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In vivo coronary effects of endothelin-1 after ischemia–reperfusion. Role of nitric oxide and prostanoids

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Abstract

To examine the effects of reperfusion after short and prolonged ischemia on the coronary action of endothelin-1, left circumflex coronary artery flow was electromagnetically measured, and 15- or 60-min occlusion of this artery followed by reperfusion was induced in anesthetized goats. In non-treated animals, during reperfusion after 15-min occlusion, the duration but not the peak of endothelin-1-induced coronary effects (0.01–0.3 nmol) was increased, and the effects of acetylcholine (3–100 ng) were unchanged. During reperfusion after 60-min occlusion, the peak and duration of endothelin-1-induced effects were increased whereas those of acetylcholine were decreased. N^{w}-nitro-L-arginine methyl esther (L-NAME) treatment did not modify the peak and duration of the coronary effects of endothelin-1 during reperfusion after both durations of occlusion. This treatment inhibited the effects of the two higher doses but not those of the two lower doses of acetylcholine during reperfusion after 15-min occlusion, and it did not modify the effects of any dose of this drug during reperfusion after 60-min occlusion. Meclofenamate treatment did not modify the coronary effects of endothelin-1 and acetylcholine during reperfusion after both durations of occlusion. These results suggest that ischemia–reperfusion increases the coronary response to endothelin-1, which is more pronounced during reperfusion after prolonged than after brief ischemia, and that this increased response is probably related to inhibition of nitric oxide release, without involvement of prostanoids.

Keywords: Coronary flow; Endothelium-dependent vasodilatation; Coronary vasoconstriction; Coronary vasodilatation; (Goat)

1. Introduction

Ischemia–reperfusion is a clinical and experimental event that can produce dysfunction of coronary vessels in addition to dysfunction of the myocardium, and this dysfunction may depend on the duration and severity of ischemia. Several lines of evidence suggest that alteration of the endothelium and endothelin-1 may play a relevant role in the pathophysiology of ischemia–reperfusion.

Results of several types of studies suggest that endothelin-1 is involved in the deleterious effects of ischemia–reperfusion (Pernow and Wang, 1997). Ischemia–reperfusion can induce increased coronary vasoconstriction in response to endothelin-1 (Watts et al., 1992; Thompson et al., 1995; Wang et al., 1995), but whether or not this increased vasoconstriction is present may depend on the duration of ischemia (Neubauer et al., 1991; Lockowandt et al., 2001). Studies on the mechanisms underlying the increased coronary response to endothelin-1 after ischemia–reperfusion are inconclusive, and this increased response has been attributed to changes in the characteristics of endothelin receptors in coronary vessels and/or alteration in the interaction of endothelin-1 with nitric oxide and prostanoids. With regard to the role of nitric oxide and prostanoids in the increased response to endothelin-1 after ischemia–reperfusion, the results reported are contradictory (Watts et al., 1992, 1995; Holm and Franco-Cereceda, 1996; Gourine et al., 2001). Therefore, to clarify the role of endothelin-1, and its interaction with nitric oxide and prostanoids during ischemia–reperfusion, more studies are needed. As ischemia–reperfusion is induced in vivo by occluding one coronary artery for different periods, and its effects on the coronary response to endothelin-1 may depend on ischemia duration, study of this response, and its interaction with nitric oxide and prostanoids during

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reperfusion after brief and prolonged ischemia, could be of interest for understanding the pathophysiology of this condition.

The objective of the present study was to examine the effects of reperfusion after brief or prolonged ischemia on the coronary response to endothelin-1 and its interaction with nitric oxide and prostanoids. Also, the functional state of the coronary endothelium after reperfusion was tested by recording the coronary response to acetylcholine. The experiments were carried out in anesthetized goats in which blood flow in the left circumflex coronary artery was electromagnetically measured, and 15- or 60-min occlusion followed by reperfusion of this artery was induced. In both cases, the coronary effects of endothelin-1, acetylcholine and sodium nitroprusside were recorded under control conditions and during reperfusion in animals non-treated and treated with the inhibitor of nitric oxide synthesis, N^{w}-nitro-L-arginine methyl esther (L-NAME), or with the inhibitor of cyclooxygenase, meclofenamate.

2. Methods

2.1. Experimental preparation

In this study 35 adult female goats (33–59 kg) were used. Anesthesia was induced 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 with room air was instituted with a Harvard respirator. A left thoracotomy in the fourth intercostal space was performed and the pericardium was opened. The proximal segment of the left circumflex coronary artery was dissected, and an electromagnetic flow probe (Biotronex) was placed on this artery to measure blood flow. A snare-type occluder was also placed around the artery, distal to the flow probe, to obtain zero-flow baseline. Systemic arterial pressure was measured through a polyethylene catheter placed in one temporal artery and connected to a Statham transducer. In each animal, coronary blood flow, systemic arterial pressure and heart rate were simultaneously recorded on a Grass model 7 polygraph. Blood samples from the temporal artery were taken periodically to measure pH, pCO_2 and pO_2 by standard electrometric methods (Radiometer, ABL™5, Copenhagen, Denmark). After termination of the experiments, the goats were killed with an overdose of i.v. thiopental sodium and potassium chloride.

