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Coronary reactivity to endothelin-1 during partial ischemia and reperfusion in anesthetized goats. Role of nitric oxide and prostanoids

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Abstract

To examine the coronary reactivity to endothelin-1 and its interaction with nitric oxide or prostanoids during partial coronary ischemia and reperfusion, left circumflex coronary artery flow was electromagnetically measured, and partial occlusion of this artery was induced for 60 min, followed by reperfusion in anesthetized goats (eight non-treated, six treated with N^{w}-nitro-L-arginine methyl esther (L-NAME) and five treated with meclofenamate). During partial occlusion, coronary vascular conductance was reduced by 24–37% ($P<0.01$), and the coronary vasodilatation in response to acetylcholine (3–100 ng) and sodium nitroprusside (1–10 μg) was much decreased in every case; the vasoconstriction in response to endothelin-1 (1–10 μg) was depressed in non-treated animals, and this depression was reversed by L-NAME and was accentuated by meclofenamate. At 30 min of reperfusion coronary vascular conductance remained decreased by 22–27% ($P<0.01$), and the vasodilatation in response to acetylcholine (3–100 ng) and sodium nitroprusside (1–10 μg), as well as the vasoconstriction with endothelin-1 (1–10 μg), were as in the control and comparable in the three groups of animals. These results suggest: (a) that during ischemia, the coronary vasodilator reserve is greatly reduced, and the vasoconstriction with endothelin-1 is blunted, with preservation of the modulatory role of nitric oxide and involvement of vasoconstrictor prostanoids in this vasoconstriction, and (2) that during reperfusion, the coronary vasodilator reserve and the coronary reactivity to acetylcholine and endothelin-1 recover, but the modulatory role of nitric oxide in this reactivity may be attenuated.

Keywords: Coronary flow; Endothelium-dependent vasodilatation; Coronary vasoconstriction; Coronary vasodilatation; Coronary vasodilator reserve

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 coronary flow reduction. The endothelium, by releasing vasodilator substances (nitric oxide, hyperpolarizing factor, prostacyclin) and vasoconstrictors (endothelin-1, thromboxan A_2), may play a main role in the regulation of vascular reactivity, and a reciprocal interaction between nitric oxide and endothelin-1 may exist (Masaki, 1995). Several lines of evidence suggest that alteration of the endothelium and endothelin-1 may play a relevant role in the pathophysiology of ischemia–reperfusion, and several aspects of this condition may be related to alterations of nitric oxide and endothelin-1 pathways. However, more studies are needed to clarify this particular issue.

Experimental data show that endothelium-dependent coronary vasodilatation is decreased during reperfusion after total (Ku, 1982; Kim et al., 1992) or partial (Nichols et al., 1994) coronary occlusion. Basal release of nitric oxide from rat hearts may be diminished after ischemia–reperfusion (Maulik et al., 1995), and studies into mechanisms of the latter have implicated the nitric oxide pathway. Administration of exogenous nitric oxide may mitigate or abolish the adverse effects of ischemia–reperfusion (Lefer, 1995). Other studies, however, showed that inhibitors of nitric oxide synthesis may protect from, rather than aggravate, the effects of ischemia–reperfusion (Matheis et al., 1992; Schulz and Wambolt, 1995). For endothelin-1, results of several types of studies suggest that this peptide is involved in deleterious effects of ischemia–reperfusion (Pernow and

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Wang, 1997). Ischemia–reperfusion can induce increased coronary vasoconstriction in response to endothelin-1 (Watts et al., 1992; Thompson et al., 1995), but whether or not this increased effect is present may depend on the severity and duration of ischemia (Neubauer et al., 1991; Lockowandt et al., 2001). The mechanisms underlying the increased coronary response to endothelin-1 after ischemia–reperfusion are not clear, and it has been attributed to decreased production of nitric oxide and prostacyclin as a result of endothelial dysfunction, and to changes in characteristics of endothelin receptors in coronary vessels (Neubauer et al., 1991; Watts et al., 1992; Thompson et al., 1995). The coronary reactivity to endothelin-1 after ischemia–reperfusion has been studied using in vivo and in vitro preparations, and coronary ischemia has been induced in vivo by occluding one coronary artery for different periods and in isolated hearts perfused with solutions under mild or severe hypoxic conditions. The study of coronary reactivity to endothelin-1, and its interaction with nitric oxide and prostanoids during partial coronary occlusion and during reperfusion after this partial ischemia could be of interest for understanding the pathophysiology of ischemia–reperfusion. Partial ischemia deserves attention because it is a condition that may be relatively frequent in patients with coronary atheromatosis and dynamic vasospasm.

