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Coronary effects of vasopressin during partial ischemia and reperfusion in anesthetized goats. Role of nitric oxide and prostanoids

María Angeles Martínez, Nuria Fernández, Belén Climent, Angel-Luis García-Villalón, Luis Monge, Elena Sanz, Godofredo Diéguez*

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

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Abstract

To examine the coronary effects of arginine–vasopressin and its interaction with nitric oxide and prostanoids during partial 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 (seven non-treated, six treated with N^{W}-nitro-L-arginine methyl esther (L-NAME) and five with meclofenamate). During partial coronary occlusion, coronary vascular conductance decreased by 20–31% ($P<0.01$), and the coronary vasodilatation in response to acetylcholine (3–100 ng) and sodium nitroprusside (1–10 μg) was much reduced in every case; the vasoconstriction in response to arginine–vasopressin (0.03–0.3 μg) was attenuated in non-treated animals; this attenuation was reversed by L-NAME and was accentuated by meclofenamate. At 30 min of reperfusion, coronary vascular conductance remained decreased by 11–25% ($P<0.05$ or $P<0.01$), and the vasodilatation in response to acetylcholine and sodium nitroprusside as well as the vasoconstriction with arginine–vasopressin was as in the control and comparable in the three groups of animals. These results suggest: (1) that, during ischemia, the coronary vasodilator reserve is greatly reduced and the vasoconstriction with arginine–vasopressin is attenuated, 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 arginine–vasopressin recover, but the modulatory role of nitric oxide in this reactivity may be attenuated.

Keywords: Coronary flow; Endothelium-dependent vasodilatation; Vasoconstriction, coronary; Vasodilatation, coronary; Vasodilator reserve, coronary

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 and vasoconstrictor substances, may play a main role in the regulation of vascular reactivity, and some studies suggest that the coronary vasoconstriction in response to arginine–vasopressin is modulated by the endothelium and nitric oxide (Myers et al., 1989; García-Villalón et al., 1996) but not by prostanoids (Maturi et al., 1991). Experimental observations suggest that the endothelium is sensitive to ischemia–reperfusion and that arginine–vasopressin may be involved in the pathophysiology of this entity (Sellke and Quillen, 1992; Schafer et al., 2002). However, more studies are needed to clarify the role of this peptide and its interaction with the endothelium in the pathophysiology of ischemia–reperfusion.

Experimental data show that endothelium-dependent coronary vasodilatation is decreased during reperfusion after total (Ku, 1982; Mehta et al., 1989; Kim et al., 1992) or partial (Yang et al., 1993; 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), but it has been also reported that inhibitors of nitric oxide synthesis may protect from, rather than aggravate, the effects of ischemia–reperfusion (Matheis et al., 1992; Schulz and

* Corresponding author. Tel.: +34-91-733-86-92 (home), +34-91-397-54-24 (business); fax: +34-91-397-53-24.
E-mail address: godofredo.dieguez@uam.es (G. Diéguez).

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Wambolt, 1995). For arginine–vasopressin, studies performed in dogs show that the coronary effects of vasopressin are increased in the ischemic myocardium, which can worsen hypoperfusion of collateral-dependent myocardium during exercise (Foreman et al., 1991). Sellke and Quillen (1992) report that the coronary action of arginine–vasopressin is augmented after ischemia alone or followed by reperfusion, and the authors suggest that this augmented effect might be related to alteration of release of nitric oxide and prostanoids. This peptide could be of interest for understanding the pathophysiology of ischemia–reperfusion as human plasma levels of arginine–vasopressin are augmented after myocardial infarction (Hart and Gokal, 1977), cardiac arrest and resuscitation (Paradis et al., 1993) and reperfusion after myocardial infarction (Schafer et al., 2002), and it can produce coronary vasoconstriction (Heyndrickx et al., 1976), which can be severe enough to cause myocardial ischemia (Maturi et al., 1991; Krajcar and Heusch, 1993).

