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European Journal of Pharmacology 315 (1996) 179–186

ejp

Coronary vasoconstriction by endothelin-1 in anesthetized goats: role of endothelin receptors, nitric oxide and prostanoids

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

Departamento de Fisiología, Facultad de Medicina, Universidad Autónoma, Arzobispo Morcillo 2, 28029 Madrid, Spain

Received 29 February 1996; revised 29 July 1996; accepted 2 August 1996

Abstract

The role of endothelin ET_A and ET_B receptors as well as of nitric oxide (NO) and prostanoids in the effects of endothelin-1 on the coronary circulation was studied in anesthetized goats, where blood flow in the left circumflex coronary artery (coronary blood flow) (electromagnetically measured), systemic arterial pressure, left ventricle pressure and dP/dt, and heart rate were recorded. Endothelin-1 (0.01–0.3 nmol), intracoronarily injected, produced marked, dose-dependent reductions in basal coronary blood flow, ranging from 5% for 0.01 nmol to 75% for 0.3 nmol; 0.1 and 0.3 nmol endothelin-1 also reduced systolic ventricle pressure and dP/dt. The effects of endothelin-1 on coronary blood flow were diminished during intracoronary infusion of BQ-123 (cyclo-(D-Asp-Pro-D-Val-Leu-D-Trp), specific antagonist for endothelin ET_A receptors, 2–16 nmol/min) in a dose-dependent way, but not during the infusion of BQ-788 (*N*-[*N*-[*N*-[(2,6-dimethyl-1-piperidinyl)carbonyl]-4-methyl-L-leucyl]-1-(methoxycarbonyl)-D-tryptophyl]-D-norleucine monosodium, specific antagonist for endothelin ET_B receptors, 2–4 nmol/min). IRL 1620 (Suc-[Glu^9, $Ala^{11,15}$]endothelin-1-(8-21), specific agonist for endothelin ET_B receptors, 0.01–0.3 nmol), intracoronarily injected, slightly reduced basal coronary blood flow only when 0.1 and 0.3 nmol were applied (maximal reduction about 25%); 0.3 nmol IRL 1620 also reduced systolic ventricle pressure and dP/dt. The effects of IRL 1620 were not modified by BQ-123 or BQ-788. N^G-nitro-L-arginine methyl ester (L-NAME, inhibitor of NO synthesis, 47 mg/kg by i.v. route) reduced resting coronary blood flow by 10% and increased mean systemic arterial pressure and systolic ventricle pressure by 22 and 20%, respectively, without changing systolic ventricle dP/dt and heart rate. With L-NAME, the reductions of coronary blood flow by endothelin-1 were potentiated ($P < 0.05$), and those by IRL 1620 were not changed ($P > 0.05$). Meclofenamate (cyclooxygenase inhibitor, 4–6 mg/kg by i.v. route) modified neither the basal values of hemodynamic variables nor the coronary effects of endothelin-1 and IRL 1620. Therefore, endothelin-1 produces marked coronary vasoconstriction, which may be mediated by endothelin ET_A receptors, with no participation of endothelin ET_B receptors. NO, but not prostanoids, may produce a basal coronary vasodilator tone and may inhibit endothelin-1-induced coronary vasoconstriction. Also, it is suggested that the coronary vasoconstriction by endothelin-1 may impair cardiac performance due to heart ischemia.

Keywords: Coronary circulation; Endothelin ET_A receptor; Endothelin ET_B receptor; IRL 1620; BQ-123; Nitric oxide (NO)

1. Introduction

Since the discovery of endothelin and its potent vasoconstrictor properties (Yanagisawa et al., 1988), this 21-amino-acid peptide has been the object of attempts by investigators to define its role in physiology and pathophysiology. This interest specially concerns the coronary circulation. Endothelin-1, the major isoform of endothelins, is produced by cardiovascular tissues, has marked effects on coronary circulation and cardiac function, and is involved in some heart diseases (Hasdai et al., 1994; Masaki, 1995). Many studies show that endothelin-1 induces a potent coronary vasoconstrictor effect in vivo and in vitro (Yanagisawa et al., 1988; Chester et al., 1989; Ezra et al., 1989; Franco-Cereceda, 1989; Diéguez et al., 1992), although there are studies showing that this peptide can also induce coronary vasodilatation (Ushio-Fukai et al., 1992; Sakuma et al., 1993).

On the basis of biological and functional studies, the vascular effects of endothelin-1 seem to be mediated by at least 2 types of endothelin receptors, which have been termed endothelin ET_A and ET_B receptors, and the relative contribution made by these 2 types of receptors to the

* Corresponding author. Tel.: (34-1) 397-5424; Fax: (34-1) 397-5317.