The objective of the present study was to examine the coronary response to endothelin-1 and its interaction with nitric oxide or prostanoids during partial coronary ischemia and its reperfusion. Also, the functional state of the coronary endothelium under both conditions was tested by recording the coronary response to acetylcholine. The experiments were carried out in anesthetized goats where blood flow in the left circumflex coronary artery was electromagnetically measured, and partial occlusion and reperfusion of this artery were induced. The coronary effects of acetylcholine, sodium nitroprusside and endothelin-1 were recorded under control conditions, during partial ischemia and 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, 19 adult female goats (31–59 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 baselines. Systemic arterial pressure was measured through a polyethylene catheter placed in one temporal artery and connected to a Statham transducer. In every 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.

2.2. Experimental protocol

After the experimental preparation was ended, and the hemodynamic variables had reached steady state, the hyperemic response to 10-s coronary occlusions was tested three times, and the coronary response to acetylcholine (3–100 μg), sodium nitroprusside (1–10 ng) and endothelin-1 (0.01–0.3 nmol) was recorded under control conditions in each animal. Then, a critical, partial occlusion of the left circumflex coronary artery was achieved with another occluder which was variable and 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. The arterial occlusion was gradually adjusted over about 15 min, and was considered adequate when the hyperemic responses to three consecutive 10-s occlusions made at 6–7-min intervals were reduced by >90% of those recorded under control conditions. This degree of occlusion was maintained for about 60 min, and during this period of ischemia the response to acetylcholine (3–100 μg), sodium nitroprusside (1–10 ng) and endothelin-1 (0.01–0.3 nmol) was assayed again. After this test during ischemia was ended, the arterial occlusion was gradually, but totally, released to permit its reperfusion, and 30 min after this release, the responses to acetylcholine, sodium nitroprusside and endothelin-1 were also tested. 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 eight non-treated goats, in six goats treated with L-NAME, and in five goats treated with meclofenamate, and in each case, the responses to the vasoactive drugs used during control, partial ischemia and reperfusion were recorded from the same animal.

Acetylcholine, sodium nitroprusside and endothelin-1 were dissolved in physiological saline, and each dose was administered in volumes 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 were administered after the end of the control tests with endothelin-1, and about 8 min before induction of coronary stenosis.

The effects of acetylcholine, sodium nitroprusside and endothelin-1 on coronary vasculature were evaluated as changes in coronary vascular conductance at their maximal effects on coronary blood flow. Coronary vascular conductance was calculated by dividing coronary blood flow in ml/min by mean systemic arterial pressure in mm Hg.

2.3. Statistical analysis

The data are expressed as means ± S.E.M. The effects of coronary occlusion and reperfusion, as well as of L-NAME and meclofenamate on the hemodynamic variables recorded and on blood gases and pH were evaluated in each case as changes in absolute values and as percentages by 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 non-treated, L-NAME-treated and meclofenamate-treated animals were compared using data expressed as percentages by applying one-way, factorial ANOVA, followed by Dunnett's test. All the effects of acetylcholine, sodium nitroprusside and endothelin-1 during ischemia and reperfusion were compared with their respective controls using changes in absolute values by applying two-way, repeated measures ANOVA, followed by Dunnett's test. Also, the effects of these drugs in each situation in the three groups of 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.

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.