The present study was performed to examine the coronary response to arginine–vasopressin 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 action of acetylcholine. The experiments were carried out in anesthetized goats where the left circumflex coronary artery flow was electromagnetically measured, and partial occlusion and reperfusion of this artery were induced. The coronary effects of acetylcholine, sodium nitroprusside and arginine–vasopressin 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, 18 adult, female goats (30–57 kg) were used. Anesthesia of the animals was induced with 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 by use of 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 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^{TM5}, Copenhagen, Denmark). After termination of the experiments, the goats were killed with a 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 responses to acetylcholine (3–100 ng), sodium nitroprusside (1–10 μg) and arginine–vasopressin (0.03–0.3 μg) were 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 or, in some cases, it was readjusted one to three times during about 60 min and, during this period of ischemia the responses to acetylcholine, sodium nitroprusside and arginine–vasopressin were assayed again. After these tests during ischemia were ended, the arterial oclussion was gradually, but totally released to permit its reperfusion, and 30 min after this release, the responses to acetylcholine, sodium nitroprusside and arginine–vasopressin 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 seven goats non-treated, in six goats treated with L-NAME, and in five goats treated with meclofenamate, and in each case, the coronary responses to the vasoactive drugs used during control, partial ischemia and reperfusion were recorded from the same animal. In every case and condition, the hemodynamic variables returned to pre-drug levels after administration of each drug.

Two non-treated animals had ventricular fibrillation and died at early reperfusion, and these two animals were eliminated from the analysis. In these two animals, the release of the arterial occlusion had been performed during about 30 s and, after this observation, the occlusion release in the animals included in the present study was performed during about 5 min. In these animals, sporadic episodes of cardiac arrhythmias were noted during the period of ische-

mia but were short lived (<1 min), and they were more frequent and of longer duration (1–3 min) during reperfusion, especially at early reperfusion. These arrhythmias occurred in six of the seven non-treated animals, in three of the six animals treated with L-NAME and in two of the five animals treated with meclofenamate. Antiarrhythmic drugs were not administered to any of the animals.

Acetylcholine, sodium nitroprusside and arginine–vasopressin were dissolved in physiological saline, and each dose was administered using 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 18–20 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 the vasoactive drugs and about 8 min before induction of coronary ischemia.

It can be estimated that the doses of L-NAME used permit to achieve plasma levels of this substance in the coronary circulation comparable to those achieved also in goats by injecting 40–47 mg/kg i.v. (Fernández et al., 1998, 2000) and they are higher than those achieved by injecting 8–10 mg/kg i.v. (García et al., 1992). In these studies, L-NAME inhibited the coronary vasodilatation produced by acetylcholine (García et al., 1992; Fernández et al., 2000) and increased the coronary vasconstriction induced with vasopressin (Fernández et al., 1998). Also, it can be estimated that the doses of meclofenamate used permit to achieve plasma concentrations of this substance higher than those used with in vitro studies (2×10^{-6}–10^{-5} M) (O'Donnell et al., 1996; García-Villalón et al., 2003), and they are used two times in rats by Walker et al. (1988). Therefore, the doses of L-NAME and meclofenamate used in the present study may be sufficient to inhibit nitric oxide synthesis or cyclooxygenase, respectively.

The effects of acetylcholine, sodium nitroprusside and arginine–vasopressin on coronary vasculature were evaluated as changes in coronary vascular conductance at their maximal effects on coronary flow. Coronary vascular conductance was calculated by dividing coronary flow in milliliter per minute 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 ischemia 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 ischemia 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 the Dunnett's test. All the effects of acetylcholine, sodium nitroprusside and arginine–vasopressin during ischemia and reperfusion were compared with their respective controls using changes in absolute values by applying two-way, repeated measures ANOVA, followed by the 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 the 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, sodium nitroprusside and [Arg^8]vasopressin acetate were from Sigma, and meclofenamate was from Parke Davis.