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PII S0014-2999(96)00625-5

vasoconstriction by endothelin-1 seems to be dependent on both vascular bed and species under investigation (for references, see Masaki, 1995). Endothelin ET_A and ET_B receptors may be located in smooth muscle cells and can mediate vascular contraction, and endothelin ET_B receptors located in the endothelium can mediate vascular relaxation in response to endothelin-1 (for references, see Masaki, 1995). The relative role played by these 2 types of endothelin receptors in the coronary effects to endothelin-1 is not yet clear. With regard to this, there are data suggesting that endothelin-1 produces coronary vasoconstriction by acting on endothelin ET_A receptors in pigs (Ihara et al., 1991; Ushio-Fukai et al., 1995) and humans (Maguire and Davenport, 1995), by acting on both endothelin ET_A and ET_B receptors in rats (Balwierczak, 1993) and dogs (Rigel and Lappe, 1993), and by acting on both endothelin ET_A and non-ET_A, non-ET_B receptors in pigs (Harrison et al., 1992) and humans (Godfraind, 1993). Also, it has been reported that endothelin ET_B receptors may mediate coronary vasodilatation in rats (Sakuma et al., 1993).

The possible involvement of nitric oxide (NO) and prostanoids in the vascular effects of endothelin-1 was also considered of interest (for references, see Masaki, 1995). Although this possibility may occur in the coronary circulation, the issue remains to be elucidated. For example, it has been reported that, in coronary vessels, endothelin-1 can release prostacyclin in rabbits (Karwatowska-Prokopczuk and Wennmalm, 1990), can produce relaxation by releasing NO but not prostacyclin in rats (Sakuma et al., 1993) and dogs (Rigel and Lappe, 1993), can produce relaxation by releasing both NO and prostacyclin in pigs (Ushio-Fukai et al., 1992), can produce coronary vasoconstriction mediated by endothelin ET_B receptors which may be opposed by an endothelin ET_B receptor-mediated NO release in dogs (Rigel and Lappe, 1993), and can produce vasoconstriction mediated by endothelin ET_A receptors, which may be counteracted by endothelin ET_B receptor-mediated NO release in rats (Wang et al., 1994).

The present study was performed to explore the role of endothelin ET_A and ET_B receptors, as well as of NO and prostanoids in the coronary vascular response to endothelin-1. To achieve this, the effects of intracoronary administration of endothelin-1 and IRL 1620 (Suc-[Glu^9, $Ala^{11,15}$]endothelin-1-(8-21), specific endothelin ET_B receptor agonist, Takai et al., 1992) on coronary blood flow were evaluated in anesthetized goats under control conditions, during intracoronary infusion of the endothelin ET_A receptor antagonist BQ-123 (cyclo-(D-Asp-Pro-D-Val-Leu-D-Trp), Ihara et al., 1992) and of 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 monosodium, Ishikawa et al., 1994), and after i.v. administration of the inhibitor of NO synthesis, N^G-nitro-L-arginine methyl ester (L-NAME), and of the inhibitor of cyclooxygenase meclofenamate. The experimental model used in the present work was one we had used to show that intracoronary injections of endothelin-1 produce marked coronary vasoconstriction (Diéguez et al., 1992) and that NO may produce a basal coronary vasodilator tone (García et al., 1992).

2. Material and methods

2.1. Experimental preparation

In this study, 34 female goats (32–59 kg) were used. The animals were anesthetized with an intramuscular injection of 10 mg/kg ketamine hydrochloride and i.v. administration of 2% thiopental sodium; supplemental doses were given as necessary for maintenance. After orotracheal intubation, artificial respiration was started with room air and a Harvard respirator.

A left thoracotomy in the 4th interspace was performed and the pericardium was opened. The proximal segment of the left circumflex coronary artery was dissected, and an electromagnetic flow transducer (Biotronex) was placed on this artery to measure blood flow. A snare-type occluder was also placed around this artery, just distal to the flow probe, to obtain flow baseline.

Systemic arterial pressure was obtained through a polyethylene catheter placed in one temporal artery and connected to a Statham transducer. In 17 of the 34 goats, the pressure from the left ventricle was also obtained by implanting a microtransducer catheter (MTC, Hugo Sachs Elektronik) introduced through the left ventricle wall. The first derivative of the left ventricle pressure (dP/dt) was measured for evaluating myocardial performance. Blood flow, systemic arterial pressure, heart rate, and pressure and dP/dt from the left ventricle were simultaneously recorded on a Grass model 7 polygraph.