The investigation conformed with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996), and the experimental procedure used in the present study was approved by the local Animal Research Committee.

2.2. Experimental protocol

After experimental preparations were completed and hemodynamic variables had reached steady state, the coronary response to endothelin-1 (0.01–0.3 nmol), acetylcholine (3–100 ng) and sodium nitroprusside (1–10 μg) was recorded under control conditions in each animal. Then, total occlusion of the left circumflex coronary artery was achieved with another occluder, which was placed around the artery immediately after the flow probe, so that this occluder was situated between the flow probe and the occluder used for obtaining zero-flow baselines. In one group of 16 animals, the arterial occlusion was maintained for 15 min, and in other group of 17 animals, the arterial occlusion was maintained for 60 min. In both cases, this occlusion was then gradually, but totally, released to permit reperfusion. This occlusion release took about 3 min in the case of 15-min occlusion and about 15 min in the case of 60-min occlusion. At 60 min after the start of occlusion release, the responses to acetylcholine, sodium nitroprusside and endothelin-1 were tested again. These drugs were injected into the left circumflex coronary artery through a needle connected to a polyethylene catheter, which pierced the artery between the two occluders. This study was performed in the group of 16 animals subjected to 15-min coronary occlusion (6 non-treated, 5 treated with L-NAME, and 5 treated with meclofenamate), and in the group of 17 animals subjected to 60-min coronary occlusion (6 non-treated, 6 treated with L-NAME and 5 treated with meclofenamate). In each case, the responses to the vasoactive drugs during control and reperfusion were recorded from the same animal.

Endothelin-1, acetylcholine and sodium nitroprusside were dissolved in physiological saline, and each dose was administered in a bolus of 0.3 ml over 5–10 s. L-NAME and meclofenamate were also dissolved in physiological saline at concentrations of 10 mg/ml. L-NAME was intracoronarily administered at a dose of 16–20 mg over 12–15 min, and meclofenamate was administered i.v. at a dose of 6–8 mg/kg body weight over 15–20 min. L-NAME or meclofenamate was administered after the end of the control tests with the vasoactive drugs used, and about 8 min before induction of coronary occlusion.

The total coronary and systemic effects of acetylcholine, sodium nitroprusside and endothelin-1 were recorded. These effects on coronary vasculature were evaluated as changes in coronary vascular conductance at their peak effects on coronary blood flow. Coronary vascular conductance was calculated by dividing coronary blood flow in milliliters per minute by mean systemic arterial pressure in milliliters of mercury.

2.3. Statistical analysis

Data are expressed as means ± S.E.M. In each of the three groups of animals, the hemodynamic variables, and

blood gases and pH before and after coronary occlusion and reperfusion, as well as before and after L-NAME and meclofenamate, were evaluated for individual animals using absolute values and applying one-way, repeated-measures analysis of variance (ANOVA) followed by Student's *t* test for paired data. The effects of coronary occlusion and reperfusion on coronary hemodynamics in L-NAME-treated and meclofenamate-treated animals were compared with those in non-treated animals using data expressed as percentages by applying one-way, factorial ANOVA, followed by Dunnett's test. The effects of endothelin-1, acetylcholine and sodium nitroprusside during reperfusion were compared with their respective control values using changes in absolute values by applying two-way, repeated-measures ANOVA (one factor was the dose of each drug, and the other factor was reperfusion vs. the corresponding control conditions), followed by Student's *t* test for paired data. Also, the effects of these drugs during reperfusion in the treated and non-treated animals were compared using absolute values by applying one-way, factorial ANOVA, followed by Dunnett's test. In each case, $P<0.05$ was considered statistically significant.

2.4. Chemicals

L-NAME, acetylcholine chloride and sodium nitroprusside were from Sigma; endothelin-1 (human, porcine) was from Peninsula Laboratories, and meclofenamate was from Parke Davis.

