooth muscle, J. Cardiovasc. Pharmacol. 22 (Suppl. 8), S140.

Marsden, P.A., N.R. Danthuluri, B.M. Brenner, B.J. Ballermann and T.A. Brock, 1989, Endothelin action on vascular smooth muscle involves inositol trisphosphate and calcium mobilization, Biochem. Biophys. Res. Commun. 158, 86.

Masaoka, H., R. Suzuki, Y. Hirata, T. Emori, F. Marumo and K. Hirakawa, 1989, Raised plasma endothelin in aneurismal subarachnoid haemorrhage, Lancet ii, 1402.

Moreland, S., D.M. McMullen, C.L. Delaney, V.G. Lee and J.T. Hunt, 1992, Venous smooth muscle contains vasoconstrictor ETB-like receptors, Biochem. Biophys. Res. Commun. 184, 100.

Riezebos, J., I.S. Watts and P.J.T. Vallance, 1994, Endothelin receptors mediating functional responses in human small arteries and veins, Br. J. Pharmacol. 111, 609.

Rigel, D.F. and R.W. Lappe, 1993, Differential responsiveness of conduit and resistance coronary arteries to endothelin A and B receptor stimulation in anesthetized dogs, J. Cardiovasc. Pharmacol. 22 (Suppl. 8), S243.

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 P.M. Vanhoutte, 1988, Heterogeneity of endothelium-dependent responses to acetylcholine in canine femoral arteries and veins, Blood Vessels 25, 75.

Saito, A., R. Shiba, S. Kimura, M. Yanagisawa, K. Goto and T. Masaki, 1989, Vasoconstrictor response of large cerebral arteries of cats to endothelin, an endothelium-derived vasoactive peptide, Eur. J. Pharmacol. 162, 353.

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

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

Seidel, C.L. and J. LaRochelle, 1987, Venous and arterial endothelia: different dilator abilities in dog vessels, Circ. Res. 60, 626.

Sumner, M.J., T.R. Cannon, J.W. Mundin, D.G. White and I.S. Watts, 1992, Endothelin ETA and ETB receptors mediate vascular smooth muscle contraction, Br. J. Pharmacol. 107, 858.

Takai, M., I. Umemura, K. Yamasaki, T. Watakabe, Y. Fujitani, K. Oda, Y. Urade, T. Inui, T. Yamamura and T. Okada, 1992, A potent and specific agonist, Suc-[Glu^{9},$Ala^{11,15}$]-endothelin-1(8–21), IRL 1620, for the ETB receptor, Biochem. Biophys. Res. Commun. 184, 953.

Vila, J.M., E. Martín de Aguilera, M.C. Martínez, M. Rodríguez, A. Irurzun and S. Lluch, 1990, Endothelin action on goat cerebral arteries, J. Pharm. Pharmacol. 42, 370.

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 three endothelin receptors mediating smooth muscle contraction and the release of EDRF, Br. J. Pharmacol. 110, 777.

Warner, T.D., J.A. Mitchell, G. De Nucci and J.R. Vane, 1989, Endothelin-1 and endothelin-3 release EDRF from isolated perfused arterial vessels of the rat and rabbit, J. Cardiovasc. Pharmacol. 13 (Suppl. 5), S85.

Yanagisawa, M., H. Kurihara, S. Kimura, Y. Tomobe, M. Kobayashi, Y. Mitsui, Y. Yazaki, K. Goto and T. Masaki, 1988, A novel potent vasoconstrictor peptide produced by vascular endothelial cells, Nature 332, 411.