2.2. Experimental protocol

Endothelin-1 and IRL 1620 (specific endothelin ET_B receptor agonist), prepared in isotonic saline, were injected directly into the circumflex coronary artery at doses of 0.01, 0.03, 0.1 and 0.3 nmol, using volumes of 0.3 ml. This was performed under the following conditions: (a) control conditions; (b) during intracoronary infusion of BQ-123 (selective endothelin ET_A receptors antagonist, 12 goats) and of BQ-788 (selective endothelin ET_B receptors antagonist, 5 goats); and (c) during i.v. administration of N^G-nitro-L-arginine methyl ester (L-NAME, inhibitor of NO synthesis, 7 goats) and of meclofenamate (inhibitor of cyclooxygenase, 7 goats). BQ-123, at rates of 2, 4, 8 and 16 nmol/min, and BQ-788, at rates of 2 and 4 nmol/min, were given intracoronarily after solution in isotonic saline at a concentration of 2–16 nmol/ml. In the case of BQ-123, the dose of 2 nmol/min was injected in 5 goats; the dose of 4 nmol/min was given to 5 goats (3 of these goats are of group of animals that received 2 nmol/min);

the doses of 8 and 16 nmol/min were given to another 5 goats, which received both doses. In the case of BQ-788, the doses of both 2 and 4 nmol/min were given to 5 goats, which received both doses. Endothelin-1 and IRL 1620 were injected 4–6 min after the start of the administration of the antagonists. Two dose-response curves for endothelin-1 and IRL 1620 were made consecutively under control conditions in 3 of the animals that then received the intracoronary infusion of BQ-123. Endothelin-1 and IRL 1620 were also tested in 3 animals before (control) and during intracoronary infusion of isotonic saline (vehicle) at a rate of 0.5–1 ml/min; these rates were the same as used for intracoronary infusion of BQ-123 and BQ-788. L-NAME, prepared in isotonic saline at a concentration of 10 mg/ml, was given first as an i.v. bolus (35 mg/kg) and was followed by an i.v. infusion at 0.05–0.1 mg/kg/min (in total each animal received 47 mg/kg of L-NAME); endothelin-1 and IRL 1620 were injected during this i.v. infusion of L-NAME, when the hemodynamic variables reached steady state. Meclofenamate, prepared in isotonic saline at a concentration of 10 mg/ml, was given i.v. by hand at a dose of 4–6 mg/kg during 5–8 min; endothelin-1 and IRL 1620 were given 20–30 min after the end of this injection of meclofenamate.

Blood samples from the temporal artery were taken periodically to measure pH, pCO_2 and pO_2 by standard electrometric methods (Radiometer, ABL 300, Copenhagen, Denmark). After termination of the experiments, the goats were killed with an overdose of i.v. thiopental sodium and potassium chloride.

2.3. 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 monosodium) from Research Biochemicals International; N^G-nitro-L-arginine methyl ester (L-NAME) from Sigma, and sodium meclofenamate from Parke-Davis.

2.4. Data analysis

The data are expressed as means ± S.E.M. The effects of the different doses of endothelin-1 and IRL 1620, as well as of BQ-123, BQ-788, L-NAME and meclofenamate on the hemodynamic variables recorded and on blood gases and pH were analyzed with an analysis of variance, followed by Dunnett's test. The action of endothelin-1 and IRL 1620 on the hemodynamic variables recorded before (control) and after the treatments used (BQ-123, BQ-788, L-NAME and meclofenamate) were evaluated by applying Student's *t*-test for paired data, and in each case the animal was used as its own control.

Under the different conditions tested, the changes in coronary blood flow were considered both as percentages and as absolute values, and the changes in the other hemodynamic variables recorded and blood gases and pH were considered only as absolute values. The changes in left ventricle pressure and dP/dt were evaluated from measurements of systolic pressure and peak systolic dP/dt, respectively, during the maximal effects of endothelin-1 and IRL 1620 on coronary blood flow.

In each case, $P < 0.05$ was considered statistically significant.

3. Results

Table 1 summarizes the values for the hemodynamic variables recorded and blood gases and pH in all the anesthetized goats used under control conditions and after treatment with BQ-123, BQ-788, L-NAME and meclofenamate.

3.1. Control conditions

Endothelin-1 (0.01–0.3 nmol, 33 goats), injected intracoronarily, produced dose-dependent decreases in basal

Table 1
Summary of values for hemodynamic variables and arterial blood gases and pH determined in the animals used, under control conditions and after treatment with BQ-123, BQ-788, N^G-nitro-L-arginine methyl ester (L-NAME) and meclofenamate