3. Results

3.1. Hemodynamic changes during occlusion and reperfusion

Tables 1 and 2 summarize the resting hemodynamic values during control conditions, coronary occlusion and reperfusion in the animals subjected to 15- or 60-min occlusion, respectively. In non-treated animals, coronary occlusion for 15 or 60 min abolished coronary flow and decreased mean arterial pressure to a similar extent (25% and 33%, respectively, $P<0.01$); it did not affect heart rate significantly. At 60 min of reperfusion, coronary flow was decreased more ($P<0.05$) after the 60-min (51%, $P<0.001$) than after 15-min (41%, $P<0.01$) occlusion; mean arterial pressure was decreased similarly (21% and 27%, $P<0.01$), and heart rate did not change (15-min occlusion, $P>0.05$) or decreased by 14% (60-min occlusion, $P<0.05$). Coronary vascular conductance decreased similarly after 15-min (28%, $P<0.05$) and after the 60-min (34%, $P<0.01$) occlusion. In animals treated with intracoronary administration of L-NAME, this drug by itself decreased coronary flow by 13–15% ($P<0.05$) without changing significantly mean arterial pressure or heart rate in both groups. In these animals, coronary occlusion of 15- and 60-min durations abolished coronary flow; mean arterial pressure did not change ($P>0.05$) or decreased by 20% ($P<0.05$), respectively, and heart rate decreased by 22% ($P<0.05$) or did not change significantly ($P>0.05$), respectively. At 60 min after reperfusion following 15- or 60-min occlusion, coronary flow decreased similarly (36% and 47%, $P<0.01$), mean arterial pressure did not change ($P>0.05$) or decreased by 16% ($P<0.05$), coronary vascular conductance decreased similarly (36% and 35%, $P<0.01$) and heart rate decreased by 17% ($P<0.05$) or did not change significantly ($P>0.05$). In animals treated with i.v. administration of meclofenamate, this drug by itself did not affect significantly the hemodynamic variables recorded. In these animals, coronary occlusion of 15- and 60-min durations abolished coronary flow, and mean arterial pressure did not change ($P>0.05$) or decreased by 13% ($P<0.05$), respectively, whereas heart rate did not change significantly ($P>0.05$). At 60 min of reperfusion after 15- or 60-min occlusion, coronary flow decreased similarly (33% and 44%, $P<0.01$), coronary vascular conductance decreased similarly (34 and 33%, $P<0.01$), mean arterial pressure did not change ($P>0.05$) or decreased by 14% ($P<0.05$), and heart rate did not change significantly ($P<0.05$).

For each occlusion duration, the coronary flow reduction during reperfusion was similar ($P>0.05$) in non-treated and

Table 1
Resting hemodynamic values obtained during control conditions, at 15 min of coronary occlusion and at 60 min of reperfusion in anesthetized goats non-treated (six animals), treated with L-NAME (five animals) and treated with meclofenamate (five animals)

	CBF (ml/min)	MAP (mm Hg)	CVC (ml/min/mm Hg)	HR (beats/min)
Non-treated				
Control	34 ± 4	93 ± 4	0.36 ± 0.05	75 ± 6
Occlusion	0	70 ± 3[a]	0	72 ± 5
Reperfusion	20 ± 3[b]	73 ± 4[a]	0.27 ± 0.04[c]	69 ± 7
L-NAME-treated				
Control	37 ± 5	97 ± 4	0.38 ± 0.04	80 ± 7
L-NAME	32 ± 4[c]	100 ± 6	0.31 ± 0.04[c]	71 ± 6
Occlusion	0	96 ± 4[d]	0	61 ± 6[b]
Reperfusion	24 ± 4[b]	95 ± 5[d]	0.25 ± 0.03[b]	67 ± 7[c]
Meclofenamate-treated				
Control	33 ± 4	102 ± 5	0.33 ± 0.04	71 ± 6
Meclofenamate	31 ± 4	103 ± 6	0.30 ± 0.04	70 ± 5
Occlusion	0	95 ± 5[d]	0	72 ± 5
Reperfusion	22 ± 3[b]	100 ± 5[d]	0.21 ± 0.03[b]	74 ± 6

Values are means ± S.E.M. CBF = coronary blood flow; MAP = mean systemic arterial pressure; CVC = coronary vascular conductance; HR = heart rate.

[a] $P<0.001$ compared with its corresponding control conditions (ANOVA and Student's *t* test for paired data).

[b] $P<0.01$ compared with its corresponding control conditions (ANOVA and Student's *t* test for paired data).

[c] $P<0.05$ compared with its corresponding control conditions (ANOVA and Student's *t* test for paired data).

[d] $P<0.05$ compared with the corresponding situation in non-treated animals (ANOVA and Dunnett's test).

Table 2
Resting hemodynamic values obtained during control conditions, at 60 min of coronary occlusion and at 60 min of reperfusion in anesthetized goats non-treated (six animals), treated with L-NAME (six animals) and treated with meclofenamate (five animals)

	CBF (ml/min)	MAP (mm Hg)	CVC (ml/min/mm Hg)	HR (beats/min)
Non-treated				
Control	29 ± 4	100 ± 4	0.30 ± 0.05	81 ± 6
Occlusion	0	78 ± 5[a]	0	73 ± 5[b]
Reperfusion	14 ± 3[a]	72 ± 5[a]	0.20 ± 0.04[a]	61 ± 7[b]
L-NAME-treated				
Control	26 ± 4	101 ± 5	0.26 ± 0.04	80 ± 7
L-NAME	22 ± 3[b]	103 ± 5	0.21 ± 0.04[b]	71 ± 6
Occlusion	0	81 ± 6[b]	0	61 ± 6[a]
Reperfusion	14 ± 3[a]	84 ± 5[b,c]	0.17 ± 0.03[a]	67 ± 7[b]
Meclofenamate-treated				
Control	37 ± 5	99 ± 5	0.37 ± 0.05	69 ± 6
Meclofenamate	35 ± 5	100 ± 5	0.36 ± 0.05	71 ± 6
Occlusion	0	87 ± 6[b]	0	69 ± 6
Reperfusion	21 ± 4[a]	85 ± 5[b,c]	0.25 ± 0.04[a]	72 ± 7

