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In vivo and in vitro action of endothelin-1 on goat cerebrovascular bed

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Abstract

This study concerned the effects and mechanisms of action of endothelin-1 on the cerebral circulation. Cerebral blood flow was electromagnetically measured in awake goats. Endothelin-1 (0.01–0.3 nmol) produced dose-dependent decreases in this flow (maximal reduction = 34%) and increases in cerebrovascular resistance (maximal increase = 74%) ($P < 0.01$). IRL 1620 (Suc-[Glu^{9}, Ala^{11,15}]endothelin-1-(8–21), agonist for endothelin ET_B receptors, 0.01–0.3 nmol) slightly decreased cerebral blood flow. The effects of endothelin-1, but not those of IRL 1620, on cerebral blood flow were diminished by 50% during infusion of the antagonist for endothelin ET_A receptors, BQ-123 (cyclo-(D-Asp–Pro–D-Val–Leu–Trp), 2 nmol min^{-1}), but not affected during infusion of the antagonist for endothelin ET_B receptors, BQ-788 (*N*-[*N*-[*N*-[(2,6-dimethyl-1-piperidinyl)carbonyl]-4-methyl-L-Leucyl-1-(methoxycarbonyl)-D-tryptophyl]-D-norleucine monosodium), 2 nmol min^{-1}). Intravenous administration of N^{W}-nitro-L-arginine methyl ester (L-NAME, 47 mg kg^{-1}) or N^{W}-nitro-L-arginine (L-NNA, 47 mg kg^{-1}) reduced basal cerebral blood flow by 39 and 33%, increased cerebrovascular resistance by 108 and 98% and mean arterial pressure by 23 and 17%, and decreased heart rate by 27 and 25%, respectively (all at least $P < 0.05$). The increases in cerebrovascular resistance (as absolute values) induced by endothelin-1 were not affected during either L-NAME or L-NNA (as absolute values and percentages). Intravenous administration of meclofenamate (5 mg kg^{-1}) did not change the cerebrovascular effects of endothelin-1 and IRL 1620. In isolated goat cerebral arteries under control, resting conditions, endothelin-1 (10^{-11}–10^{-7} M) induced concentration-dependent contractions ($EC_{50} = 4.78 \times 10^{-9}$ M; maximal contraction = 3177 ± 129 mg), whereas IRL 1620 (10^{-11}–10^{-7} M) produced no effect. This contraction produced by endothelin-1 was competitively blocked by BQ-123 (10^{-7}–3×10^{-6} M), and was not affected by BQ-788 (10^{-6} and 10^{-5} M). L-NAME (10^{-4} M), meclofenamate (10^{-5} M), indomethacin (10^{-5} M), L-NAME (10^{-4} M) plus meclofenamate (10^{-5} M) and phosphoramidon (10^{-4} M) did not affect the contraction in response to endothelin-1. Endothelium removal increased the response to endothelin-1, as well as the BQ-123 antagonism against endothelin-1 (pA_2 values, 7.62 vs. 6.88; $P < 0.01$). In both intact and de-endothelized arteries precontracted with prostaglandin $F_{2\alpha}$, endothelin-1 induced a further contraction, and IRL 1620 caused no effect. These results suggest that: (1) endothelin-1 produces cerebral vasoconstriction by activating endothelin ET_A receptors probably located in smooth muscle; (2) endothelin ET_B receptors, nitric oxide and prostanoids might be not involved in the cerebrovascular action of endothelin-1, and (3) endothelium removal may increase cerebrovascular reactivity by increasing sensitivity of endothelin ET_A receptors to endothelin-1.

Keywords: Cerebral circulation; Endothelin receptor; Endothelium; Nitric oxide (NO); Prostanoid

1. Introduction

Endothelin-1 is a potent vasoconstrictor 21-amino acid peptide that may be involved in regulation of the cerebral circulation under normal and some pathological conditions. This was suggested on the basis that the cerebrovascular endothelium constitutively produces endothelin-1 (Adner et al., 1994). Concentrations of this peptide in cerebrospinal fluid of humans are about seven times higher than in plasma (Hoffman et al., 1989), and the concentration in cerebrospinal fluid is elevated in patients with cerebrovascular spasm after subarachnoid hemorrhage (Suzuki, 1990). Also, its concentration in brain tissue and plasma is elevated after brain ischemia (Bian et al., 1994). The effects of this peptide on the cerebral circulation, however, are not well established and controversial results are reported for these effects. Also, knowledge of the mechanisms of endothelin-1 action on cerebral vasculature is confusing as experiments exploring this issue are still

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scarce and the results reported are contradictory. There is agreement that isolated cerebral arteries and veins of several species, including humans, contract in response to endothelin-1 (García et al., 1991b; Hardebo et al., 1989; Yanagisawa et al., 1988). In vivo studies, however, yield discrepant results as this peptide can produce vasoconstriction (Armstead et al., 1989; Kobari et al., 1994; Willette et al., 1990), vasodilatation (Kobari et al., 1994; Willette et al., 1990) or no effect (Kadel et al., 1990) in the cerebral circulation. With regard to endothelin receptors, it has been suggested that the cerebral vasoconstriction in response to endothelin-1 may be mediated (Adner et al., 1994; Gulati et al., 1996; Patel et al., 1996; Sagher et al., 1994; Salom et al., 1993) or not (Kobari et al., 1994) by endothelin ET_A receptors. The role of endothelin ET_B receptors in the cerebrovascular effects of endothelin-1 has been less studied, and some reports suggest that these receptors mediate cerebral vasodilatation in response to the peptide (Kitazono et al., 1995; Schilling et al., 1995; Patel et al., 1996). About the role of the endothelium, the results reported are contradictory as the contraction induced by endothelin-1 in cerebral arteries can be endothelium-independent (García et al., 1991b; Yanagisawa et al., 1988) or endothelium-dependent (Jansen et al., 1989; Saito et al., 1989). Some experiments suggest that prostanoids mediate the cerebral vasoconstriction in response to endothelin-1 (Armstead et al., 1989), but others suggest that prostanoids are not involved in this vasoconstriction (García et al., 1991b). Studies on the role of nitric oxide in the cerebral vasoconstriction induced by endothelin-1 are particularly scarce, and it has been reported that inhibition of nitric oxide synthesis does not affect the cerebral vasoconstriction in response to endothelin-1 (Moreau et al., 1995).

The present study was performed to analyze the effects, and especially the mechanisms of action, of endothelin-1 on the cerebral circulation. The experiments were carried out using in vivo and in vitro preparations of the goat cerebral vasculature. In vivo experiments were performed in awake goats where blood flow to one brain hemisphere can be electromagnetically measured, and relatively small doses of drugs can be injected directly into the cerebral circulation (Reimann et al., 1972). In vitro experiments were made with isolated cerebral arteries from goats. The arteries were mounted in organ baths and examined under different experimental conditions. In these letter experiments, we examined the role of endothelin ET_A and ET_B receptors as well as the role of the endothelium, of nitric oxide and prostanoids in the effects of endothelin-1 on the cerebral vasculature. We have previously observed, also in goats, that endothelin-1 produces cerebral vasoconstriction in vivo and in vitro (Diéguez et al., 1992; García et al., 1991a). For one of these studies (Diéguez et al., 1992), we have reported that the in vitro cerebral vasoconstriction in response to endothelin-1 was endothelium-independent, an idea that must be revised in view of the results of the present study.

2. Materials and methods

2.1. In vivo studies

Some 15 female goats, ranging in weight from 30 to 52 kg, were used. In this species, each internal maxillary artery, a branch of the external carotid artery, provides the total blood flow to each cerebral hemisphere via the rete mirabile; the vertebral arteries do not contribute to brain blood flow, and the extracranial internal carotid artery is absent (Daniel et al., 1953; Reimann et al., 1972). The circle of Willis in the goat is similar to that of humans except that the blood flows in a caudal direction in the basilar artery (Daniel et al., 1953; Reimann et al., 1972). Analysis of the distribution of radioactively labeled microspheres in the cerebral circulation of the goat after the surgical procedure described by Reimann et al. (1972) indicates that nearly all the blood carried by the internal maxillary artery passes directly to cerebral tissue (Miletich et al., 1975). Extracerebral blood flow is minimal, $< 5\%$ of total flow.