	Control	BQ-123	BQ-788	L-NAME	Meclofenamate
CBF (ml/min)	31 ± 1.27 (34)	30 ± 3.13 (12)	28 ± 3.54 (5)	26 ± 2.74 [a] (5)	29 ± 2.52 (7)
MAP (mmHg)	89 ± 2.44 (34)	86 ± 4.15(12)	95 ± 4.74 (5)	110 ± 2.71 [b] (7)	87 ± 5.55 (7)
SVP (mmHg)	102 ± 3.65 (17)	100 ± 6.23 (5)	106 ± 5.84 (2)	130 ± 4.74 [b] (5)	99 ± 7.68 (5)
dP/dt (mmHg/s)	1354 ± 86 (17)	1217 ± 101 (5)	1298 ± 123 (2)	1410 ± 105 (5)	1350 ± 108 (5)
HR (beats/min)	77 ± 2.04 (34)	74 ± 3.60 (12)	83 ± 4.79 (5)	77 ± 4.81 (7)	77 ± 4.92 (7)
pO_2	99 ± 3.03 (34)	96 ± 4.18 (12)	102 ± 6.63 (5)	103 ± 5.24 (7)	102 ± 6.30 (7)
pCO_2	24 ± 1.01 (34)	26 ± 4.02 (12)	24 ± 4.50 (5)	22 ± 3.71 (7)	25 ± 4.85 (7)
pH	7.39 ± 0.01 (34)	7.39 ± 0.01 (12)	7.38 ± 0.02 (5)	7.39 ± 0.02 (7)	7.40 ± 0.02 (7)

Values are means ± S.E.M.; the number of animals used is given parentheses.
CBF, coronary blood flow; MAP, mean systemic arterial pressure; SVP, systolic left ventricle pressure; dP/dt, peak systolic left ventricle pressure.
[a] $P < 0.05$ and [b] $P < 0.01$ compared with that obtained in the corresponding animals under control conditions.

coronary blood flow in every animal, with a maximal reduction of about 75 ± 2%. In 17 of these goats, we found that the doses of 0.1 and 0.3 nmol of endothelin-1 also reduced, slightly but significantly, systolic ventricular pressure and peak systolic ventricular dP/dt during the maximal effect on coronary blood flow. After this maximal effect and when coronary blood flow was recovering, 0.1 and 0.3 nmol of endothelin-1 increased systemic arterial pressure, systolic ventricle pressure and peak systolic ventricle dP/dt in most of the animals (these latter effects have been not considered in the present work).

IRL 1620 (0.01–0.3 nmol, 19 goats), injected intracoronarily, produced small decreases in resting coronary blood flow only when 0.1 and 0.3 nmol were injected (the maximal reduction was about 25 ± 2%), and the doses of 0.01 and 0.03 nmol did not affect the resting coronary blood flow significantly. In 5 of these goats, we found that the dose of 0.3 nmol of IRL 1620 also reduced, slightly but significantly, systolic ventricle pressure and peak systolic ventricle dP/dt, coinciding with its maximal effect on coronary blood flow. Systemic arterial pressure and heart rate were not affected significantly by IRL 1620.

In 18 goats given both endothelin-1 and IRL 1620, we observed that the maximal reduction in coronary blood flow was about 3 times greater with endothelin-1 than with IRL 1620 (Table 2).

3.2. Effects of endothelin ET_A and ET_B receptor antagonists

Intracoronary administration of both BQ-123 (endothelin ET_A receptor antagonist) and BQ-788 (endothelin ET_B receptor antagonist) at the doses used did not affect resting coronary blood flow or the other hemodynamic variables recorded. Table 1 shows the values for hemodynamic variables and for blood gases and pH after grouping the results obtained at all rates used for BQ-123 and BQ-788.

The effects of endothelin-1 (0.01–0.3 nmol, 12 goats) on coronary blood flow, but not those of IRL-1620 (0.01–0.3 nmol, 9 goats), were significantly diminished in a dose-dependent way during intracoronary infusion of BQ-123 (2–16 nmol/min) as compared with the effects produced under control conditions. The dose of 2 nmol/min of BQ-123 (5 goats) did not affect significantly, the dose of 4 nmol/min (5 goats) blocked significantly the effects of 0.01 and 0.03 nmol of endothelin-1 on coronary blood flow, and the doses of 8 and 16 nmol/min (5 goats) blocked significantly the effects of all endothelin-1 doses tested on coronary blood flow. Fig. 1 shows the results with endothelin-1 under control conditions and during intracoronary infusion of only the doses of 4 and 16 nmol/min of BQ-123; for clarity, the results obtained during infusion of 2 and 8 nmol/min of BQ-123 are not displayed in the figure. The effects of both endothelin-1 (6 goats) and IRL 1620 (5 goats) on left ventricular pressure and dP/dt as well as on systemic arterial pressure and heart rate were not significantly different before and during treatment with BQ-123 (2 and 4 nmol/min).

During intracoronary infusion of the 2 doses used of BQ-788 (2 and 4 nmol/min), the effects of both endothelin-1 (0.01–0.3 nmol, 5 goats) and IRL 1620 (0.01–0.3 nmol, 5 goats) on the hemodynamic variables recorded were not significantly different from those recorded under control conditions (Fig. 1). This antagonist for endothelin ET_B receptors, at the concentration of 3×10^{-6} M, was able to shift to the right in a parallel way about 13-fold the contraction induced by IRL-1620 (10^{-11}–10^{-6} M) in resting isolated pulmonary arteries from 2 rabbits (these data are not shown).