Values are means ± S.E.M. CBF = coronary blood flow; MAP = mean systemic arterial pressure; CVC = coronary vascular conductance; HR = heart rate.

[a] $P<0.01$ compared with its corresponding control conditions (ANOVA and Student's t test for paired data).

[b] $P<0.05$ compared with its corresponding control conditions (ANOVA and Student's t test for paired data).

[c] $P<0.05$ compared with the corresponding situation in non-treated animals (ANOVA and Dunnett's test).

L-NAME-treated animals, whereas it was less pronounced ($P<0.05$) in meclofenamate-treated than in non-treated animals. Hypotension during 15-min occlusion and reperfusion was present in non-treated but not ($P>0.05$) in L-NAME- and meclofenamate-treated animals. Hypotension during 60-min occlusion and reperfusion was less ($P<0.05$) pronounced in L-NAME- and meclofenamate-treated animals than in non-treated animals. The reduction of coronary vascular conductance during reperfusion was similar ($P>0.05$) after both occlusion durations, in both non-treated and treated animals.

Systemic blood gases and pH did not change significantly during ischemia and reperfusion as compared with control conditions in the animals subjected to 15- or 60-min coronary occlusion (these data are not shown).

3.2. Coronary response during reperfusion

Fig. 1 displays actual recordings showing the coronary effects of endothelin-1 and acetylcholine obtained under control conditions and during reperfusion after 15- or 60-min occlusion in two non-treated goats.

Under control conditions, endothelin-1 (0.01–0.3 nmol) produced dose-dependent decreases in coronary vascular conductance in each animal. During reperfusion after 15-min occlusion in non-treated and L-NAME-treated animals, the peak effects of endothelin-1 (0.01–0.3 nmol) on coronary vascular conductance were not significantly distinct to, but the duration of these effects was longer ($P<0.05$) than, those under the corresponding control conditions. During reperfusion, these endothelin-1 effects were similar in non-treated and L-NAME-treated animals (Fig. 2). During reperfusion after 60-min occlusion in non-treated and L-NAME-treated animals, the peak and duration of the coronary effects of endothelin-1 were significantly higher ($P<0.05$, or $P<0.01$) than under the corresponding control conditions. These endothelin-1 effects were comparable in the two groups of animals (Fig. 2). In animals treated with meclofenamate, during reperfusion after 15-min occlusion, the peak coronary effects of endothelin-1 (0.01–0.3 nmol) were comparable to, but the duration of these effects was longer ($P<0.05$) than, those under the corresponding control conditions. During reperfusion, these endothelin-1 effects were similar to those during reperfusion in non-treated and L-NAME-treated animals (Fig. 2). During reperfusion after 60-min occlusion, the peak and duration of the coronary effects of endothelin-1 were higher ($P<0.05$, or $P<0.01$) than under the corresponding control conditions. These endothelin-1 effects were similar to those during reperfusion in non-treated and L-NAME-treated animals (Fig. 2).

In the animals subjected to 15- or 60-min occlusion, endothelin-1 at the doses of 0.1 and 0.3 nmol slightly but significantly increased mean arterial pressure, and this was comparable under reperfusion and control conditions, in treated and non-treated animals. These effects of endothelin-1 occurred after its maximal effects on coronary flow.

In each animal under control conditions, acetylcholine (3–100 ng) and sodium nitroprusside (1–10 μg) produced dose-dependent increases in coronary vascular conductance. In non-treated animals, during reperfusion after 15-min occlusion the effects (peak and duration) of acetylcholine (3–100 ng) (Fig. 3) and sodium nitroprusside (1–10 μg) (Fig. 4) on coronary vascular conductance were comparable to those under the corresponding control conditions. During reperfusion after 60-min occlusion, the peak and duration of the coronary effects of both drugs were lower ($P<0.05$, or $P<0.01$) than under the corresponding control conditions. In L-NAME-treated animals, during reperfusion after 15-min occlusion, the peak effects of the two higher doses (30 and 100 ng), but not those of the two lower doses of acetylcholine (Fig. 3) and those of sodium nitroprusside (Fig. 4) on coronary vascular conductance, were reduced with regard to the corresponding control conditions. The effects of the two higher doses of acetylcholine were also lower than those during reperfusion in non-treated animals. During reperfusion after 60-min occlusion, the peak and duration of the coronary effects of acetylcholine (Fig. 3) and sodium nitroprusside (Fig. 4) were lower ($P<0.05$, or $P<0.01$) than those under the corresponding control conditions, and they were not distinct to those during reperfusion in non-treated animals. In meclofenamate-treated animals, during reperfusion after 15-min occlusion, the