2.4. Chemicals

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

3. Results

3.1. Hemodynamic changes during ischemia and reperfusion

The resting hemodynamic values during control, partial ischemia and reperfusion are summarized in Table 1. Under control conditions, the resting values for coronary flow and coronary vascular conductance were comparable in the three groups of animals. In eight non-treated animals, coronary occlusion decreased coronary flow by 45% ($P<0.001$), mean arterial pressure by 16% ($P<0.01$) and coronary vascular conductance by 35% ($P<0.001$); it did not affect heart rate significantly. At 30 min after the start of reperfusion, coronary flow remained decreased by 35% ($P<0.001$), mean arterial pressure by 10% ($P<0.05$) and coronary vascular conductance by 27% ($P<0.01$); heart rate was not significantly different from the control. In six animals treated with intracoronary administration of L-NAME, this drug by itself decreased coronary flow by 13% ($P<0.05$) without changing significantly mean arterial pressure or heart rate; in these animals coronary occlusion decreased coronary flow by 26% ($P<0.01$) and coronary vascular conductance by 24% ($P<0.01$), without changing significantly mean arterial pressure and heart rate. At 30 min after reperfusion coronary flow remained decreased by 31% ($P<0.01$) and coronary vascular conductance by 22% ($P<0.01$), whereas mean arterial pressure and heart rate were not significantly distinct from the control conditions. In five animals with i.v. administration of meclofenamate, this drug by itself did not affect significantly coronary flow, mean arterial pressure or heart rate; in these animals coronary occlusion decreased coronary flow by 32% ($P<0.01$) and coronary vascular conductance by 37% ($P<0.01$), whereas mean arterial pressure and heart rate did not change significantly. At 30 min of reperfusion, coronary flow remained decreased by 22% ($P<0.01$) and

Table 1
Resting hemodynamic values obtained during control conditions, partial coronary ischemia and at 30 min of reperfusion in anesthetized goats non-treated (eight 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	37 ± 4	89 ± 3	0.41 ± 0.04	77 ± 5
Ischemia	20 ± 3[a]	75 ± 3[a]	0.27 ± 0.03[a]	75 ± 7
Reperfusion	24 ± 3[a]	80 ± 4[b]	0.30 ± 0.04[a]	71 ± 5
L-NAME-treated				
Control	34 ± 4	92 ± 4	0.37 ± 0.04	67 ± 5
L-NAME	29 ± 3[b]	94 ± 5	0.31 ± 0.03[b]	61 ± 5
Ischemia	25 ± 4[b]	93 ± 4[c]	0.28 ± 0.04[b]	62 ± 6
Reperfusion	23 ± 4[b]	85 ± 5	0.29 ± 0.04[b]	66 ± 6
Meclofenamate-treated				
Control	32 ± 3	98 ± 5	0.33 ± 0.04	72 ± 6
Meclofenamate	31 ± 4	96 ± 5	0.32 ± 0.04	80 ± 7
Ischemia	22 ± 3[a]	100 ± 5[c]	0.21 ± 0.03[a]	72 ± 5
Reperfusion	25 ± 4[a]	99 ± 4[c]	0.26 ± 0.04[a]	69 ± 6

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

[a] $P<0.01$ compared with its corresponding control.

[b] $P<0.05$ compared with its corresponding control.

[c] $P<0.05$ compared with the corresponding situation in non-treated animals.

coronary vascular conductance by 22% ($P<0.01$), whereas mean arterial pressure and heart rate were comparable to those under control conditions.

The decreases in coronary vascular conductance induced by coronary occlusion were comparable in non-treated and in meclofenamate-treated animals and were lower in L-NAME-treated animals. These decreases during reperfusion were comparable in the three groups of animals.

Systemic blood gases and pH did not change significantly during ischemia and reperfusion as compared with control conditions in the three groups of animals (these data are not shown).

3.2. Coronary response during ischemia

In eight non-treated, six L-NAME-treated and five meclofenamate-treated animals, acetylcholine (3–100 ng) and sodium nitroprusside (1–10 μg) produced dose-dependent increases in coronary vascular conductance under control conditions, and these effects were much reduced during coronary ischemia. The coronary effects of these two drugs during ischemia were comparable in the three groups of animals (Fig. 1). Acetylcholine and sodium nitroprusside did not produce systemic effects.