The effects of endothelin-1 and IRL 1620 on the hemodynamic variables recorded were not different when these

Table 2
Summary of the hemodynamic effects of endothelin-1 (0.01–0.3 nmol) and of IRL 1620 (0.01–0.3 nmol) found in anesthesized goats under control conditions, where both substances were given intracoronarily

Endothelin-1 (nmol)	CBF		MAP (mmHg)	SVP (mmHg)	dP/dt (mmHg/s)	HR (beats/min)
	ml/min	$-\Delta\%$				
Control	31 ± 2.25	0	87 ± 3.54	100 ± 5.79	1295 ± 129	81 ± 2.92
0.01	29 ± 2.27	4 ± 1.00	87 ± 2.99	101 ± 5.64	1319 ± 126	81 ± 2.81
0.03	27 ± 2.12	15 ± 1.81 [b]	89 ± 4.15	102 ± 7.18	1195 ± 150	82 ± 2.78
0.1	20 ± 1.67 [a]	38 ± 2.98 [b]	88 ± 4.63	95 ± 5.92 [a]	1135 ± 107 [a]	83 ± 2.79
0.3	9 ± 1.42 [b]	74 ± 3.21 [b]	87 ± 5.26	91 ± 5.79 [a]	1005 ± 103 [a]	82 ± 3.43
IRL 1620 (nmol)						
Control	33 ± 2.32	0	88 ± 4.30	101 ± 5.54	1275 ± 130	82 ± 3.02
0.01	34 ± 2.32	0 ± 0	88 ± 4.30	102 ± 5.71	1290 ± 128	82 ± 3.01
0.03	33 ± 2.35	2 ± 1.00	89 ± 4.45	101 ± 5.54	1305 ± 130	83 ± 3.03
0.1	30 ± 2.21	11 ± 1.36 [b]	89 ± 4.35	100 ± 5.75	1298 ± 131	83 ± 3.09
0.3	25 ± 2.05	26 ± 2.33 [b]	89 ± 4.22	96 ± 6.00 [a]	1127 ± 148 [a]	83 ± 3.12

Values are means ± S.E.M. The values for CBF, MAP and HR are for 18 goats, and the values for SVP and dP/dt are for only 6 of the 18 goats.
CBF, coronary blood flow; MAP, mean systemic arterial pressure; SVP, systolic left ventricle pressure; dP/dt, peak systolic left ventricle dP/dt; HR, heart rate.
$-\Delta\%$, reduction in percentage. [a] $P < 0.05$ and [b] $P < 0.01$ compared with its control.

substances were tested twice consecutively under control conditions in 3 goats. In addition, during intracoronary infusion of isotonic saline (the vehicle used for BQ-123 and BQ-788) in 3 goats, the effects of both endothelin-1 and IRL 1620 on the hemodynamic variables recorded were similar to those recorded immediately before under control conditions (these data are not shown).

3.3. Effects of L-NAME and meclofenamate

L-NAME, given i.v. to 7 goats, reduced resting coronary blood flow by 10% ($P < 0.05$), increased mean systemic arterial pressure by 22% ($P < 0.01$) and systolic ventricular pressure by 20% ($P < 0.01$), and did not change significantly the peak systolic ventricular dP/dt, heart rate, blood gases and pH (Table 1). During this treatment, the effects of endothelin-1 (0.01–0.3 nmol, 6 goats) on coronary blood flow, both as percentages (Fig. 2) and as absolute values, were significantly increased with respect to those found under control conditions. Absolute values (ml/min) for the reductions of coronary blood flow by endothelin-1 during L-NAME treatment and control conditions, respectively, were: 4 ± 0.69 vs. 1.5 ± 0.56 (0.01 nmol), 8 ± 1.12 vs. 5 ± 0.78 (0.03 nmol), 16 ± 1.32 vs. 11 ± 1.62 (0.1 nmol) and 25 ± 2.81 vs. 16 ± 0.81 (0.3 nmol) ($P < 0.05$). The reductions of coronary blood flow by IRL-1620 (0.01–0.3 nmol, 3 goats) were comparable for L-NAME treatment and control conditions (not shown). In 5 goats, the effects of 0.1 and 0.3 nmol of endothelin-1 on systolic ventricular pressure and peak systolic ventricular dP/dt were more pronounced during L-NAME treatment than under control conditions.