The surgery has been described elsewhere (Reimann et al., 1972). Briefly, the extracerebral vessels from one of the internal maxillary arteries were ligated and thrombosed with 1000 NIH units of thrombin (thrombin, topical; Parke Davis, Detroit, MI) dissolved in 1 ml of 0.9% NaCl solution. This maneuver produces an almost immediate obliteration of the ethmoidal, ophthalmic, and buccinator arteries and thus eliminates blood flow to the eye and other facial structures. This is confirmed on recovery from surgery by the presence of ipsilateral blindness. However, obliteration of the extracerebral vessels from the internal maxillary artery does not cut off vascular supply to half of the face. The areas supplied by the ethmoidal, buccinator, dental, and temporal arteries are nourished by anastomotic channels that are normally in a state of dynamic balance but in which the direction of blood flow can be quickly changed, depending on the pressure differential from one side of the union to the other (Daniel et al., 1953; Reimann et al., 1972). There is no necrosis, and the functions related to these areas such as eating, drinking, and rumen are intact. Obliteration and thrombosis of the ophthalmic artery permanently cuts off vascular supply to the ipsilateral eye. This procedure becomes necessary for the successful isolation of the cerebral circulation (Miletich et al., 1975; Reimann et al., 1972). The ipsilateral blindness that ensues does not seem to alter normal behavior and physical condition of the animals.

An electromagnetic flow transducer (Biotronex) was placed on the internal maxillary artery to measure blood flow to the ipsilateral cerebral hemisphere. A polyethylene catheter (PE-90) inserted in the temporal artery permitted the injection of drugs directly into the internal maxillary artery in the awake goat; the same catheter was used to measure arterial blood pressure with a Statham transducer (P23 ID). A snare-type occluder was placed on the external

carotid, close to the temporal artery, to obtain zero-flow baselines. The external connecting leads from the flow transducer and occluder and the temporal artery catheter were led out subcutaneously and secured to a horn of the goat.

Heart rate was measured from the arterial pressure pulse with a ratemeter. Flow measurements were made with a Biotronex electromagnetic flowmeter (model BL-610). Cerebral blood flow, arterial blood pressure, and heart rate were recorded on a Dynograph Recorder R611 (SensorMedics). Cerebrovascular resistance was calculated by dividing mean systemic arterial pressure in mm Hg by cerebral blood flow in milliliter per minute.

The experiments on the unanesthetized goat were started 2–3 days after the operative procedure, at which time the goats had fully recovered and were in good condition. The various measurements were made with the goat unrestrained in a large cage, except for a Lucite stock, fitting loosely around the neck, that limited forward and backward motion. Once placed in the cage the animal stood quietly during the experiments and showed no signs of disturbance.

Endothelin-1 (human, porcine) at doses of 0.01, 0.03, 0.1 and 0.3 nmol was injected in volumes of <0.5 ml directly into the internal maxillary artery of the unanesthetized animals, with cerebral blood flow, systemic arterial pressure, and heart rate simultaneously and continuously recorded. Each dose was administered at 6- to 40-min intervals, and the experiments were performed under control conditions and during treatment with the antagonist for endothelin ET_A receptors, BQ-123 (Cyclo-(D-Asp–Pro–D-Val–Leu–D-Trp), Ihara et al., 1992), the antagonist for endothelin ET_B receptors, BQ-788 (*N*-[*N*-[*N*[2,6-dimethyl-1-piperidinyl) carbonyl]-4-methyl-L-Leucyl]-1-(methoxycarbonyl)-D-tryptophyl)-D-norleucine monosodium), Ishikawa et al., 1994), the inhibitors of nitric oxide synthesis, N^W-nitro-L-arginine methyl ester (L-NAME) or N^W-nitro-L-arginine (L-NNA), as well as with the cyclooxygenase inhibitor, meclofenamate.

BQ-123 (2 nmol min^{-1}, seven animals) or BQ-788 (2 nmol min^{-1}, five animals) was administered by infusion through the catheter placed into the internal maxillary artery, and endothelin-1 was injected during the infusion of these antagonists. L-NAME was first administered by i.v. bolus of 35 mg kg^{-1} and when the hemodynamic parameters being recorded were stable, an additional i.v. infusion was administered at a rate of 0.15–0.20 mg kg^{-1} min^{-1} (in total the animals received 47 mg kg^{-1} of L-NAME). Endothelin-1 was injected during this i.v. infusion of L-NAME to five animals. L-NNA was administered in the same way and in the same doses as L-NAME, and endothelin-1 was injected during the i.v. infusion of L-NNA to four goats. To allow comparison with the effects of endothelin-1, we also examined the effects of noradrenaline (0.3, 1, 3 and 9 μg) on cerebral blood flow in a separate group of animals under control conditions and during L-NAME treatment (five goats). In these animals, noradrenaline was also injected directly into the internal maxillary artery, and L-NAME was administered as indicated above. Meclofenamate was administered as i.v. bolus of 2 mg kg^{-1} over 3–4 min, and then i.v. infused at a rate of 1 mg kg^{-1} min^{-1} (in total the animals received 5 mg kg^{-1} of meclofenamate). Endothelin-1 was injected during the i.v. infusion of meclofenamate in five animals.

IRL 1620, an agonist for endothelin ET_B receptors ((Suc-[Glu^9, $Ala^{11,15}$]endothelin-1-(8–21), Takai et al., 1992) at doses of 0.01, 0.03, 0.1 and 0.3 nmol was also injected in volumes of <0.5 ml into the internal maxillary artery. This was performed in awake goats under control conditions and during the administration of BQ-123 (2 nmol min^{-1}, six animals), of BQ-788 (2 and 4 nmol min^{-1}, five animals), of L-NAME (47 mg kg^{-1}, four animals) and of meclofenamate (5 mg kg^{-1}, five animals). These antagonists for endothelin ET_A and ET_B receptors, L-NAME and meclofenamate were injected as indicated above.

In each animal, experiments and measurements were performed throughout 5–16 days. During this period, at least three control dose–response curves for endothelin-1 and IRL 1620 were determined in each animal on different days, and the responses to these peptides after each treatment were examined 3–24 h after the control dose–response curve had been obtained. The treatments with L-NAME and L-NNA were given to different animals, and each of the treatments was tested at intervals of 1–3 days depending on the type of treatment applied. In our experiments we found that the responses to endothelin-1 had recovered to control conditions about 3 h after the treatment with the antagonists, and 2–3 days after the treatment with L-NAME or L-NNA. For each animal only one control dose–response curve for endothelin-1 and IRL 1620 was considered, which was obtained by averaging the results obtained for control dose–response curves recorded on different days. This average control dose–response curve was then used for comparisons with the results obtained with various treatments in the same animal.

Arterial blood pH, pCO_2 and pO_2 were measured by standard electrometric methods (Radiometer, ABL 300, Copenhagen) before and under the various experimental conditions.

After termination of the experiments the goats were killed with an overdose of i.v. thiopental sodium.

2.2. In vitro studies

The brain of 39 goats were removed after the animals were killed with i.v. injections of 2% thiopental sodium and a saturated solution of KCl. Branches of the middle cerebral artery were dissected free and cut into cylindrical segments, 3 mm in length and 300–500 μm in external diameter, and mounted in an organ bath for isometric tension recording. Two stainless steel pins, 150 μm in

diameter, were introduced through the arterial lumen; one pin was fixed to the organ bath wall, and the other was connected to a strain gauge. The recording system included a Universal transducing cell (UC 3), a Statham microscale accessory (U15), and a Beckman type RS recorder. Each arterial segment was set up in a 4-ml organ bath containing modified Krebs–Henseleit solution of the following composition (in mM): 115 NaCl, 4.6 KCl, 1.2 KH_2PO_4, 1.2 $MgSO_4$, 2.5 $CaCl_2$, 25 $NaHCO_3$, 11.1 glucose, and 0.01 disodium EDTA. The solution was equilibrated with 95% O_2–5% CO_2 to a pH of 7.3–7.4. The temperature was held at 37°C.