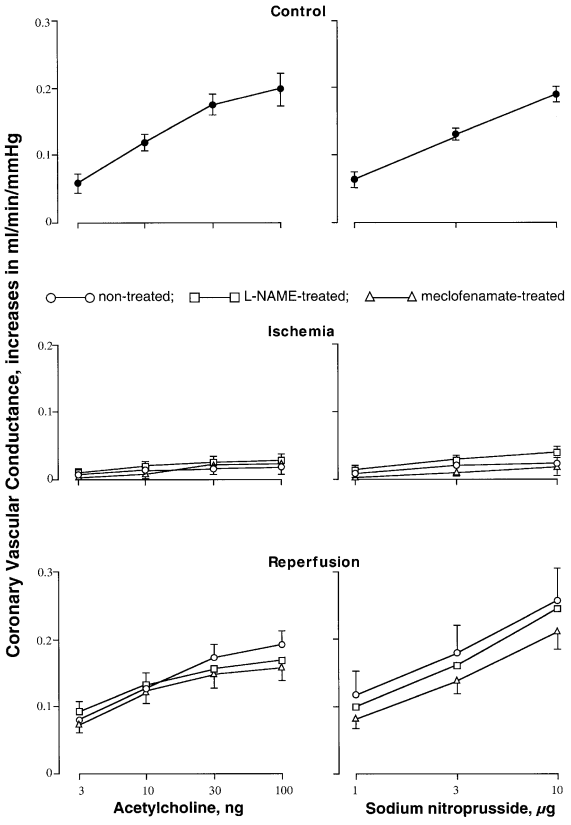

Fig. 1. Summary of the effects of acetylcholine (left panels) and sodium nitroprusside (right panels) on coronary vascular conductance obtained under control conditions (top, averages of the control effects in the three groups of animals), during coronary ischemia (middle) and during reperfusion (bottom) in anesthetized goats non-treated (eight animals), treated with L-NAME (six animals) and treated with meclofenamate (five animals).

Under control conditions, in the three groups of animals, endothelin-1 (0.01–0.3 nmol) produced dose-dependent decreases in coronary vascular conductance. During ischemia, these decreases were significantly lower than under control conditions in eight non-treated animals, were not significantly different from the corresponding control conditions in six L-NAME-treated animals, and were much lower than under control conditions in five meclofenamate-treated animals. During ischemia, the coronary effects of endothelin-1 in L-NAME-treated animals were significantly greater, and in meclofenamate-treated animals they were significantly less than in non-treated animals (Fig. 2).

Endothelin-1 at the doses of 0.1 and 0.3 nmol also increased mean arterial pressure in non-treated and treated animals by 6 ± 2 and 12 ± 4 mm Hg ($P>0.05$), respectively, under control conditions, and this increase was similar during coronary ischemia in the three groups of animals. These effects of endothelin-1 were present after their maximal effects on coronary flow.

3.3. Coronary response after reperfusion

In eight non-treated, six L-NAME-treated and five meclofenamate-treated animals, the increases in coronary vascular conductance induced by acetylcholine (3–10 ng) and sodium nitroprusside (1–10 μg) after reperfusion were not significantly different from those under the corresponding control conditions, and under reperfusion they were similar in the three groups of animals (Fig. 1). As occurred under control conditions and during ischemia, acetylcholine and sodium nitroprusside did not affect systemic variables in non-treated and treated animals.

In eight non-treated animals, the effects of endothelin-1 (0.01–0.3 nmol) on coronary vascular conductance tended to be greater during reperfusion, but they were not significantly different from those recorded under control conditions (Fig. 2). In six animals treated with L-NAME, the reductions of coronary vascular conductance induced by 0.01 and 0.3 nmol of endothelin-1 were not significantly different, and those with 0.03 and 0.1 nmol were significantly higher during reperfusion than those found during the corresponding control conditions. The reductions by all doses of endothelin-1 during this reperfusion were similar to those found during reperfusion in non-treated animals (Fig. 2). In five animals treated with meclofenamate, the effects on coronary vascular conductance produced by endothelin-1 (0.01–0.3 nmol) were not significantly different during this reperfusion and their corresponding controls, and were comparable to those found during reperfusion in non-treated animals (Fig. 2).

As occurred under control conditions and during ischemia, endothelin-1 at the doses of 0.1 and 0.3 nmol also increased mean arterial pressure by 5 ± 2 and 14 ± 4 mm