3. Results

3.1. Hemodynamic changes during ischemia and reperfusion

The resting hemodynamic values obtained during control, 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 seven non-treated animals, coronary occlusion decreased coronary flow by 39% ($P<0.01$), mean arterial pressure by 14% ($P<0.05$) and coronary vascular conductance by 28% ($P<0.01$); it did not affect heart rate significantly. At 30 min after the start of reperfusion, coronary flow remained decreased by 33% ($P<0.01$), mean arterial pressure by 12% ($P<0.05$) and coronary vascular conductance by 25% ($P<0.01$); heart rate was not significantly different from the control. In six animals treated with intracoronary administration of L-NAME, this drug by itself decreased basal coronary flow by 14% ($P<0.05$) without changing significantly mean arterial pressure and heart rate; in these animals, coronary occlusion decreased coronary flow by 27% ($P<0.01$) and coronary vascular conductance by 20% ($P<0.01$), without changing significantly mean arterial pressure and heart rate. At 30 min after reperfusion, coronary flow remained decreased by 16% ($P<0.05$) and coronary vascular conductance by 11% ($P<0.05$), whereas mean arterial pressure and heart rate were not significantly distinct from the control conditions. In this group of animals, coronary vascular conductance at 30 min of reperfusion was not significantly

Table 1
Resting hemodynamic values obtained during control conditions, partial coronary ischemia and at 30 min of reperfusion in anesthetized goats non-treated (seven animals), treated with L-NAME (six animals) and treated with meclofenamate (five animals)

	CBF (ml/min) (beats/min)	MAP (mm Hg)	CVC (ml/mi/mm Hg)	HR
Non-treated				
Control	34 ± 3	91 ± 4	0.38 ± 0.04	70 ± 5
Ischemia	21 ± 3[a]	77 ± 3[a]	0.27 ± 0.03[a]	73 ± 6
Reperfusion	23 ± 3[a]	79 ± 3[a]	0.29 ± 0.03[a]	75 ± 5
L-NAME-treated				
Control	38 ± 4	93 ± 4	0.41 ± 0.05	76 ± 6
L-NAME	33 ± 3[b]	90 ± 4	0.37 ± 0.04[b]	72 ± 5
Ischemia	28 ± 3[a,c]	88 ± 4[c]	0.33 ± 0.04[a]	69 ± 7
Reperfusion	32 ± 3[b,c]	87 ± 4[c]	0.37 ± 0.05[b]	71 ± 6
Meclofenamate-treated				
Control	30 ± 3	100 ± 4	0.31 ± 0.04	76 ± 6
Meclofenamate	30 ± 3	97 ± 4	0.32 ± 0.04	79 ± 7
Ischemia	21 ± 3[a]	103 ± 5[c]	0.21 ± 0.03[a]	69 ± 7
Reperfusion	24 ± 4[a]	102 ± 6[c]	0.24 ± 0.03[a]	71 ± 6

Values are means ± S.E.M. n = number of animals. 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 values.

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

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

distinct to that found after L-NAME administration (before ischemia). In five animals treated with i.v. administration of meclofenamate, this drug by itself did not affect significantly hemodynamic variables; in these animals, coronary occlusion decreased coronary flow by 30% ($P < 0.01$) and coronary vascular conductance by 31% ($P < 0.01$), whereas mean arterial pressure and heart rate did not change significantly. At 30 min of reperfusion, coronary flow remained decreased by 21% ($P < 0.05$) and coronary vascular conductance by 23% ($P < 0.01$), whereas mean arterial pressure and heart rate were comparable to those under control conditions. In this group of animals, coronary vascular conductance at 30 min of reperfusion was also lower than after meclofenamate administration (before ischemia).

The decrements in coronary vascular conductance found during both coronary occlusion and reperfusion were comparable ($P > 0.05$) in non-treated and meclofenamate-treated animals, and were lower ($P < 0.05$) in L-NAME-treated animals.

Systemic blood gases and pH did not change significantly during coronary occlusion 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 seven non-treated, six L-NAME-treated and five meclofenamate-treated animals, acetylcholine (3–100 ng) and sodium nitroprusside (1–10 μg) induced dose-dependent increases in 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 cause systemic effects.

Under control conditions, arginine–vasopressin (0.03–0.3 μg) produced dose-dependent decreases in coronary vascular conductance in the three groups of animals. During ischemia, these decreases were significantly lower than under control conditions in seven non-treated animals, were not significantly different from the corresponding control conditions in six L-NAME-treated animals and were much lower than in control conditions in five meclofenamte-treated animals. During ischemia, the coronary effects of arginine–vasopressin in L-NAME-treated animals were significantly greater and, in meclofenamate-treated animals, they were significantly less than in non-treated animals (Fig. 2).