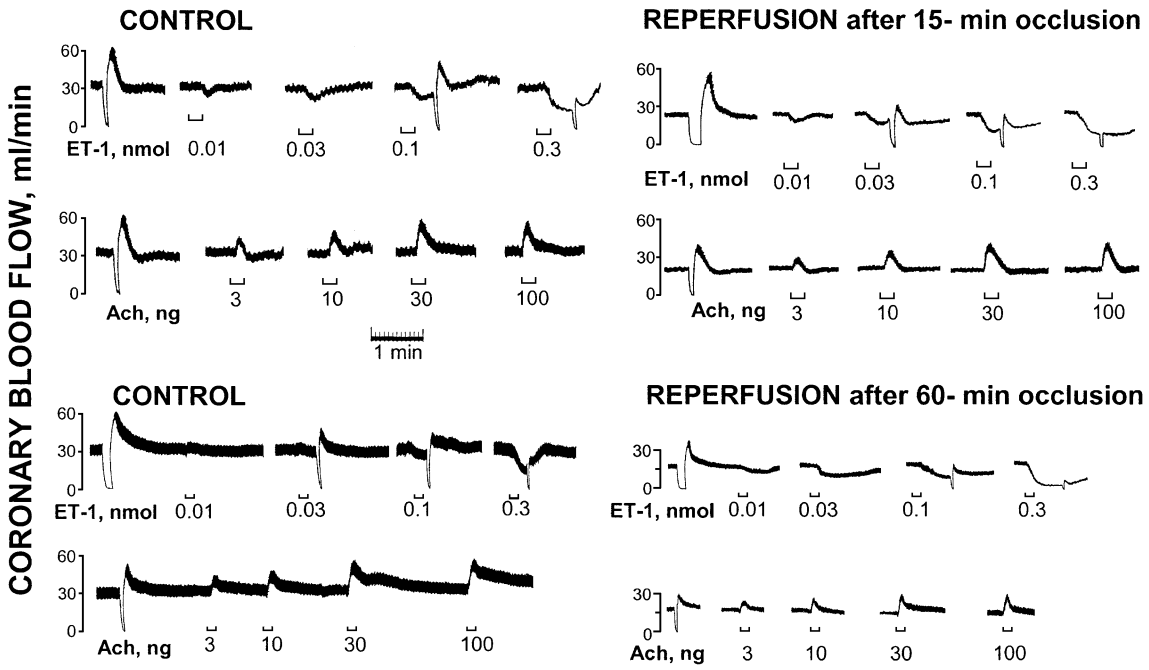

Fig. 1. Actual recordings showing the effects of endothelin-1 (ET-1) and acetylcholine (Ach) on coronary flow under control conditions (left, top and bottom) and during reperfusion following 15-min occlusion (right, top) and 60-min occlusion (right, bottom) in two non-treated goats.

coronary effects (peak and duration) of acetylcholine (Fig. 3) and sodium nitroprusside (Fig. 4) were similar to those under the corresponding control conditions, and to those during reperfusion in non-treated animals. During reperfusion after 60-min occlusion, the peak and duration of the coronary effects of acetylcholine (Fig. 3) and sodium nitroprusside (Fig. 4) were lower ($P<0.05$ or $P<0.01$) than those under the corresponding control conditions, and they

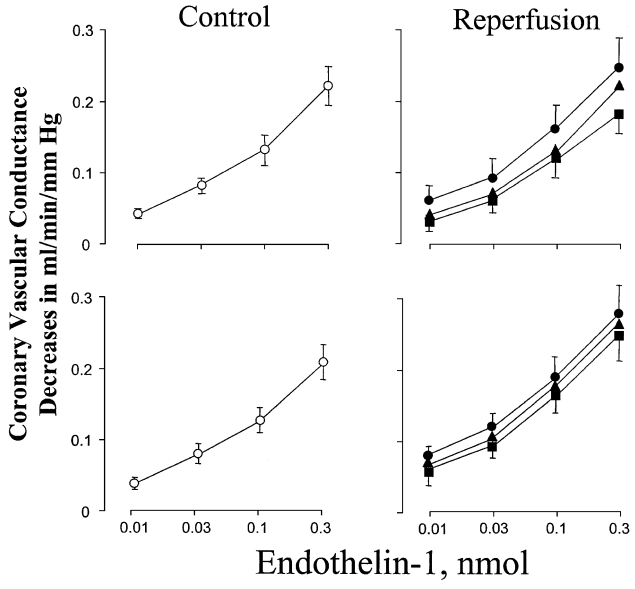

Fig. 2. Summary of the effects of endothelin-1 on coronary vascular conductance obtained under control conditions (left, averages of the control effects in the three groups of animals) and during reperfusion (right) after 15-min (top) and 60-min (bottom) occlusion in anesthetized goats non-treated (●—●), treated with L-NAME (▲—▲) and treated with meclofenamate (■—■). Animals subjected to 15-min occlusion and reperfusion: six non-treated, five treated with L-NAME and five treated with meclofenamate. Animals subjected to 60-min occlusion and reperfusion: six non-treated, six treated with L-NAME and five treated with meclofenamate.