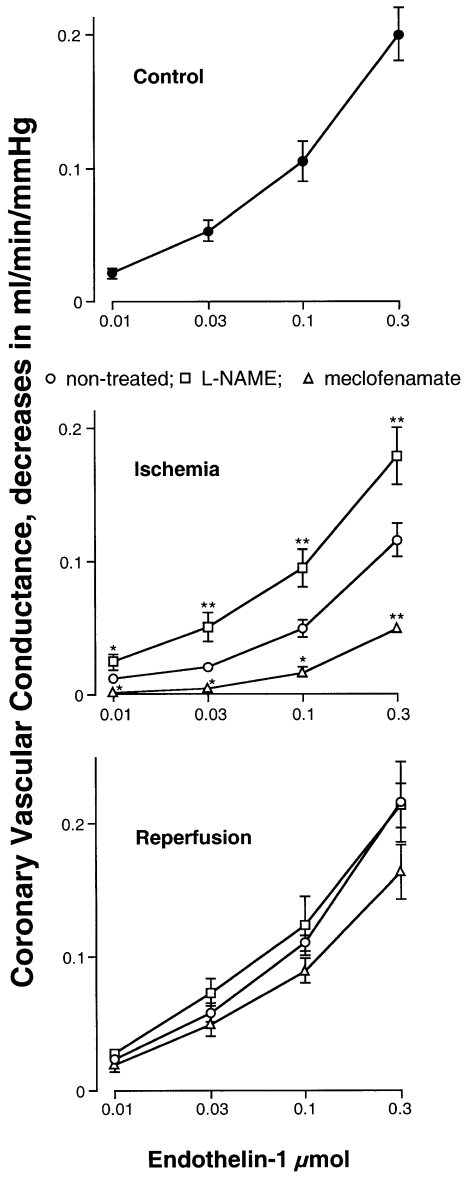

Fig. 2. Summary of the effects of endothelin-1 on coronary vascular conductance obtained under control conditions (top, averages of the control effects in the three groups of animals), during coronary ischemia (middle) and during reperfusion (bottom) in anesthetized goats non-treated (eight animals), treated with L-NAME (six animals) and treated with meclofenamate (five animals). *$P<0.05$ and **$P<0.01$ for difference between non-treated and L-NAME or meclofenamate-treated animals.

Hg ($P>0.05$), respectively, during reperfusion. These effects were similar in the three groups of animals and were also present after their maximal effects on coronary flow.

4. Discussion

The present study was carried out to examine the coronary effects of endothelin-1 and its interaction with nitric oxide and prostanoids during both partial coronary ischemia and its reperfusion. Also, the functional state of the coronary endothelium was tested by examing the coronary effects of acetylcholine under both conditions. 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). We will first comment on the results during ischemia, and then on those during reperfusion.

Partial coronary occlusion reduced coronary vascular conductance, and this reduction was similar in non-treated and meclofenamate-treated animals, and was less pronounced in L-NAME-treated animals. Moderate hypotension was also present during ischemia only in non-treated animals. The presence of hypotension in non-treated animals might be related to an increased release of nitric oxide and vasodilator prostanoids during ischemia, which may have been inhibited by L-NAME or meclofenamate, respectively. 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 reported previously (García et al., 1992, 1996; Fernández et al., 1998) as did others (Bassenge, 1995). The lower reduction of coronary hemodynamics during ischemia in L-NAME-treated animals may be related in part to this drug itself reducing the resting coronary flow which may have reduced the hyperemic response, and consequently ischemia may have been achieved with a lesser reduction of flow. 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). This drug also did not modify the effects of ischemia on coronary hemodynamics as compared with the effect in non-treated animals.

During ischemia the vasodilator responses to both acetylcholine and sodium nitroprusside were markedly decreased, to a similar degree in the three groups of animals. This finding may be expected, as coronary occlusion probably produced coronary vasodilatation, thus reducing the capacity of the coronary vasculature to further dilate in response to vasodilator stimuli under these conditions. Therefore, coronary vasodilator reserve may be diminished during partial ischemia. The coronary response to endothelin-1 was attenuated during ischemia in non-treated animals, and this attenuation was reversed by L-NAME and was accentuated by meclofenamate. The results with L-NAME suggest that inhibition of nitric oxide synthesis potentiates the coronary vasoconstriction in response to endothelin-1 during partial ischemia as occurs in anesthetized goats under normal conditions (García et al., 1996). Therefore, our results with L-NAME suggest that the modulatory role of nitric oxide in the coronary reactivity to endothelin-1 may be preserved at least in part