Arginine–vasopressin at the highest dose used also increased mean arterial pressure by 10 ± 4 mm Hg ($P < 0.05$)

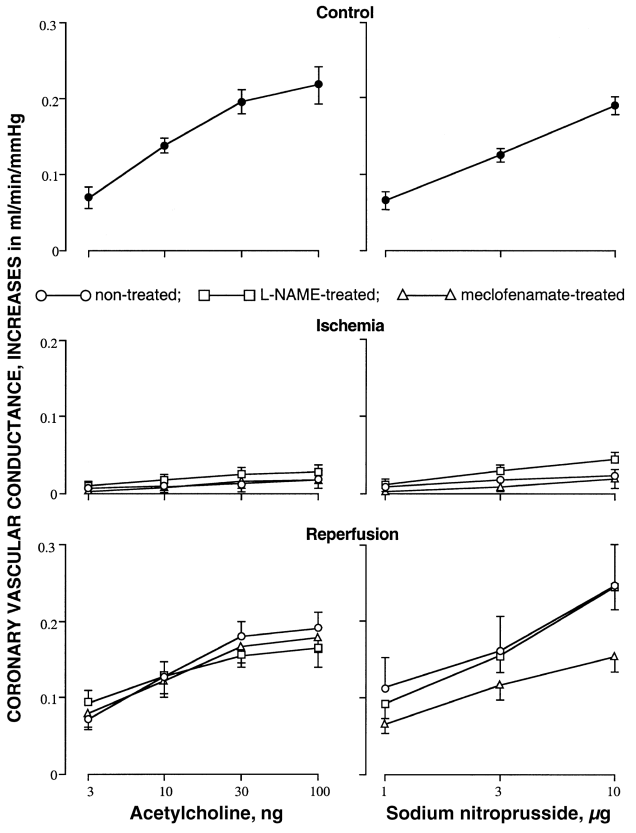

Fig. 1. Summary of the effects of acetylcholine (left panels) and sodium nitroprusside (right panels) on coronary vascular conductance obtained under control conditions (top, averages the control effects in the three groups of animals), during coronary ischemia (middle) and during reperfusion (bottom)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

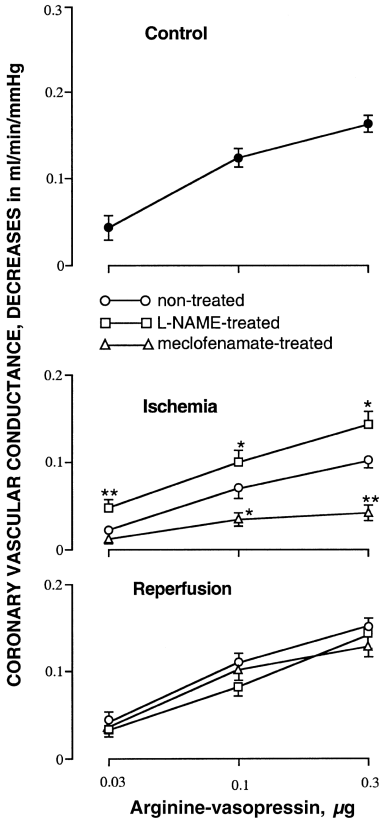

Fig. 2. Summary of the effects of arginine–vasopressin on coronary vascular conductance obtained under control conditions (top, averages the control effects in the three groups of animals), during coronary ischemia (middle) and during reperfusion (bottom) in anesthetized goats non-treated (seven animals), 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-treated or meclofenamate-treated animals.

under control conditions and this increase was similar during ischemia in the three groups of animals. These effects of arginine–vasopressin were present after their maximal effects on coronary flow.