Some arterial segments were mounted in the organ bath immediately after the arteries were removed from the animals (fresh arteries), and others were mounted 24 h after the arteries were removed from the animals (24-h stored arteries). These 24-h stored arteries were placed in cold saline immediately after they were removed from the animals, and were then stored at a temperature of 4°C.

A resting tension of 1 g was applied to each vascular segment and equilibrated for 60–90 min before experiments began. The resting tension of 1 g was selected as we have previously found that this tension is the optimal one to contract in response to 100 mM KCl, after the arteries were subjected to resting tensions of 0.3–1.5 g. Cumulative concentration–response curves for endothelin-1 (10^{-11} to 10^{-7} M) and for IRL 1620 (10^{-11}–10^{-7} M) were made for cerebral arteries under resting conditions or precontracted with prostaglandin $F_{2\alpha}$ (10^{-6}–10^{-5} M).

In resting arteries, the effects of endothelin-1 were evaluated under control conditions and after treatment with BQ-123 (10^{-7}–3×10^{-6} M), BQ-788 (10^{-6} and 10^{-5} M), meclofenamate (10^{-5} M), indomethacin (cyclooxygenase inhibitor, 10^{-5} M), L-NAME (10^{-4} M), L-NAME (10^{-4} M) plus meclofenamate (10^{-5} M) or phosphoramidon (10^{-4} M). The effects of endothelin-1 were also tested in resting arteries after endothelium removal, and not treated or treated with BQ-123 (3×10^{-8}–10^{-6} M) or BQ-788 (10^{-6} and 10^{-5} M). The effects of IRL 1620 were evaluated in resting arteries under control conditions and after endothelium removal. As this agonist for endothelin ET_B receptors had no effect on arteries, it was not tested in the presence of blocking agents.

For precontracted cerebral arteries with prostaglandin $F_{2\alpha}$(10^{-6}–10^{-5} M), the effects of endothelin-1 (10^{-11}–10^{-7} M) and IRL 1620 (10^{-11}–10^{-7} M) were recorded in intact and de-endothelized arteries.

Each treatment was applied to the organ bath for 20–25 min, except for phosphoramidon that was added about 90 min before endothelin-1 or IRL 1620 was tested. The endothelium was removed by gentle rubbing of the internal surface of the arteries with a roughened steel rod. The presence of endothelium or the adequacy of endothelial removal was tested functionally at the end of the experiments by recording the relaxation response to acetylcholine (10^{-7} and 10^{-6} M) of arteries precontracted with prostaglandin $F_{2\alpha}$ or endothelin-1. The response to acetylcholine (10^{-7}–10^{-6} M) was tested in intact fresh and 24-h stored arteries, with and without endothelium, precontracted with prostaglandin $F_{2\alpha}$ or endothelin-1.

For comparison with the effects of endothelin-1, we also recorded the effects of noradrenaline (10^{-8}–10^{-3} M) in fresh, resting cerebral arteries under the following conditions: control, treated with L-NAME (10^{-4} M), deprived of endothelium, and treated with phentolamine (3×10^{-8}–10^{-6} M) (this treatment was performed in arteries with and without endothelium).

Each arterial segment, either under control conditions or after the various treatments, was used for only one concentration–response curve for endothelin-1, IRL 1620 or noradrenaline.

The effective concentrations eliciting 50% of the maximal response (EC_{50}) to endothelin-1, IRL 1620 and noradrenaline were obtained from the curves under the different conditions tested. The concentration–response curves for endothelin-1 and IRL 1620 were obtained by calculation of the effects of these substances on each arterial segment relative to their maximal effect on the corresponding arterial segment. For noradrenaline the curves were obtained by calculation of the effects of this amine in each arterial segment relative to those produced by KCl (100 mM) in the corresponding arterial segment.

2.3. Drugs used

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-eucyl]-1-(methoxycarbonyl)-D-tryptophyl]-D-norleucine monosodium), (*N*-((-(6-Doxy-a-L-mannopyranosyl)-L-leucyl)-L-tripto-phan)(phosphoramidon) from Research Biochemicals International; L-NAME, L-NNA, indomethacin, norepinephrine bitartrate, and phentolamine hydrochloride from Sigma, and sodium meclofenamate from Parke Davis. All drugs were prepared in saline immediately before the beginning of the experiments.

2.4. Data analysis

A repeated measure analysis of variance followed by Dunnett's test was applied to the entire group of data obtained with endothelin-1, IRL 1620 and noradrenaline in the in vivo experiments under the different conditions tested. The effects of these substances on cerebral blood flow and cerebrovascular resistance were evaluated based on both changes in absolute values from the corresponding basal value, and percentage change, taking the corresponding basal value as 100 percent. To compare the in vivo effects of endothelin-1, IRL 1620 and noradrenaline under control conditions and under the various experimental

conditions, Student's *t*-test for paired data was applied using animals as their own control. Results of the in vitro experiments were analyzed by means of Student's *t*-test for unpaired data; in each case, arteries examined under control conditions and the various experimental conditions and removed from the same animals were compared. The data for pA_2 for the blocking action of BQ-123 on endothelin-1-induced responses, and for the blocking action of phentolamine on the noradrenaline-induced responses obtained in intact and de-endothelized arteries were evaluated by applying an analysis of variance, followed by Dunnett's test. $P < 0.05$ was considered significant.

3. Results

3.1. In vivo studies

The basal values for hemodynamic variables and blood gases and pH in 15 awake goats under control conditions were: cerebral blood flow = 61 ± 3 ml, mean systemic arterial pressure = 101 ± 4 mmHg, heart rate = 73 ± 6 beats min^{-1}; pCO_2 = 37 ± 2 mmHg, pO_2 = 86 ± 4 mmHg, and pH = 7.40 ± 0.01.

3.1.1. Control conditions

Endothelin-1 (0.01–0.3 nmol) produced dose-dependent decreases in cerebral blood flow and increases in cerebrovascular resistance. For 10 goats the reductions in cerebral blood flow ranged from 9% (for 0.01 nmol) ($P < 0.05$) to 34% (for 0.3 nmol) ($P < 0.01$) and the increases in cerebrovascular resistance ranged from 13% (0.01 nmol) ($P < 0.05$) to 74% (0.3 nmol)($P < 0.01$). Only higher doses (0.1 and 0.3 nmol) produced slight hypertension and bradycardia. The effects of this peptide on cerebral blood flow persisted for 4–25 min depending on the doses and were evident before systemic effects, when these occurred.

IRL 1620 (agonist for endothelin ET_B receptors, 0.01–0.3 nmol) induced small reductions of cerebral blood flow, which became significant ($P < 0.05$) only with the higher doses (0.1 and 0.3 nmol). In nine goats, the reductions of cerebral blood flow by IRL 1620 ranged from 2% (for 0.01 nmol) to 12% (for 0.3 nmol). This endothelin ET_B receptor agonist did not affect systemic arterial pressure and heart rate, as the values for these variables after IRL 1620 administration were not significantly different from those measured under basal conditions.

3.1.2. Effects of endothelin ET_A and ET_B receptors antagonists

BQ-123 (antagonist for endothelin ET_A receptors, 2 nmol min^{-1}, seven goats) and BQ-788 (antagonist for endothelin ET_B receptors, 2 nmol min^{-1}, five goats), injected by infusion through the internal maxillary artery, did not change resting cerebral blood flow, arterial pressure, heart rate, and blood gases or pH. During the infusion of BQ-123, the reduction of cerebral blood flow caused by endothelin-1 was diminished by about 50% (Fig. 1), and the systemic hypertensive effects of this peptide were not affected. During the infusion of BQ-788, the effects of endothelin-1 on cerebral blood flow and systemic arterial pressure were not significantly different from those under control conditions (Fig. 1).