during partial, moderate ischemia in our experimental conditions. Our data obtained with meclofenamate show that this drug inhibited the coronary vasoconstriction in response to endothelin-1 during partial ischemia, feature not seen in anesthetized goats under normal conditions (García et al., 1996). This suggests that vasoconstrictor prostanoids are involved in the vasoconstriction with endothelin-1 during ischemia, and this does not occur under normal conditions in goats (García et al., 1996) and other species (Rigel and Lappe, 1993). The present results do not explain the observed attenuated coronary effects of endothelin-1 during partial ischemia. The presence of tachyphylaxis can be reasonably excluded as the response to this peptide was restored during reperfusion. We can speculate that this attenuation might be related to the following factors: (1) decreased sensitivity of endothelin ET_A receptors and/or decreased reactivity of the wall of coronary vessels as a consequence of the probably low intracoronary pressure, hypoxia and acidosis in the ischemic area, as it is generally accepted that these factors may decrease vascular reactivity. In isolated rat hearts perfused with Krebs–Henseleit solution under mild hypoxia, the coronary vasoconstriction in response to endothelin-1 was attenuated (Neubauer et al., 1991); and (2) increased production of nitric oxide as a consequence of myocardial ischemia (Lecour et al., 2001), and this nitric oxide may have inhibited the coronary vasoconstriction in response to endothelin-1 (Masaki, 1995). As the damaging effects of hypoxia on cells appear to be energy-dependent (Buderus et al., 1989) and because endothelium and vascular smooth muscle exhibit very low basal energy and oxygen requirements, and flow during ischemia was moderately reduced, it is unlikely that hypoxic damage to the endothelium and smooth muscle of coronary vessels was marked enough to affect the response to vasoconstrictors during ischemia in our experiments.

At 30 min of reperfusion, coronary flow and coronary vascular conductance remained decreased to the same extent in the three groups of animals, and this was accompanied by hypotension only in non-treated animals. From our study, it is apparent that the non-reflow phenomenon was present during reperfusion in non-treated and treated animals, and this phenomenon has been observed during reperfusion after total (Forman et al., 1989) but not after partial (Nichols et al., 1994) coronary occlusion. The mechanisms of the no-reflow phenomenon are not totally understood, and several factors have been suggested to be involved (Ku, 1982). After reperfusion, the vasodilator effects of acetylcholine and sodium nitroprusside were as in control conditions, indicating that the diminished vasodilator reserve recorded during ischemia recovers during reperfusion. Nichols et al. (1994) report a reduced flow reserve during reperfusion after subtotal coronary occlusion in anesthetized dogs, and the reason for this difference between this study and ours is not apparent. We also found that L-NAME and meclofenamate did not affect the coronary vasodilator reserve as they did not alter the effects of acetylcholine and sodium nitroprusside during reperfusion. L-NAME failed to reduce the effects of acetylcholine during reperfusion, which contrasts with the effect in anesthetized goats under normal conditions where L-NAME did inhibit this coronary action (García et al., 1992). This suggests that during reperfusion after partial ischemia, the mediator role of nitric oxide in the vasodilatation with acetylcholine may be reduced, probably because ischemia induces endothelial dysfunction. Tsao et al. (1990) found that, in cats, endothelial dysfunction did not occur before reperfusion, at least in ischemic episodes of up to 90 min, and that endothelial dysfunction occurred very early after reperfusion. Results of other studies suggest that the endothelium is very sensitive to ischemia–reperfusion (Nicklas and Gips, 1989; Kim et al., 1992). Loss of vascular reactivity to endothelium-dependent drugs after ischemia–reperfusion may be due to several factors, including depletion of endogenous stores of nitric oxide, enhanced inactivation of nitric oxide, or both (Miller and Vanhoutte, 1985). Experiments with dog isolated coronary arteries suggest that the relaxation in response to acetylcholine of arteries exposed to ischemia and reperfusion is mediated by nitric oxide and endothelium-dependent hyperpolarizing factor, and that this factor may be a reserve system activated by ischemia–reperfusion (Chan and Woodman, 1999). Based on our study, we can speculate that some other factors distinct from nitric oxide are involved in the coronary vasodilatation with acetylcholine during reperfusion after partial ischemia, thus preserving this vasodilatation during reperfusion. This other factor is probably not a product of the cyclooxygenase pathway as meclofenamate did not affect the coronary action of acetylcholine during reperfusion.