3.3. Coronary response during reperfusion

In seven non-treated and five meclofenamate-treated animals, the effects on coronary vascular conductance induced by acetylcholine (3–100 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 non-treated and meclofenamate-treated animals (Fig. 1). In six animals treated with L-NAME, the effects on coronary vascular conductance by the two lower doses of acetylcholine were comparable, and those by the two highest doses were lower under reperfusion than under control conditions, but under reperfusion the effects of all doses were comparable to those found during reperfusion in non-treated and meclofenamate-treated animals (Fig. 1). In these L-NAME-treated animals, the responses to sodium nitroprusside (1–10 μg) were similar under reperfusion and control conditions and, under reperfusion, they were similar to those under reperfusion in non-treated and meclofenamate-treated animals (Fig. 1).

In non-treated, L-NAME-treated and meclofenamate-treated animals, the effects of arginine–vasopressin (0.03–0.3 μg) on coronary vascular conductance were similar under reperfusion and the corresponding control conditions, and under reperfusion they were comparable in the three groups of animals (Fig. 2).

As occurred under control conditions and ischemia, acetylcholine and sodium nitroprusside did not alter systemic variables during reperfusion in non-treated and treated animals, and arginine–vasopressin, at the highest dose used, increased mean systemic arterial pressure by 12 ± 4 mm Hg during reperfusion. This effect of arginine–vasopressin was comparable in the three groups of animals and it was present after its maximal effect on coronary flow.

4. Discussion

The present study was performed to examine the coronary reactivity to arginine–vasopressin during both partial coronary occlusion and its reperfusion, analyzing the role of nitric oxide and prostanoids in this reactivity. Also, the functional state of the coronary endothelium was tested by examining the coronary action of acetylcholine under both conditions. The coronary effects of the vasoactive drugs used have been 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).

With regard to the control values, coronary vascular conductance was decreased during partial coronary occlusion and it remained decreased after 30 min of reperfusion and, under both circumstances, this reduction was similar in non-treated and meclofenamate-treated animals and was less pronounced in L-NAME-treated animals. Moderate systemic hypotension was present during ischemia and reperfusion only in non-treated animals. This hypotension might be related to increased release of nitric oxide and/or vasodilator prostanoids during ischemia and reperfusion, which may have been inhibited by L-NAME or meclofenamate, respectively. Increased release of nitric oxide (Lecour et al., 2001) and of vasodilator prostanoids (Cocker et al., 1981) as consequence of myocardial ischemia or ischemia–reperfusion has been reported. From the present study it is apparent that the non-reflow phenomenon was present during reperfusion after partial ischemia in non-treated and meclofenamate-treated animals, and it is less clear in L-NAME-treated animals. This phenomenon has been found during reperfusion after total (Forman et al., 1989) but not after partial (Nichols et al., 1994) coronary occlusion. The mechanisms of the non-reflow phenomenon are not totally understood

and several factors have been suggested to be involved (Ku, 1982). Under control conditions, L-NAME by itself reduced resting coronary flow without changing systemic arterial pressure and heart rate, suggesting that nitric oxide may produce a basal vasodilator tone in the coronary circulation under normal conditions as we previously reported (García et al., 1992; Fernández et al., 1998, 2002) 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. As coronary vascular conductance at 30 min of reperfusion was not distinct from that found after L-NAME administration (before ischemia), but it did was lower than after meclofenamate administration (before ischemia), it can be suggested that non-reflow was not present during reperfusion after L-NAME treatment. One explanation for this may be that non-reflow in non-treated animals is due to reperfusion inhibits nitric oxide release, and this feature can not occur when synthesis of nitric oxide is previously inhibited with L-NAME. Another explanation may be that L-NAME, by blocking both constitutive and inducible nitric oxide synthases, inhibits the nitric oxide release and the subsequent formation of free radicals during reperfusion, thus diminishing the aggregation of neutrophils and vessel wall damage, and avoiding obstruction of coronary microvessels. Meclofenamate did not modify the effects of ischemia and reperfusion on coronary hemodynamics, suggesting that prostanoids are not involved in these effects.