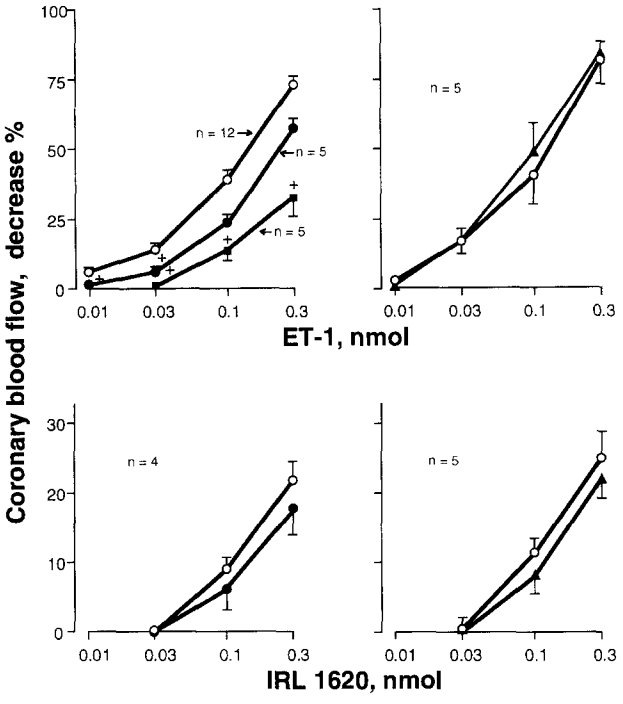

Fig. 1. (Top) Summary of the effects of intracoronary injections of endothelin-1 on coronary blood flow of anesthetized goats under control conditions (○ ——— ○) and during intracoronary infusion of BQ-123 (left) at doses of 4 nmol/min (● ——— ●) and 16 nmol/min (■ ——— ■) and of BQ-788 (right) at the dose of 4 nmol/min (▲ ——— ▲). (Bottom) summary of the effects of IRL 1620 on coronary blood flow of anesthetized goats under control conditions (○ ——— ○) and during intracoronary infusion of 4 nmol/min of BQ-123 (left) (● ——— ●) and of BQ-788 (right) (▲ ——— ▲). n = number of animals used in each experiment. $^{+}$ $P < 0.01$ between treatment with BQ-123 and its corresponding control.

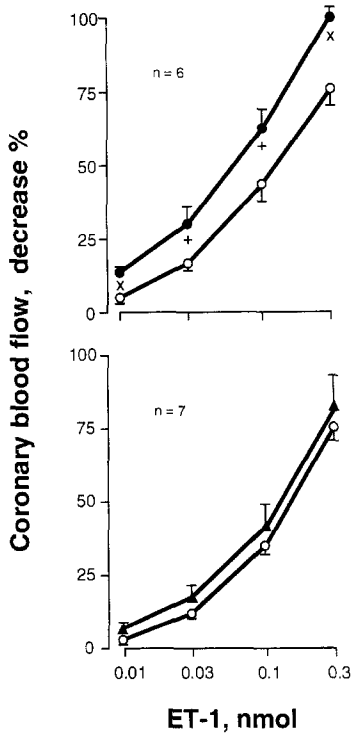

Fig. 2. Summary of the effects of intracoronary injections of endothelin-1 on coronary blood flow of anesthetized goats under control conditions (○ ——— ○) and after i.v. administration of N^G-nitro-L-arginine methyl ester (L-NAME) (top) (● ——— ●) and meclofenamate (bottom) (▲ ——— ▲). n = number of animals used in each experiment. $^{+}$ $P < 0.05$ and $^{\wedge}$ $P < 0.01$ between control and treated with L-NAME.

Meclofenamate, given i.v. to 7 goats, did not affect the hemodynamic variables recorded or blood gases and pH as compared with control conditions (Table 1). After administration of this drug, the effects of both endothelin-1 (0.01–0.3 nmol, 7 goats) (Fig. 2) and IRL 1620 (0.01–0.3 nmol, 3 goats) (not shown) on the hemodynamic variables recorded were not significantly different from those found under control conditions.

4. Discussion

The present results showed that endothelin-1 produces a dose-dependent, marked coronary vasoconstriction in the goat, which confirms previous results, also with goats, from our laboratory (Diéguez et al., 1992) and are in line with results obtained for pigs by others (Yanagisawa et al.,

1988; Ezra et al., 1989) and in humans (Chester et al., 1989; Franco-Cereceda, 1989). We did not observe increases in coronary blood flow with any of the endothelin-1 doses injected, suggesting that under our experimental conditions this peptide does not produce vasodilatation in goat coronary vasculature.