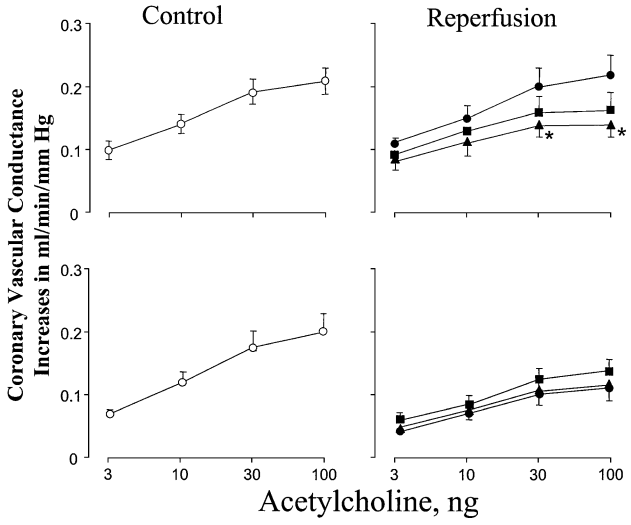

Fig. 3. Summary of the effects of acetylcholine on coronary vascular conductance obtained under control conditions (left, average of the control effects in the three groups of animals) and during reperfusion (right) after 15-min (top) and 60-min (bottom) occlusion in anesthetized goats non-treated (●—●), treated with L-NAME (▲—▲) and treated with meclofenamate (■—■). The number of animals subjected to 15- or 60-min occlusion and reperfusion, non-treated, treated with L-NAME and treated with meclofenamate is the same as in Fig. 2. *$P<0.05$ for differences during reperfusion between non-treated and L-NAME-treated animals (ANOVA and Dunnett's test).

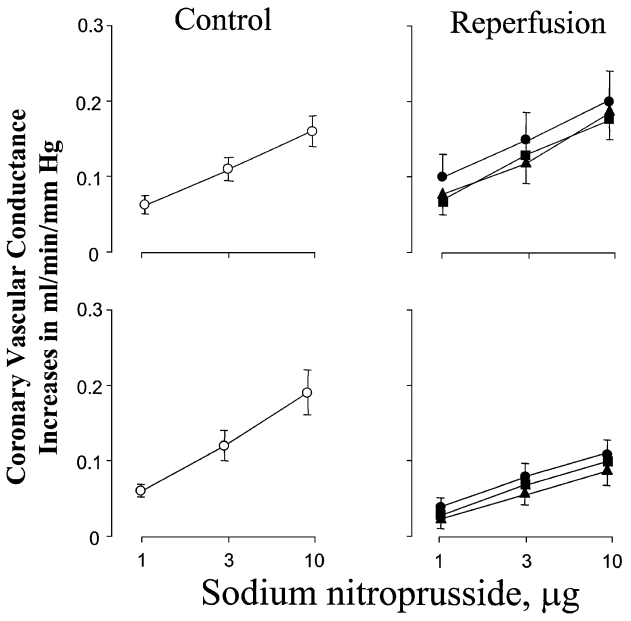

Fig. 4. Summary of the effects of sodium nitroprusside on coronary vascular conductance obtained under control conditions (left, averages of the control effects in the three groups of animals) and during reperfusion (right) after 15-min (top) and 60-min (bottom) occlusion in anesthetized goats non-treated (●—●), treated with L-NAME (▲—▲) and treated with meclofenamate (■—■). The number of animals subjected to 15- or 60-min occlusion and reperfusion, non-treated, treated with L-NAME and treated with meclofenamate is the same as in Fig. 2.

were similar to those during reperfusion in non-treated and L-NAME-treated animals.

In the animals subjected to 15- or 60-min occlusion, acetylcholine and sodium nitroprusside did not produce systemic effects under control conditions or during reperfusion in non-treated and treated animals.

4. Discussion

The present study shows that the coronary response to endothelin-1 is increased after ischemia–reperfusion, and that this increase is more pronounced during reperfusion after 60-min than after 15-min occlusion. This increased coronary response to endothelin-1 is accompanied by a decreased release of nitric oxide, which is also more pronounced during reperfusion after 60-min than after 15-min occlusion. First, we will comment on the effects of ischemia–reperfusion on hemodynamic variables, and then on the effects on the coronary response. The coronary effects of the vasoactive drugs used were analyzed by using the changes in coronary vascular conductance because these probably reflect better the in vivo vascular effects, especially when blood flow is the variable mainly affected (Lautt, 1989).