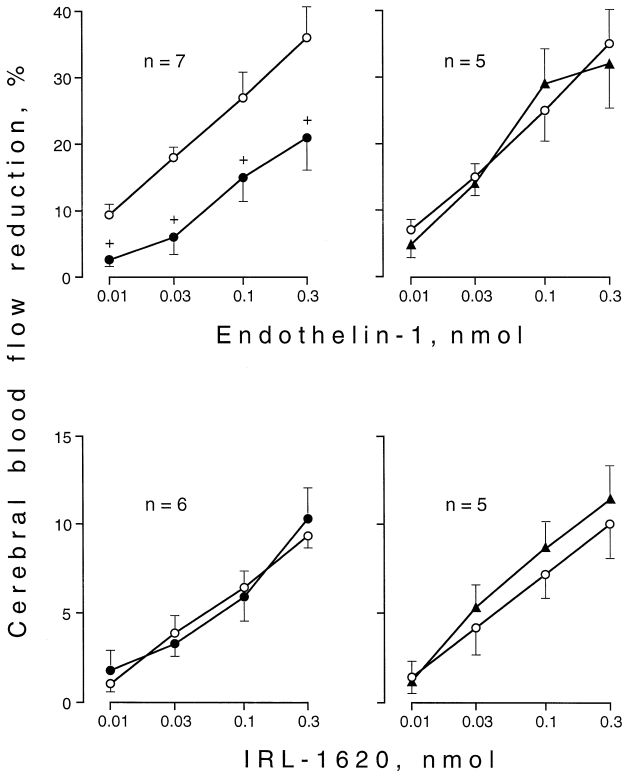

Fig. 1. Top: effects of endothelin-1 on cerebral blood flow of awake goats under control conditions (○——○) and after treatment with BQ-123 (2 nmol min^{-1}, ●——●) (left) and after treatment with BQ-788 (2 nmol min^{-1}, ▲——▲) (right). Bottom: effects of IRL 1620 on cerebral blood flow of awake goats under control conditions (○——○) and after treatment with BQ-123 (2 nmol min^{-1}, ●——●) (left) and after treatment with BQ-788 (4 nmol min^{-1}, ▲——▲) (right). $^{+}P < 0.01$ compared with its control. n = Number of animals.

The effects of IRL 1620 on cerebral blood flow during the intraarterial infusion of BQ-123 (2 nmol min^{-1}, six goats) or BQ-788 (2 and 4 nmol min^{-1}, five goats) did not differ significantly from those recorded under control conditions (Fig. 1).

3.1.3. Effects of L-NAME, L-NNA and meclofenamate

L-NAME (five goats) or L-NNA (four goats) themselves reduced resting cerebral blood flow by 39 and 33% ($P < 0.01$), increased cerebrovascular resistance by 108 and 98% ($P < 0.01$) mean systemic arterial pressure by 23 and 17% ($P < 0.05$), and decreased heart rate by 27 and 25% ($P < 0.05$), respectively; these substances did not change significantly either blood gases or pH. The effects of endothelin-1 on cerebrovascular resistance during L-NAME

Table 1
Increases in cerebrovascular resistance (CVR) expressed as absolute values (mmHg ml^{-1} min^{-1}) and as percentages, as well as decreases in cerebral blood flow (CBF) as absolute values (ml min^{-1}) and as percentages induced by endothelin-1 (ET-1) in awake goats under control conditions (10 goats) and after treatment with L-NAME (five goats), L-NNA (four goats) or meclofenamate (five goats)

	0.01		0.03		0.1		0.3 nmol of ET-1	
	CVR	CBF	CVR	CBF	CVR	CBF	CVR	CBF
In absolute								
Control	0.21 ± 0.03	6 ± 0.46	0.38 ± 0.05	11 ± 1.03	0.72 ± 0.08	16 ± 1.94	1.29 ± 0.09	22 ± 4.00
L-NAME	0.19 ± 0.05	2 ± 0.69[b]	0.47 ± 0.07	4.5 ± 0.56[b]	0.78 ± 0.18	6.5 ± 1.08[b]	1.54 ± 0.23	10 ± 1.78[b]
L-NNA	0.30 ± 0.08	3 ± 0.51[a]	0.60 ± 0.11	5 ± 0.52[b]	0.94 ± 0.29	7 ± 0.61[b]	1.62 ± 0.47	9 ± 1.44[a]
Meclofenamate	0.16 ± 0.06	5 ± 0.83	0.36 ± 0.08	8.5 ± 1.13	0.70 ± 0.19	15 ± 2.04	1.43 ± 0.31	22 ± 4.3
In percentage								
Control	11 ± 1.45	9 ± 0.67	22 ± 2.49	17 ± 0.99	39 ± 4.07	24 ± 1.86	74 ± 7.97	31 ± 2.04
L-NAME	6 ± 1.52[a]	6 ± 1.04	16 ± 1.64[a]	12 ± 1.17[a]	25 ± 4.87[a]	17 ± 2.98[a]	48 ± 9.76[a]	27 ± 4.43
L-NNA	9 ± 2.68	8 ± 1.93	19 ± 3.91	15 ± 2.33	34 ± 7.01	22 ± 2.36	53 ± 11.89	27 ± 3.09
Meclofenamate	9 ± 1.96	9 ± 1.87	20 ± 3.88	13 ± 3.32	43 ± 7.26	24 ± 2.19	83 ± 9.64	33 ± 3.81

Values are means ± S.E.M. with regard to the corresponding basal values.
Basal values for CVR (mmHg ml^{-1} min^{-1}) under control conditions and after L-NAME, L-NNA or meclofenamate, respectively, were: 1.61 ± 0.08, 3.26 ± 0.29, 3.22 ± 0.31 and 1.65 ± 0.14.
Basal values for CBF (ml min^{-1}) under control conditions and after L-NAME, L-NNA or meclofenamate, respectively, were: 61 ± 3, 38 ± 3, 39 ± 3 and 60 ± 6.
[a] $P < 0.05$ and [b] $P < 0.01$ compared with its control.

treatment, in absolute but not in percentage terms, and during L-NNA treatment, both in absolute and in percentage terms, were similar to those found under control conditions (Table 1). The effects of endothelin-1 on cerebral blood flow during L-NAME treatment, as absolute values and as percentages, and during L-NNA treatment, only as absolute values, were lower than under control conditions (Table 1). L-NAME and L-NNA did not modify significantly the effects of endothelin-1 on systemic arterial pressure.

During L-NAME treatment, the effects of IRL 1620 on cerebral blood flow were not significantly different from those under control conditions (not shown).

Noradrenaline (0.3–9 μg, five goats) produced dose-dependent decreases in cerebral blood flow and increases in cerebrovascular resistance without affecting significantly either heart rate or systemic arterial pressure. The effects of noradrenaline on cerebral blood flow (as percentages but not as absolute values) and on cerebrovascular resistance (both as percentages and as absolute values; these results are not shown) were significantly increased during L-NAME as compared with those found under control conditions. Absolute values (ml min^{-1}) for the reductions of cerebral blood flow caused by noradrenaline under control conditions and L-NAME treatment, respectively, were: 7 ± 1.02 vs. 7 ± 1.17 (0.3 μg), 10 ± 1.25 vs. 11 ±

Table 2
Values for EC_{50} and maximal contraction (E_{max}) obtained with endothelin-1 (10^{-11}–10^{-6} M) in resting fresh cerebral arteries under control conditions and after treatment with BQ-123 (10^{-6} M), BQ-788 (10^{-6} M), L-NAME (10^{-4} M), meclofenamate (10^{-5} M), indomethacin (10^{-5} M), L-NAME (10^{-4} M) plus meclofenamate (10^{-6} M), and phosphoramidon (10^{-4} M)