The coronary effects of endothelin-1 during reperfusion in treated and non-treated animals were not different from those found under control conditions, indicating that these effects apparently recover during reperfusion after the attenuation found during ischemia. Experiments performed in isolated rat hearts indicate that, during reoxygenation after severe hypoxia or during reperfusion after short (10 min) total ischemia, the coronary vasoconstriction in response to endothelin-1 was unaltered, but during reperfusion after a longer (30 min) total ischemia this vasoconstriction was augmented (Neubauer et al., 1991). Also, studies in pigs show that reperfusion after 10 min of coronary occlusion decreases the endothelium-dependent coronary vasodilatation but does not alter the coronary vasoconstriction with endothelin-1, whereas reperfusion after 60 min of coronary occlusion both decreases endothelium-dependent vasodilatation and increases the vasoconstriction induced by this peptide, suggesting that endothelium regulation of coronary vascular reactivity is more sensitive, and precedes changes in vascular smooth muscle function after ischemia–reperfusion (Lockowandt et al., 2001). Therefore, the effects of ischemia–reperfusion on the coronary vasoconstriction in response to endothelin-1 may be dependent on the severity and duration of ischemia. The increased coronary reactivity

to endothelin-1 after ischemia–reperfusion has been attributed to a reduced endothelin ET_B receptor-mediated release of nitric oxide or prostacyclin as a result of endothelial dysfunction (Watts et al., 1992), and to either increased endothelin-1 binding sites or loss of counteracting vasodilator mechanisms such as prostaglandins and/or nitric oxide (Neubauer et al., 1991). Other studies, however, show that inhibition of nitric oxide or prostacyclin formation does not affect the coronary contractile response to endothelin-1 (Wang et al., 1995), and that loss of endothelin ET_B receptor-mediated vasodilatation does not fully account for the enhanced endothelin-1-mediated coronary vasoconstriction (Thompson et al., 1995) after ischemia–reperfusion. Our data with L-NAME indicate that this drug failed to potentiate the coronary effects of endothelin-1 during reperfusion, which contrasts with effects observed during ischemia (present study) and under normal conditions (García et al., 1996), suggesting that the modulatory role of nitric oxide in the coronary response to endothelin-1 may be attenuated during reperfusion. As inhibition of nitric oxide synthesis with N^G-nitro-L-arginine may result in increased production of prostacyclin in rabbit hearts exposed to ischemia (Aitchinson and Coker, 1999), we cannot exclude that this feature also occurred in our experiments after treatment with L-NAME, thus prostacyclin might have counteracted and masked the possible potentiating action of L-NAME on the coronary response to endothelin-1. Our hypothesis about the decreased modulatory role of nitric oxide in the coronary effects of endothelin-1 is consistent with the idea that the mediator role of nitric oxide in the response to acetylcholine is diminished during reperfusion. Therefore, reperfusion after partial ischemia, but not partial ischemia alone, may impair endothelial function, thus altering the coronary reactivity to stimuli that are mediated (e.g., acetylcholine) or modulated (e.g., endothelin-1) by nitric oxide. Based on experiments with isolated rat coronary arteries, it is suggested that endothelial dysfunction is produced by reperfusion after ischemia but not by ischemia alone (Richard et al., 1994a,b). In meclofenamate-treated animals, the coronary response to endothelin-1 during reperfusion was comparable to that found during reperfusion in non-treated animals. This suggests that prostanoids may be not involved in the coronary effects of this peptide during reperfusion, as may occur under normal conditions in anesthetized goats (García et al., 1996) and other species (Rigel and Lappe, 1993), but contrasts with what occurs during ischemia where vasoconstrictor prostanoids may be involved (present results).

In conclusion, the present results suggest: (1) that during partial ischemia, the coronary vasodilator reserve is greatly reduced, and the vasoconstriction in response to endothelin-1 is blunted, with preservation of the modulatory role of nitric oxide and probable involvement of vasoconstrictor prostanoids in this vasoconstriction, and (2) that during reperfusion, the coronary vasodilator reserve and the coronary reactivity to acetylcholine and endothelin-1 recover, but the modulatory role of nitric oxide in this reactivity may be attenuated. The blunted effects of ischemia on the coronary action of endothelin-1 do not mean that this peptide is not involved in deleterious effects of ischemia–reperfusion on coronary vasculature, and the endothelial function in modulating coronary reactivity may be very sensitive to reperfusion after partial, moderate ischemia.

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