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 in the ischemic area, thus reducing the capacity of coronary vasculature to further dilate in response to vasodilator stimuli under these conditions. These data confirm previous studies from our laboratory (Fernández et al., 2002) and suggest that the coronary vasodilator reserve is decreased during partial ischemia. The coronary effects of arginine–vasopressin were attenuated during ischemia in non-treated animals, and this attenuation was reversed by L-NAME and was accentuated by meclofenamate. We did not measure poststenotic vascular pressure and therefore we can not know the resting values of poststenotic vascular resistance. However, as resistance induced by the mechanical stenosis remains constant, the changes in coronary vascular conductance produced by arginine–vasopressin probably represent the effects of this peptide on poststenotic coronary vessels, and they indicate that under these circumstances these vessels are less responsive than under normal conditions to vasopressin.

Foreman et al. (1991) report that in dogs vasopressin does not produce effects on coronary vessels of the normal myocardium, but it constricts vessels of the collateral-dependent region developed after coronary occlusion, and the authors suggest that this difference may result from altered endothelium-derived relaxing factor in vessels of collateral zone. Sellke and Quillen (1992) observed that the response to arginine–vasopressin of canine isolated small coronary arteries, but not of large arteries, was increased after ischemia, and the authors suggest that this augmented response is related to upregulation of vasopressin receptors, and alteration in the release of nitric oxide and prostanoids. Our study was performed in goats and we did not examine the development of collateral vessels, but data obtained by others in this species show that collateral blood flow after occlusion is negligible (Lipovetsky et al., 1983; Brown et al., 1991). Also, as we induced a moderate ischemia, development of collaterals during this ischemia may be less probable. Therefore, the effects of vasopressin during ischemia in our experiments are more probably due to its action on poststenotic, innate vessels. The augmented response to vasopressin during ischemia observed by Foreman et al. (1991) may be due, at least in part, to the presence of collateral vessels with impaired endothelial function, and the probable absence of collaterals in our experimental model may explain the absence of increased response to this peptide. It has been tested that isolated collateral arteries from the dog coronary circulation exhibit increased response to vasopressin but not to endothelin-1 (Rapps et al., 1997). Also, differences in the degree of ischemia, and perhaps in species used, may influence vascular response. Foreman et al. (1991) and Sellke and Quillen (1992) performed their studies in dogs and provoked severe ischemia, and this latter may have altered endothelial function thus increasing the coronary response vasopressin. In our study, moderate ischemia was induced and this probably did not affect the release of nitric oxide as suggested by our data with L-NAME. This substance potentiated the coronary action of arginine–vasopressin during ischemia as occurs in anesthetized goats under normal conditions (Fernández et al., 1998), suggesting that the modulatory role of nitric oxide in the coronary response to this peptide may be preserved at least in part during partial coronary occlusion. The data with meclofenamate indicates that this drug inhibited the coronary effects of arginine–vasopressin during ischemia, feature not seen under normal conditions in anesthetized goats (Fernández et al., 1998) and in anesthetized dogs (Maturi et al., 1991). Therefore, vasoconstrictor prostanoids may be involved in the coronary vasoconstriction in response to arginine–vasopressin during coronary ischemia, and this does not occurs under normal conditions in goats (Fernández et al., 1998) and other species (Maturi et al., 1991). The present results, however, do not explain the observed attenuated coronary effects of arginine–vasopressin during partial ischemia. The presence of tachyphylaxis can be reasonably excluded as the response recovered during reperfusion in the same animals. As the attenuation of the coronary action of arginine–vasopressin during partial ischemia was also observed with endothelin-1 (Fernández et al., 2002), we can speculate that this attenu-

ation might be related to factors that inhibit in unspecific manner the coronary vasoconstriction (for example, low intracoronary vascular pressure and/or acidosis in the ischemic area, increased production of nitric oxide as consequence of myocardial ischemia). We can not exclude, however, a decreased sensitivity of vasopressin V_1 receptors in coronary vessels as consequence of the possible increased production of this peptide during coronary ischemia (Wu et al., 1980; Schafer et al.,2002). 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 is significant to affect the response to vasoconstrictors during ischemia in our experiments.