	EC_{50} (M)		E_{max} (mg)
	Mean	95% confidence interval	
Control ($n = 34$)	4.78×10^{-9}	$3.65–6.25 \times 10^{-9}$	3177 ± 129
BQ-123 ($n = 10$)	3.04×10^{-8}[a]	$2.46–3.77 \times 10^{-8}$[a]	3036 ± 214
BQ-788 ($n = 6$)	4.21×10^{-9}	$1.78–9.96 \times 10^{-9}$	3292 ± 211
L-NAME ($n = 11$)	3.29×10^{-9}	$1.62–8.48 \times 10^{-9}$	3520 ± 237
Meclofenamate ($n = 9$)	3.02×10^{-9}	$1.85–4.95 \times 10^{-9}$	3351 ± 194
Indomethacin ($n = 8$)	5.81×10^{-9}	$1.05–8.92 \times 10^{-9}$	3297 ± 244
L-NAME + Meclofenamate ($n = 8$)	2.93×10^{-9}	$1.74–4.87 \times 10^{-9}$	3698 ± 298
Phosphoramidon ($n = 8$)	2.70×10^{-9}	$1.61–4.54 \times 10^{-9}$	2777 ± 137

Values are means ± S.E.M.
[a] $P < 0.01$ compared with its corresponding control.
n = Number of arterial segments.

1.30 (1 μg), 15 $\pm$ 1.31 vs. 13 $\pm$ 1.39 (3 μg) and 18 $\pm$ 1.47 vs. 17 $\pm$ 1.36 (9 μg) (all $P > 0.05$). As percentages, these reductions, respectively were: 13 $\pm$ 1.93 vs. 21 $\pm$ 3.18 (0.3 μg), 19 $\pm$ 2.80 vs. 39 $\pm$ 3.51 (1 μg), 26 $\pm$ 2.96 vs. 36 $\pm$ 3.67 (3 μg) and 30 $\pm$ 2.78 vs. 46 $\pm$ 382 (9 μg) (all at least $P < 0.05$).

Meclofenamate, injected i.v. (5 mg kg^{-1}, five goats), did not affect significantly the resting cerebral blood flow, cerebrovascular resistance and heart rate, and decreased slightly, but significantly the systemic arterial pressure ($P < 0.05$). This substance did not affect significantly either blood gases or pH. After meclofenamate treatment, the effects of endothelin-1 (0.01–0.3 nmol) (Table 1) and IRL 1620 (0.01–0.3 nmol) (not shown) on cerebral blood flow, cerebrovascular resistance and systemic arterial pressure were not significantly different from those under control conditions.

3.2. In vitro studies

3.2.1. Control conditions

In fresh arteries under resting tension, endothelin-1 (10^{-11}–10^{-7} M) produced a slowly developing, then sustained contraction which was concentration-dependent, in every arterial segment tested (Table 2).

IRL 1620 (10^{-11}–10^{-7} M) produced no effect in eight fresh, resting segments from four goats (not shown).

In fresh arteries precontracted with prostaglandin $F_{2\alpha}$ (10^{-6}–10^{-5} M; induced tone = ~ 1150 mg), endothelin-1 (10^{-11}–10^{-7} M, six segments from three animals) did not cause relaxation, and even induced further contraction when it was applied at concentrations higher than 3×10^{-10} M (not shown). IRL 1620 (10^{-11}–10^{-7} M, nine segments from four animals) produced no effects in fresh arteries precontracted with prostaglandin $F_{2\alpha}$ (10^{-6}–10^{-5} M; induced tone = ~ 1200 mg) (not shown).

In fresh arteries precontracted with prostaglandin $F_{2\alpha}$ or endothelin-1 (induced tone = ~ 1250 mg for 18 segments from five goats), acetylcholine (10^{-7} and 10^{-6} M) induced relaxation in all segments tested (maximal relaxation = 228 $\pm$ 41 mg).

3.2.2. Effects of endothelin ET_A and ET_B receptor antagonists

The antagonist for endothelin ET_A receptors, BQ-123 (10^{-7}–3×10^{-6} M), and the antagonist for endothelin ET_B receptors, BQ-788 (10^{-6} and 10^{-5} M), themselves caused no changes in the resting tension of fresh arteries.

BQ-123 produced concentration-dependent rightward parallel displacement of the concentration–response curve for endothelin-1. Schild analysis of BQ-123 antagonism against endothelin-1 yielded a pA_2 value of 6.88 (slope of 0.98, which was not significantly different from unity) (Fig. 2; Table 2). BQ-788 did not significantly modify either the EC_{50} values or the maximal response of the concentration–response curve for endothelin-1 (Fig. 2; Table 2).

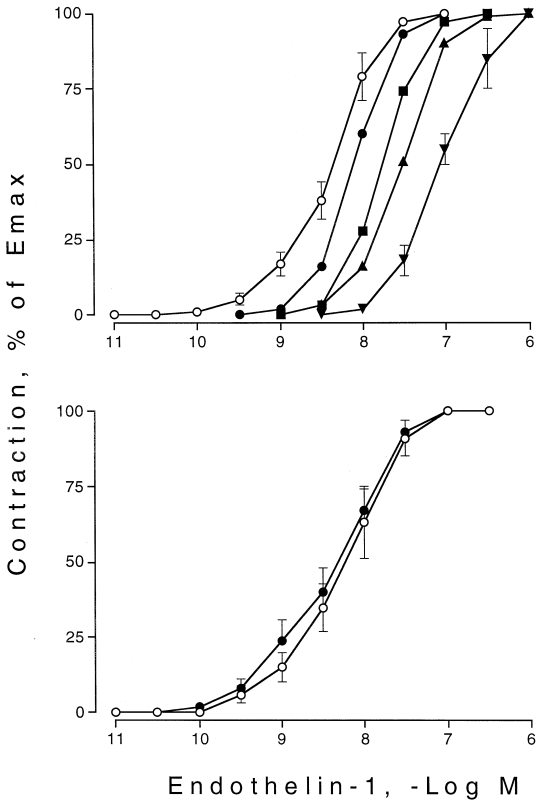

Fig. 2. Top: concentration–response curve for endothelin-1 obtained in fresh intact cerebral arteries under control conditions (○ ——— ○, eight segments) and after treatment with BQ-123 at concentrations of 10^{-7} M (● ——— ●, eight segments), 3×10^{-7} M (■ ——— ■, 10 segments), 10^{-6} M (▲ ——— ▲, 10 segments) and 3×10^{-6} M (▼ ——— ▼, five segments). Bottom: concentration–response curve for endothelin-1 obtained in fresh intact cerebral arteries under control conditions (○ ——— ○, six segments) and after treatment with 10^{-6} M BQ-788 (● ——— ●, six segments).

3.2.3. Effects of L-NAME, meclofenamate, indomethacin and phosphoramidon

L-NAME (10^{-4} M; 11 fresh segments from five goats) itself had no effect on resting tension (five segments) or produced a contraction of about 500 mg (six segments). The effects of endothelin-1 (10^{-11}–10^{-7} M) were not significantly different in the segments with and without this contraction caused by L-NAME (not shown), and also were not significantly different from effects obtained in the arteries under control conditions (Table 2). L-NAME (10^{-4} M) did increase the contraction in response to noradrenaline in seven fresh arteries from four goats as compared with the effects in control arteries: EC_{50} values were 3.24×10^{-7} vs. 7.02×10^{-7} M ($P < 0.05$) and the maximal contraction was 2773 $\pm$ 311 (212 $\pm$ 22% of the contraction to 100 mM KCl) vs. 1475 $\pm$ 178 mg (111 $\pm$ 8% of the contraction to 100 mM KCl) ($P < 0.05$).

Meclofenamate (10^{-5} M; nine fresh segments from five goats) or indomethacin (10^{-5} M; eight fresh segments from four goats) itself induced no changes in resting tension of the arteries. These substances did not affect significantly the concentration–response curve for endothelin-1, as compared to that recorded under control conditions (Table 2).