Total coronary occlusion of 15- and 60-min durations abolished coronary flow in non-treated and treated animals as expected, and during both occlusion durations, it was accompanied by hypotension. This hypotension was prevented in the case of brief occlusion, or attenuated in the case of longer occlusion, by L-NAME or meclofenamate treatment. During reperfusion, coronary vascular conductance and systemic arterial pressure were decreased, and the degree of reduction for each of these variables was similar during reperfusion after both occlusion durations. This hypotension, but not the reduction in coronary vascular conductance, was prevented in the case of brief occlusion, or attenuated in the case of longer occlusion, by treatment with L-NAME and meclofenamate. The presence of hypotension during both occlusion durations and reperfusion in non-treated animals, and its prevention or attenuation in treated animals, might be related, at least in part, to an increased release of nitric oxide and/or vasodilator prostanoids during ischemia–reperfusion, which may have been inhibited by L-NAME and meclofenamate, respectively. An increased release of nitric oxide (Lecour et al., 2001) and of prostanoids (Cocker et al., 1981) as a consequence of myocardial ischemia or ischemia–reperfusion has been reported. Under control conditions, L-NAME by itself reduced resting coronary flow without changing arterial pressure and heart rate, suggesting that nitric oxide may produce a basal vasodilator tone in the coronary circulation under normal conditions, as we and others have reported previously (Bassenge, 1995; García et al., 1992, 1996; Fernández et al., 1998). Meclofenamate did not affect resting hemodynamic variables, suggesting that prostanoids are not involved in the regulation of coronary vascular tone under basal conditions, as was reported previously (García et al., 1996; Fernández et al., 1998). The decreased coronary vascular conductance indicates that the non-reflow phenomenon was present, and of similar magnitude during reperfusion after both ischemia durations in non-treated and treated animals. The non-reflow phenomenon has been observed during reperfusion after total coronary occlusion (Forman et al., 1989), but the mechanisms underlying this phenomenon are not totally understood (Ku, 1982). The changes found in coronary vascular conductance indicate that L-NAME and meclofenamate do not interfere with the mechanisms involved in non-reflow after ischemia–reperfusion.

It has been reported that coronary flow is closely matched by the rate of myocardial oxygen consumption, and that several factors may be involved in this matching (Tune et al., 2002). Therefore, it is possible that the reduction of coronary vascular conductance found during reperfusion after both ischemia durations, as well as in non-treated and treated animals, is related to changes in myocardial oxygen consumption as a consequence of probable changes in factors that match coronary flow and myocardial oxygen consumption in these circumstances. Also, the prevention or attenuation of hypotension by L-NAME during ischemia and reperfusion might be related to changes in myocardial oxygen consumption and myocardial function produced by the direct action of this drug, which was

intracoronarily administered, on the heart. These are only hypothetical considerations as we did not measure myocardial oxygen consumption, and, therefore, we cannot analyze its role in the changes in coronary and systemic hemodynamics after ischemia–reperfusion in our experiments.

The peak coronary effects of endothelin-1 were not altered, but the duration of these effects was increased in non-treated animals during reperfusion after brief ischemia, and both the peak and duration of these effects were increased during reperfusion after prolonged ischemia. This indicates that ischemia–reperfusion increases the coronary action of endothelin-1 and suggests that this increase is related to the duration of ischemia. This agrees with studies of isolated rat hearts (Neubauer et al., 1991) and anesthetized pigs (Lockowandt et al., 2001). To analyze the mechanisms underlying the increased coronary response to endothelin-1 after ischemia–reperfusion, several experimental approaches have been used, and the results reported are inconclusive (Neubauer et al., 1991; Watts et al., 1992; Wang et al., 1995; Holm and Franco-Cereceda, 1996; Brunner et al., 1997; Gourine et al., 2001; Lockowandt et al., 2001). From experiments performed with isolated rat hearts, it has been reported that the increased response to endothelin-1 might be related to the loss of counteracting vasodilator mechanisms such as prostaglandins and/or endothelium-derived relaxing factor (Neubauer et al., 1991), that it may be due to endothelial dysfunction with reduced ability to modulate or limit the constriction to endothelin-1 (Watts et al., 1992) and that it is unrelated to endothelial dysfunction (Wang et al., 1995). Also, experiments with isolated rat hearts indicate that during ischemia–reperfusion, endothelin-1 does not compromise nitric oxide synthesis (Brunner et al., 1997), and experiments with anesthetized pigs show that the cardioprotective effects of endothelin-1 ETA receptor antagonism in ischemia–reperfusion are mediated via a mechanism related to nitric oxide (Gourine et al., 2001). However, Lockowandt et al. (2001), from experiments with anesthetized pigs, suggest that an alteration in the release of nitric oxide/prostacyclin is probably not the cause of the increased response to endothelin-1, because during reperfusion after 10-min ischemia, the response to this peptide remained unchanged when the response to acetylcholine was reduced. Our data with L-NAME indicate that this drug did not affect the coronary effects of endothelin-1 during reperfusion after brief and prolonged ischemia, which contrasts with the effects observed in anesthetized goats under normal conditions, where L-NAME potentiated the coronary vasoconstriction in response to this peptide (García et al., 1996). This suggests that the modulatory role of nitric oxide in the coronary response to endothelin-1 may be attenuated during reperfusion after brief and prolonged ischemia. This attenuation is probably more accentuated during reperfusion after prolonged than after brief ischemia because the response to endothelin-1 was greater after 60-min than after 15-min occlusion.