The use of endothelin isopeptides, and of specific agonists and antagonists has allowed the differentiation of 2 types of endothelin receptors, termed endothelin ET_A and ET_B receptors (for references, see Masaki, 1995). In our experiments, we observed that the effects of endothelin-1 and IRL 1620 on the hemodynamic variables recorded were consistent when they were tested twice consecutively under control conditions, and that they were not changed during intracoronary infusion of isotonic saline (the vehicle for BQ-123 and BQ-788). On the other hand, we observed that the administration of BQ-123 (specific antagonist for endothelin ET_A receptors) and BQ-788 (specific antagonist for endothelin ET_B receptors) did not modify the resting coronary blood flow, suggesting that endothelin-1 may be not produced in sufficient amounts to participate in the regulation of the basal coronary blood flow. We also found that BQ-123 inhibited in a dose-dependent way, whereas BQ-788 did not affect, the coronary blood flow reduction by endothelin-1. On the other hand, IRL 1620 (specific agonist for endothelin ET_B receptors) also reduced coronary blood flow, but this reduction was evident only when the 2 highest doses used were injected, and the maximal reduction was about 3 times lower with IRL 1620 than with endothelin-1. Neither BQ-123 nor BQ-788 modified the effects of IRL 1620 on coronary blood flow. Therefore, our results suggest that the coronary vasoconstriction induced in goats by endothelin-1 is produced by activation of endothelin ET_A receptors, and that endothelin ET_B receptors are mostly not involved in this vasoconstriction, as the reduction of coronary blood flow by this peptide was inhibited by BQ-123 in a dose-dependent way, but not by BQ-788. As the effects of IRL 1620 on coronary blood flow were present only when relatively high doses of this substance were injected and they were not affected by BQ-788 or BQ-123, it is suggested that IRL 1620 could produce coronary vasoconstriction by mechanisms different from activation of endothelin ET_A and ET_B receptors. These results with IRL 1620, together with those for endothelin-1, suggest that endothelin ET_B receptors may not be functionally present in the goat coronary circulation.

Studies concerned with the types of endothelin receptors involved in coronary vascular effects of endothelin-1 have yielded discrepant results, which could be related in part to the different species and experimental approaches used by investigators; species such as the human (Godfraind, 1993; Maguire and Davenport, 1995), pig (Ihara et al., 1991; Harrison et al., 1992; Ushio-Fukai et al., 1995), dog (Rigel and Lappe, 1993) and rat (Balwierczak, 1993) have been used. The results of all these studies, however, are consistent in that endothelin ET_A receptors are involved in the endothelin-1-induced coronary vasoconstriction, and they differ as to the role played by endothelin ET_B receptors in the coronary vascular effects of this peptide. The presence of endothelin ET_A receptors in human coronary arteries has been established using techniques such as mRNA encoding (Davenport et al., 1993), autoradiography (Bax et al., 1993) and radioligand binding assay (Davenport et al., 1994). Results of some of the functional studies suggest, as do the results from goats presented herein, that endothelin ET_A receptors are the endothelin receptors that mediate the coronary vasoconstriction induced by endothelin-1 in humans (Davenport et al., 1993; Bax et al., 1994; Maguire and Davenport, 1995), pigs (Ushio-Fukai et al., 1995) and rats (Wang et al., 1994). This feature seems to occur not only in coronary vessels, but also in other vascular beds (for references, see Masaki, 1995). With regard to endothelin ET_B receptors, radioligand binding studies show that human coronary arteries show no (Bax et al., 1993), or very few(Davenport et al., 1993) endothelin ET_B receptors. Functional studies suggest that endothelin ET_B receptors are not involved in, or contribute only slightly to the vasoconstriction of human (Davenport et al., 1993; Bax et al., 1994) and porcine (Ihara et al., 1991) coronary arteries in response to endothelin-1. Balwierczak (1993) suggested that endothelin-1 produces rat coronary vasoconstriction by activating both endothelin ET_A and ET_B receptors, on the basis that IRL 1620 caused vasoconstriction and that the effect was not affected by BQ-123. This author, however, did not test whether or not the coronary effects of endothelin-1 and of IRL 1620 were inhibited by an antagonist of endothelin ET_B receptors. Bax et al. (1994) found that $[Ala^{1,3,11,15}]$endothelin-1, another endothelin ET_B agonist, produced small contractions in human coronary arteries only when high concentrations were applied, and suggested that this observation argues against the presence of functional contractile endothelin ET_B receptors in these arteries. As the presence of mRNA encoding for endothelin ET_B receptors has been shown in smooth muscle cells of human coronary arteries (Davenport et al., 1993), Bax et al. (1994) suggested that these endothelin ET_B receptors may not play a major role in the coronary vasoconstriction in response to endothelin-1, but may be involved in other still unknown effects.