L-NAME (10^{-4} M) plus meclofenamate (10^{-5} M) in eight fresh segments from four goats did not affect the arterial contraction caused by endothelin-1 (10^{-11}–10^{-7} M) with regard to that recorded in control arteries (Table 2).

Phosphoramidon (10^{-4} M; eight fresh segments from three goats) did not change the response to endothelin-1, in comparison to that found in the corresponding control arteries (Table 2).

3.2.4. Effects of endothelium removal

Under resting tension, fresh arteries with endothelium removed (27 segments from 15 goats) exhibited a concentration-dependent contraction in response to endothelin-1 (10^{-11}–10^{-7} M) that was displaced in parallel to the left about four times with regard to the contraction obtained in fresh intact arteries ($P < 0.05$) (Fig. 3). For these segments without endothelium, Schild analysis for the antagonism of the BQ-123 (3×10^{-8}–10^{-6} M) against endothelin-1 yielded a pA_2 value of 7.62 (slope of 0.93, which was not significantly different from unity). This pA_2 value was significantly ($P < 0.01$) higher than that obtained for fresh intact arteries ($pA_2 = 6.88$) (Fig. 3). BQ-788 (10^{-6} M, six segments from three animals) did not affect significantly the response to endothelin-1 of the arteries without endothelium (Fig. 3).

IRL 1620 (10^{-11}–10^{-7} M) had no effects in five fresh resting segments without endothelium from three goats, as occurred in fresh intact arteries (not shown).

Under resting conditions, 24-h stored arterial segments with and without (16 segments from eight animals) endothelium exhibited a similar response to endothelin-1 (10^{-11}–10^{-7} M), but the concentration-dependent re-

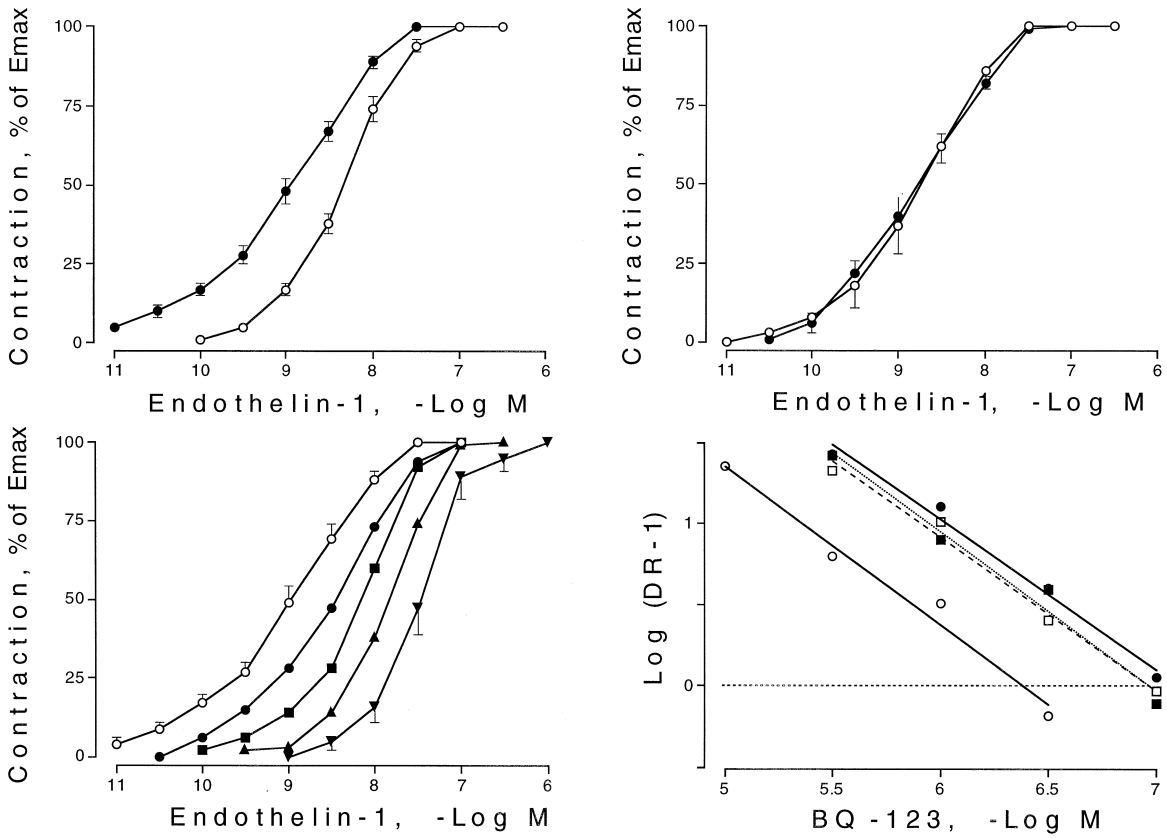

Fig. 3. Left: (a) top, concentration–response curve for endothelin-1 obtained in fresh, intact (○ ——— ○, 34 segments) and fresh, de-endothelized (● ——— ●, 27 segments) cerebral arteries, and (b) bottom, concentration–response curve for endothelin-1 obtained in fresh de-endothelized, non-treated cerebral arteries (○ ——— ○, 10 segments) and fresh de-endothelized cerebral arteries treated with BQ-123 at concentrations of 3×10^{-8} M (● ——— ●, five segments), 10^{-7} M (■ ——— ■, eight segments), 3×10^{-7} M (▲ ——— ▲, eight segments) and 10^{-6} M (▼ ——— ▼, six segments). Right: (a) top, concentration–response curve for endothelin-1 obtained in fresh de-endothelized, non-treated cerebral arteries (○ ——— ○, six segments) and de-endothelized cerebral arteries treated with 10^{-6} M BQ-788 (● ——— ●, six segments) and (b) bottom, Schild plot for BQ-123 at concentrations of 3×10^{-8}–3×10^{-6} M on contractions induced by endothelin-1 on fresh intact (○, 41 segments) and fresh de-endothelized (●, 37 segments) cerebral arteries, as well as in 24-h stored intact (□, 32 segments) and 24-h stored de-endothelized (■, 32 segments) cerebral arteries.

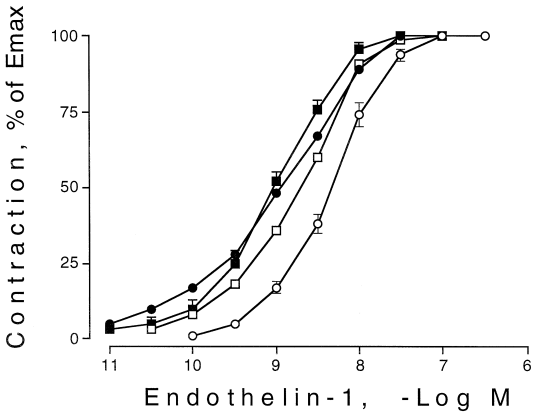

Fig. 4. Concentration–response curve for endothelin-1 obtained in fresh cerebral arteries (intact arteries, 34 segments, ○ ——— ○; de-endothelized arteries, 27 segments, □ ——— □), and in 24-h stored cerebral arteries (intact arteries, 27 segments, ● ——— ●; de-endothelized arteries, 16 segments, ■ ——— ■).

sponse of these arteries (both with and without endothelium) to the peptide was shifted to the left in comparison to that obtained in fresh intact arteries (Fig. 4). Schild analysis of the BQ-123 antagonism against endothelin-1 yielded a similar pA_2 value in 24-h stored de-endothelized ($pA_2 = 7.50$; slope of 0.90; 6–7 segments for each dose, from four animals) and intact ($pA_2 = 7.47$; slope of 0.96; 6–8 segments for each dose, from four animals) arteries ($P > 0.05$). Also, we found that the pA_2 value for the antagonism of BQ-123 against endothelin-1 in 24-h stored intact arteries ($pA_2 = 7.47$) was significantly higher than that found in fresh intact arteries ($pA_2 = 6.88$, $P < 0.05$), but was not significantly different from that for fresh arteries without endothelium ($pA_2 = 7.62$, $P > 0.05$). Fig. 3 (right, bottom) shows the Schild plot for BQ-123 on the contractions caused by endothelin-1.