After reperfusion, the vasodilator effects of acetylcholine and sodium nitroprusside were as in control conditions, and these effects were not modified by L-NAME or meclofenamate. This indicates that the diminished coronary vasodilator reserve found during ischemia recovers during reperfusion, and that this recovery is not affected by treatment with L-NAME or meclofenamate, confirming previous studies from our laboratory (Fernández et al., 2002). Our data with L-NAME indicate, however, that this drug failed to reduce the effects of acetylcholine during reperfusion, and this contrasts with that recorded in anesthetized goats under normal conditions where L-NAME did inhibit these coronary effects (García et al., 1992). Thus, it is suggested that during reperfusion after partial ischemia the mediator role of nitric oxide in the vasodilatation to acetylcholine may be reduced, probably because ischemia–reperfusion induces endothelium dysfunction as we have previously reported (Fernández et al., 2002). 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). Data from 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 to contribute to endothelium-dependent coronary vasodilatation (Chan and Woodman, 1999). Based in our study, we can speculate that some other factors distinct to 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 cyclooxygenase pathway as meclofenamate did not affect the coronary action of acetylcholine during reperfusion.

The coronary action of arginine–vasopressin during reperfusion in non-treated and treated animals was similar to that found under control conditions, indicating that this action apparently recovers during reperfusion after the attenuation found during ischemia. Sellke and Quillen (1992) report that the response to arginine–vasopressin of canine isolated small coronary arteries was increased similarly after 1 h of ischemia alone or followed by 1 h of reperfusion. Our study, on the contrary, suggests that 1 h of ischemia alone and followed by 30 min of reperfusion produces different effects on the coronary reactivity to arginine–vasopressin. The different results between the study of Sellke and Quillen (1992) and ours may be due to differences in the experimental approach used in both studies (in vitro vs. in vivo), the degree of coronary ischemia induced, and species used. Also, it may be that all or part of the canine small vessels used by Sellke and Quillen (1992) were of the collateral dependent zone, which seem to exhibit increased coronary response to vasopressin (Foreman et al., 1991; Rapps et al., 1997). Our data show that meclofenamate did not modify the coronary action of arginine–vasopressin after reperfusion, suggesting that prostanoids are not involved in the coronary action of this peptide during reperfusion as may occur under normal conditions (Fernández et al., 1998; Maturi et al., 1991), but it differs from partial ischemia where vasoconstrictor prostanoids may be involved (present study). The absence of effects of L-NAME on the coronary action of arginine–vasopressin found during reperfusion contrasts to that found during ischemia (present results) and in anesthetized goats under normal conditions (Fernández et al., 1998), where L-NAME did potentiate the coronary vasoconstriction in response to this peptide. This suggests that the modulatory role of nitric oxide in the coronary effects of arginine–vasopressin present under ischemia (present results) and normal conditions (Myers et al., 1989; García-Villalón et al., 1996; Fernández et al., 1998) may be attenuated during reperfusion after partial ischemia. This hypothesis is consistent with the idea that the mediator role of nitric oxide in the response to acetylcholine is attenuated during reperfusion. Something similar seems to occur with endothelin-1 as reperfusion after partial ischemia, but not ischemia alone, also attenuated the modulatory role of nitric oxide in coronary vasoconstriction in response to endothelin-1 (Fernández et al., 2002). Therefore, the present study and the previous one (Fernández et al., 2002) suggest that reperfusion after partial ischemia, but not partial ischemia alone, may impair endothelial function. Experiments with isolated coronary arteries from cats (Tsao et al., 1990) and rats (Richard et al., 1994a,b) suggest that endothelial dysfunction is produced by reperfusion after ischemia but not by ischemia alone.

In conclusion, the present study suggests: (1) that, during partial coronary ischemia, the coronary vasodilator reserve is greatly reduced and the coronary action of arginine–vasopressin is attenuated, 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 coronary reactivity to acetylcholine and arginine–vasopressin recover, but the modulatory role of nitric oxide in this reactivity may be attenuated. The significance of these results may be that the endothelial function in modulating coronary reactivity to acetylcholine and arginine–vasopressin is very sensitive to reperfusion after partial, moderate ischemia, and that arginine–vasopressin may be not excluded as a factor involved in the adverse effects of ischemia–reperfusion on coronary vasculature.

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