It seems that NO or prostacyclin may mediate or modulate the vascular response to endothelin-1 (for references, see Masaki, 1995). There are data suggesting that endothelin-1 can release NO in the coronary vasculature of pigs with vasodilatation as a result (Ushio-Fukai et al., 1992), as it also does in dogs (Rigel and Lappe, 1993) and rats (Sakuma et al., 1993) whereas it counteracts the vasoconstriction in rats (Wang et al., 1994) in response to this peptide. In our experiments, we found that neither endothelin-1 nor IRL 1620 produced coronary vasodilatation, nor did BQ-788 potentiate the coronary vasoconstrictor effects

of endothelin-1, which is consistent with reports concerning rats (Balwierczak, 1993) and humans (Davenport et al., 1993; Bax et al., 1994; Maguire and Davenport, 1995). We did find that the reduction of coronary blood flow by endothelin-1 was potentiated during the effects of L-NAME, an inhibitor of NO synthesis; this suggested that under normal conditions NO could inhibit the coronary vasoconstriction induced in goats by endothelin-1. Our data do not allow us to distinguish whether this NO corresponds to basal release and /or to an increased release in the goat coronary circulation after endothelin-1 stimulation. L-NAME reduced resting coronary blood flow and produced hypertension, which confirms previous results from our laboratory, also obtained with goats (García et al., 1992) and from other laboratories, for humans (Chester et al., 1990) and dogs (Chu et al., 1991), suggesting that NO is released and produces vasodilator tone in the coronary circulation under basal conditions. Wang et al. (1994) observed that blockade of endothelin ET_B receptors with IRL 1038 potentiated the rat coronary vasoconstriction in response to endothelin-1, an effect that did not occur after L-NAME treatment. These authors, thus, suggested that NO release is increased by activation of endothelin ET_B receptors and counteracts the coronary vasoconstriction in response to endothelin-1. If an increased NO release also occurred in our experiments with endothelin-1, it could not have been endothelin ET_B receptor-mediated as BQ-788 did not potentiate the coronary vasoconstriction in response to endothelin-1.

With regard to prostacyclin, it has been reported that this prostanoid can be released in the rabbit coronary vasculature (Karwatowska-Prokopczuk and Wennmalm, 1990) or can mediate pig coronary vasodilatation (Ushio-Fukai et al., 1992) in response to endothelin-1. Other investigators, however, suggest that prostanoids, including prostacyclin, are not involved in the coronary vascular effects of endothelin-1 in rats (Sakuma et al., 1993; Wang et al., 1994) and dogs (Rigel and Lappe, 1993). We observed that meclofenamate did not change the resting coronary blood flow and did not affect the reduction of coronary blood flow caused by endothelin-1 and IRL 1620. Thus, our results are in line with those suggesting that prostanoids are not involved in the coronary vascular effects of endothelin-1 and do not play a major role in the regulation of coronary blood flow under basal conditions. Also, as meclofenamate, by inhibiting cyclooxygenase, affects production not only of prostaglandins but also of thromboxane A_2, and as meclofenamate can also antagonize the binding of thromboxane A_2 to its receptor (Wilkes et al., 1992) as well as inhibit the lipoxygenase pathway (Stadler et al., 1994), our results suggest that eicosanoids may be not involved in the goat coronary vasoconstriction in response to endothelin-1.

With regard to cardiac performance, it has been reported that endothelin-1 exerts a positive inotropic effect on atria and papillary muscle preparations from rats (Hu et al., 1988), and that both endothelin ET_A and ET_B binding sites have been demonstrated in the human left ventricle (Bax et al., 1993). However, it has been observed that endothelin-1 induces primarily a decrease in contractility in whole heart preparations of rabbits (Karwatowska-Prokopczuk and Wennmalm, 1990) and rats (Wang et al., 1994). We found that higher doses of endothelin-1 reduced both systolic pressure and peak systolic dP/dt in the left ventricle, coinciding with their maximal effects on coronary blood flow. These results suggest that the simultaneous decreases in cardiac performance and coronary blood flow were closely related, and, thus, the observed reduction in heart contractility may have been a consequence of coronary ischemia after the coronary vasoconstriction caused by endothelin-1. This suggestion is supported by the the finding that IRL 1620 also slightly reduced cardiac contractility when coronary blood flow was sufficiently reduced, and agrees with the report by Wang et al. (1994) where it is suggested that the reduction in contractility in whole rat heart preparations evoked by endothelin-1 is secondary to the reduction in coronary flow in response to this peptide. Treatment with the endothelin ET_A antagonist, BQ-123 (2 and 4 nmol/min), did not modify the effects of endothelin-1 on cardiac performance, probably because this peptide still caused marked coronary vasoconstriction in spite of the presence of these doses of BQ-123.

In conclusion, the present data showed that endothelin-1 evokes a marked coronary vasoconstriction in vivo in goats and that the vasoconstriction may be mediated by activation of endothelin ET_A receptors with no participation of endothelin ET_B receptors. NO, but not prostanoids, may produce a basal coronary vasodilator tone and may modulate the coronary vascular effects of endothelin-1 in goats by inhibiting its coronary vasoconstrictor action. Our data also suggest that coronary vascular effects of endothelin-1 may impair cardiac performance as a consequence of heart ischemia.

Acknowledgements

We are grateful to Mrs. H. Fernández-Lomana and M.E. Martínez for technical assistance. This work was supported in part by FIS (93/338), CICYT (SAF93/0085) and CAM (AE 236/95).

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