In fresh arteries both without endothelium and precontracted with prostaglandin $F_{2\alpha}$ (10^{-6}–10^{-5} M; induced tone for five segments from three goats = ~ 1300 mg), the effects of endothelin-1 (10^{-11}–10^{-7} M) were not significantly different from those recorded in precontracted fresh intact arteries (not shown). IRL 1620 (10^{-11}–10^{-6} M) produced no effects in fresh arteries without endothelium and precontracted with prostaglandin $F_{2\alpha}$ (10^{-6} M; induced tone for five segments from three goats = ~ 1300 mg), as occurred in precontracted fresh intact arteries (not shown).

In both fresh (42 segments from 12 goats) and 24-h stored (12 segments from four goats) arteries deprived of endothelium and precontracted with prostaglandin $F_{2\alpha}$ or endothelin-1 (induced tone = ~ 1250 mg), acetylcholine (10^{-7} and 10^{-6} M) produced a small contraction or no response. In 24-h stored arteries with endothelium and precontracted with prostaglandin $F_{2\alpha}$ or endothelin-1 (induced tone = ~ 1300 mg for 18 segments from five goats), acetylcholine (10^{-7} and 10^{-6} M) produced a contraction in nine segments (maximal contraction = 122 ± 31 mg), no response in six segments and a small relaxation in three segments (maximal relaxation = 50 ± 10 mg).

In fresh, resting arteries without endothelium, noradrenaline (10^{-8}–10^{-3} M; 12 arteries from nine goats) caused a concentration-dependent contraction, and this contraction, relative to the contraction produced by 100 mM KCl, was higher, and the sensitivity was similar to that obtained in fresh resting intact arteries (10 segments from nine goats). The mean EC_{50} values were 4.32×10^{-7} M (fresh de-endothelized arteries) and 7.0210^{-7} M (fresh intact arteries) ($P > 0.05$). The contraction produced by 100 mM KCl in fresh de-endothelized and in intact arteries, respectively, was 1162 ± 149 and 1318 ± 171 mg ($P > 0.05$). With regard to the response to KCl, the maximal contraction in response to noradrenaline was $178 \pm 17\%$ (fresh de-endothelized arteries) and $111 \pm 8\%$ (fresh intact arteries) ($P < 0.05$). For fresh arteries without endothelium, Schild analysis for the antagonism of phentolamine against noradrenaline yielded a pA_2 value of 7.40 (slope of 1.13, which was not significantly different from unity), and this pA_2 value was similar to that obtained for fresh intact arteries ($pA_2 = 7.65$) ($P > 0.05$).

4. Discussion

The present results obtained from awake goats and with isolated goat cerebral arteries under control conditions confirmed previous observations from our laboratory (Diéguez et al., 1992; García et al., 1991a), and indicate that endothelin-1 produces marked cerebral vasoconstriction. In vitro cerebral vasoconstriction in response to endothelin-1 is a common finding (García et al., 1991b; Hardebo et al., 1989; Yanagisawa et al., 1988), and our in vitro results suggest that the sensitivity of goat cerebral arteries to endothelin-1 is similar, not only to that reported for cerebral arteries from humans (Hardebo et al., 1989) and dogs (García et al., 1991b), but also to that reported for human omental arteries (Riezebos et al., 1994).

As the blood supply to the goat brain occurs via an intracranial network of medium-sized muscular arteries (carotid rete), it raises the question of whether or not the observed effects of endothelin-1 on cerebral blood flow in the awake goat could reflect the overall response of retial and brain vasculature to this peptide. We observed earlier (García et al., 1991a) that isolated retial arteries exhibit a much lower sensitivity and contractile response to endothelin-1 than isolated cerebral arteries, thus suggesting that cerebral vessels rather than retial vessels should be the main site where endothelin-1 acts for reducing cerebral blood flow in the awake goat. The in vivo vasoconstrictor effects of endothelin-1 on the cerebral vasculature have also been tested in anesthetized goats where the carotid

rete was avoided by measuring blood flow in the middle cerebral artery and injecting the peptide directly into this artery (Diéguez et al., 1992). Therefore, endothelin-1 can produce cerebral vasoconstriction, and our in vivo observations agree with results reported by others who also used in vivo preparations and intraarterial injection of endothelin-1 (Clozel and Clozel, 1989; Kobari et al., 1994; Willette et al., 1990).

With regard to the type of endothelin receptors, we observed that BQ-123, an endothelin ET_A receptor antagonist, but not BQ-788, an endothelin ET_B receptor antagonist, reduced the effects on cerebral blood flow, and produced a competitive blockade of the effects in isolated cerebral arteries caused by endothelin-1. These data suggest that the in vivo and in vitro cerebral vasoconstriction produced by this peptide is mediated by activation of endothelin ET_A rather than of endothelin ET_B receptors. A competitive antagonism of BQ-123 for endothelin-1-induced vasoconstriction has also been found by others in cerebral (Adner et al., 1994; Fernández et al., 1995; Sagher et al., 1994; Salom et al., 1993) and non-cerebral (Adner et al., 1994; Ihara et al., 1992; Riezebos et al., 1994) blood vessels. 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 = 0.98).

We also observed that IRL 1620, a specific agonist for endothelin ET_B receptors, produced a relatively small reduction of cerebral blood flow, which was not affected by BQ-123 or BQ-788, and that it produced no effect on resting isolated cerebral arteries. These data for IRL 1620, together with the lack of blocking effect of BQ-788 on the in vivo and in vitro action of endothelin-1, suggest that cerebral vessels of the goat lack, or have a low concentration of, endothelin ET_B receptors for mediating contraction. The small reduction of cerebral blood flow produced by IRL 1620 may have been related to an unspecific effect of this substance on the cerebral vasculature. As endothelin-1 and IRL 1620 failed to increase cerebral blood flow and to relax the isolated arteries with an extrinsic active tone, it is also suggested that the goat cerebral vasculature also may lack endothelin ET_B receptors for mediating vasodilatation.

Experiments performed in vivo (Kitazono et al., 1995; Patel et al., 1996) and in vitro (Schilling et al., 1995) suggest that the cerebral vasculature has endothelin ET_B receptors and their activation produces vasodilatation. In one of these studies (Patel et al., 1996) it is indicated that activation of endothelin ET_B receptors is achieved when the agonist for these receptors BQ-3020 is applied adventitially but not when it is infused by the intracarotid route. The authors of this study suggest that BQ-3020 is ineffective after intracarotid infusion because of the presence of the blood–brain barrier, which prevents access of this agonist to the abluminal surface of cerebral arterioles where endothelin ET_B receptors would be located.

Ihara et al. (1992) have reported from binding studies that the binding affinity of BQ-123 for endothelin ET_A receptors in porcine aortic smooth muscle cells is IC_{50} = 7.3 nM, and Ishikawa et al. (1994) have reported that the binding affinity of BQ-788 for endothelin ET_B receptors in human Girardi heart cells is IC_{50} = 1.2 nM. These two reports suggest that the affinity of BQ-788 is relatively greater than that of BQ-123 for its corresponding receptors. Assuming that this behavior of the two antagonists was also present in our experimental preparation, the dose of BQ-788 administered in the in vivo experiments should have been sufficient to modify the effects of endothelin-1 and IRL 1620 on cerebral blood flow if functional endothelin ET_B receptors are present in the cerebral vasculature. Also, as the antagonist for endothelin ET_A receptors, BQ-123, with a peptide structure comparable to that of BQ-788, was able to inhibit the endothelin-1 effects and, therefore, to gain access to endothelin ET_A receptors of the cerebral vasculature to exert its effects, one may expect that BQ-788 can also gain access to endothelin ET_B receptors. In consequence, as BQ-788 did not modify the effects of endothelin-1 and IRL 1620 on cerebral blood flow, we suggest that endothelin ET_B receptors, if they are present, are not of functional significance in the mediation of the cerebrovascular effects of these two agonists. Studies with in vitro rat brain slices suggest that endothelin ET_A receptors are primarily responsible for the cerebral vasoconstrictor effect of endothelin-1 (Sagher et al., 1994). We have recently reported that, in dog cerebral veins, the endothelin ET_A receptor subtype may be the main mediator of the constriction in response to endothelin-1, and that the endothelin ET_B receptor subtype may be absent or present only in a very low concentration (Fernández et al., 1995). As suggested by the present data, the cerebrovascular bed may be mainly equipped with endothelin ET_A receptors for mediating contraction, and these receptors are probably located on smooth muscle cells as endothelin-1 also produced contraction in de-endothelized arteries that was competitively blocked by BQ-123.