Our hypothesis about the decreased modulatory role of nitric oxide in the coronary effects of endothelin-1 is consistent with the present data for acetylcholine. The coronary effects of acetylcholine and sodium nitroprusside during reperfusion after 15-min occlusion were not altered, but during reperfusion after 60-min occlusion they were reduced. The effects of sodium nitroprusside were not affected by L-NAME and meclofenamate, indicating that coronary vasodilator capacity is decreased during reperfusion after prolonged but not after brief ischemia, and that it is not affected by these treatments. These data agree with those reported by others indicating that the coronary vasodilator capacity is preserved during reperfusion after brief ischemia (Kim et al., 1992) but not after prolonged ischemia (Nichols et al., 1994; Lockowandt et al., 2001). We also found that L-NAME did not affect the coronary effects of the two lower doses of acetylcholine during reperfusion after 15-min occlusion. This contrasts with the effect observed in anesthetized goats under normal conditions, where L-NAME did inhibit the coronary action of this neurotransmitter (García et al., 1992). Therefore, the mediator role of nitric oxide in the coronary action of acetylcholine may be reduced during reperfusion after short ischemia, probably because this condition induces endothelial dysfunction. With regard to interpretation of the data for acetylcholine during reperfusion after 60-min ischemia, we must be cautious because in this situation, the coronary vasodilatation in response to both sodium nitroprusside and acetylcholine was reduced. However, as L-NAME did not affect the coronary action of acetylcholine during reperfusion, we can infer that the mediator role of nitric oxide in this action of acetylcholine is diminished, and that this diminution might be more pronounced than during reperfusion after 15-min ischemia. If this inference is accepted, it can be suggested that the magnitude of the reduction in nitric oxide release and of endothelial dysfunction is related to the duration of ischemia, as occurred with the increased response to endothelin-1. Data from other laboratories have shown that reperfusion after brief ischemia produces endothelial dysfunction without morphological damage, whereas longer ischemia induces also morphological damage of the endothelium (Kim et al., 1992). Also, it has been reported that endothelial function is decreased during reperfusion after relatively prolonged periods of coronary occlusion (Van Benthuysen et al., 1987; Mehta et al., 1989; Pearson et al., 1990; Ehring et al., 1995). Discrepant data, however, have been obtained during reperfusion after shorter periods (<30 min) of coronary occlusion, when endothelial dysfunction has been observed (Kim et al., 1992; Lockowandt et al., 2001) or not (Winn and Ku, 1992; Ehring et al., 1995). We have reported previously that the role of nitric oxide in the coronary cholinergic vasodilatation is also reduced during reperfusion after 1 h of partial ischemia in anesthetized goats (Fernández et al., 2002). The loss of vascular reactivity to endothelium-dependent drugs after ischemia–reperfusion may be due to several factors, includ-

ing depletion of endogenous stores of nitric oxide, enhanced inactivation of nitric oxide or both (Miller and Vanhoutte, 1985).

In meclofenamate-treated animals, the coronary response to endothelin-1 during reperfusion after both brief and prolonged ischemia was comparable to that found during reperfusion in non-treated animals. This indicates that meclofenamate did not affect the coronary response to endothelin-1 during reperfusion, as occurs under normal conditions in anesthetized goats (García et al., 1996) and other species (Rigel and Lappe, 1993). Therefore, prostanoids are probably not involved in the coronary effects of this peptide during reperfusion after brief and prolonged ischemia. This is consistent with the effect observed during reperfusion after partial ischemia, where prostanoids may be not involved, and differs from that seen during partial ischemia, where vasoconstrictor prostanoids may be involved, in the coronary response to endothelin-1 (Fernández et al., 2002). Meclofenamate neither affected the coronary effects of acetylcholine during reperfusion after brief and prolonged ischemia, and this feature is consistent with the observed effect in anesthetized goats under normal conditions (García et al., 1992).

In conclusion, the present results suggest: (1) that the coronary response to endothelin-1 is increased after ischemia–reperfusion and is more pronounced after prolonged (60 min) than after brief (15 min) ischemia, and (2) that this increased response is related to inhibition of nitric oxide release and does not involve prostanoids.

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