With regard to the role of the endothelium, we have previously reported that reactivity of isolated goat cerebral arteries was endothelium-independent (Diéguez et al., 1992). This idea, however, must be revised, as suggested by the present in vitro results that endothelium removal did increase the reactivity of goat cerebral arteries to endothelin-1. Thus, the results of our present study suggest that, under normal conditions, endothelium may blunt the response of cerebral arteries to endothelin-1, as reported by others for cerebral (Jansen et al., 1989; Saito et al., 1989) and non-cerebral (Warner et al., 1989) arteries. The reason for the discrepancy between the present results and those previously reported by us (Diéguez et al., 1992) may be due to the fact that in that study (Diéguez et al., 1992) we grouped, and in the present one we separated, the results obtained for fresh and 24-h stored arteries. We have now found that the fresh arteries without endothelium were

more sensitive to endothelin-1 than were fresh intact arteries. This feature was no seen in 24-h stored arteries, as in this case arteries with and without endothelium exhibited similar reactivity to endothelin-1. This observation, along with the data for the antagonism of BQ-123 against endothelin-1 in the arteries under the various conditions (see the pA_2 values for fresh and 24-h stored arteries with and without endothelium, Fig. 3, right, bottom, and Fig. 4) suggest that 24-h-stored arteries behave like fresh arteries deprived of endothelium in their response to endothelin-1. It is also suggested that storage conditions and/or the period elapsed between removal of the arteries and the experiment might, by attenuating the normal inhibitory role of the endothelium in the cerebrovascular reactivity to endothelin-1, affect the response to endothelin-1 in the same way as does endothelium removal. This suggestion is supported by the present results with acetylcholine which induced a contraction or no response in most of the 24-h stored intact arteries tested, as occurred in fresh de-endothelized arteries. This suggests the possibility that storage of the arteries for some time may affect the sensitivity of the endothelium to stimulation and/or may affect its capacity to release relaxing factors such as nitric oxide.

Our observations with endothelin-1 and BQ-123 in intact and de-endothelized fresh arteries suggest that endothelium removal may increase the sensitivity to endothelin ET_A receptors to endothelin-1. This did not occur with noradrenaline, as endothelium removal did not affect either the sensitivity of the arteries to this amine or the pA_2 values of the antagonism for phentolamine against noradrenaline. The increased sensitivity of endothelin ET_A receptors for endothelin-1 after endothelium removal is probably not related to supersensitivity as a consequence of possible decreases in the local concentration of endothelin-1 in smooth muscle, as phosphoramidon, an inhibitor of endothelin synthesis, did not affect the response to the peptide. Thus, we suggest that endothelium removal may increase the affinity of endothelin ET_A receptors for endothelin-1, or may lead to expression of a subtype of endothelin receptors in smooth muscle that are functionally close to endothelin ET_A receptors. This subtype of receptors would have relatively high affinity and potency for endothelin-1 as suggested by characteristics of the concentration–response curve for this peptide in de-endothelized cerebral arteries (see Fig. 3).

With regard to nitric oxide, we found that L-NAME and L-NNA reduced resting cerebral blood flow, which is in line with the idea that nitric oxide produces a cerebral vasodilator tone under basal conditions (Faraci, 1993; Fernández et al., 1993). We also found that L-NAME reduced, both as absolute values and percentages, and L-NNA reduced only as absolute values, the effects of endothelin-1 on cerebral blood flow as compared to those found under control conditions. However, when changes in cerebrovascular resistance were considered, we observed that neither L-NAME, based on absolute values, nor L-NNA, based both on absolute values and percentages, modified the increases in resistance caused by endothelin-1 as compared to those found under control conditions. As cerebrovascular resistance may reflect the overall hemodynamic changes better than does cerebral blood flow, it is probable that these inhibitors of nitric oxide synthesis did not affect, at least clearly, the in vivo cerebrovascular action of endothelin-1. The in vitro data are probably consistent with this as L-NAME caused no effect on the response of cerebral arteries to endothelin-1. Therefore, although we must be cautious with their interpretation, these observations are not clearly in favor of the hypothesis that nitric oxide is involved in the cerebral vasoconstriction induced by endothelin-1. If nitric oxide were involved, the inhibition of its synthesis should cause an increase in the in vivo and in vitro cerebrovascular reactivity to endothelin-1. It has been observed that in anesthetized cats, treatment with a combination of indomethacin and L-NAME increased the cerebral vasoconstriction in response to endothelin-1 (Granstam et al., 1993). Results of studies with human omental arteries (Riezebos et al., 1994) and goat coronary arteries (García et al., 1996) suggest that nitric oxide may inhibit vasoconstriction caused ty endothelin-1. In the present study we observed that L-NAME did increase the in vivo and in vitro vasoconstrictor response to noradrenaline. These in vivo results with noradrenaline indicate that the reduction of basal cerebral blood flow after L-NAME does not limit the capacity of the cerebrovascular bed to constrict in response to vasoconstrictors. The same results also suggest that, under normal conditions, nitric oxide may inhibit the cerebral vasoconstriction caused by the adrenergic neurotransmitter. Thus, the role of nitric oxide in the cerebral vasoconstriction in response to endothelin-1 and that in noradrenaline may differ, which has been also suggested for the renal vasculature of conscious dogs (Fitzgerald et al., 1995). Studies with isolated basilar arteries from cats suggest that nitric oxide is involved in the cerebral vasodilatation in response to endothelin-1 (Moreau et al., 1995). Further, results for rat basilar artery in vivo (Kitazono et al., 1995) and in vitro (Schilling et al., 1995) suggest that activation of endothelin ET_B receptors produces dilatation mediated by nitric oxide.

About the role of prostanoids, our in vivo and in vitro data with meclofenamate and indomethacin show that these cyclooxygenase inhibitors did not change either resting cerebral blood flow or resting tension of isolated cerebral arteries, nor did they modify the cerebral vasoconstriction in response to endothelin-1. This confirms previous observations from our laboratory (Diéguez et al., 1992) and suggests that cyclooxygenase products such as prostanoids are probably not involved in the endothelin-1-induced cerebral vasoconstriction. This also agrees with observations in cerebral arteries of cats (Saito et al., 1989) and dogs (García et al., 1991b), and in dog cerebral veins (Fernández et al., 1995), as well as in human omental

arteries (Riezebos et al., 1994) and porcine coronary arteries (Yanagisawa et al., 1988). On the contrary, Armstead et al. (1989) have suggested that cerebral vasoconstriction induced by endothelin in piglets may be mediated by prostanoids. The reason for the discrepancy between this latter study (Armstead et al., 1989) and others (Fernández et al., 1995; García et al., 1991b; Saito et al., 1989; present results) is not obvious, but may be related to the different species and experimental approaches used.

In summary, the present in vivo and in vitro results suggest that: (1) endothelin-1 produces marked cerebral vasoconstriction by activation of endothelin ET_A receptors, probably located in smooth muscle cells; (2) endothelin ET_B receptors, nitric oxide and prostanoids might be not involved in the cerebrovascular action of endothelin-1, and (3) removal of the endothelium may increase cerebrovascular reactivity by increasing the sensitivity of endothelin ET_A receptors to endothelin-1.

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