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Role of nitric oxide in vascular tone and in reactivity to isoproterenol and adenosine in the goat coronary circulation

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Abstract

The present study examined the role of nitric oxide in coronary vascular tone and in the coronary vasodilatation in response to β-adrenoceptor stimulation and adenosine. In anesthetized goats, the effects of intracoronary and i.v. administration of the inhibitor of nitric oxide synthesis, N^{w}-nitro-L-arginine methyl ester (L-NAME), and those of isoproterenol, adenosine and acetylcholine on coronary blood flow, measured electromagnetically in the left circumflex coronary artery, were recorded. Intracoronary infusion of L-NAME (30–40 μg kg^{-1} min^{-1}, four goats) reduced resting coronary blood flow by 14 ± 3% ($P < 0.05$) without changing arterial pressure and heart rate. L-NAME (40 mg kg^{-1}, eight goats) i.v. reduced resting coronary blood flow by 19 ± 4% ($P < 0.05$), increased mean systemic arterial pressure by 22 ± 3% ($P < 0.01$) and decreased heart rate by 10 ± 2% ($P < 0.05$). These effects of L-NAME were partially, but significantly reversed by L-arginine (six goats). Isoproterenol (10–100 ng, eight goats), adenosine (0.3–10 μg, seven goats) and acetylcholine (3–100 ng, five goats), injected intracoronarily, increased coronary conductance in a dose-dependent way and, under control conditions, these increases for isoproterenol, ranged from 32 ± 5% to 82 ± 12%; for adenosine, 6 ± 2% to 174 ± 22%; and for acetylcholine, 39 ± 5% to 145 ± 15%. During i.v. L-NAME the increases in coronary conductance induced by isoproterenol and acetylcholine were significantly reduced by about 50 and 60% ($P < 0.05$), respectively, whereas those induced by adenosine were significantly increased further (about 30–100%, $P < 0.05$). During L-NAME plus L-arginine, the effects of isoproterenol, acetylcholine and adenosine on coronary conductance were not significantly different from those under control conditions. Therefore, it is suggested that in the coronary circulation: (a) nitric oxide may produce a basal vasodilator tone under normal conditions; (b) nitric oxide may be an intermediate in the vasodilatation due to β-adrenoceptor stimulation and acetylcholine, and (c) the vasodilatation due to adenosine is potentiated during reduction of nitric oxide production.

Keywords: Coronary blood flow; Coronary vasodilatation; Coronary vasodilator tone; β-Adrenoceptor stimulation

1. Introduction

It is generally accepted that β-adrenoceptor stimulation and adenosine (Feigl, 1983) as well as nitric oxide (Moncada et al., 1991) produce coronary vasodilatation, and these factors may be of great relevance in the regulation of the coronary circulation. Nitric oxide is synthetized by the coronary endothelium from L-arginine under basal and stimulated conditions, through receptor-dependent and receptor-independent mechanisms, and its synthesis can be inhibited by several L-arginine analogues (Moncada et al., 1991; Bassenge, 1995). Moreover, nitric oxide may produce vasodilatation by direct stimulation of soluble guanylate cyclase and increase of cGMP, and by direct activation of potassium channels in vascular smooth muscle cells (Moncada et al., 1991; Bolotina et al., 1994; Bassenge, 1995; Mistry and Garland, 1998).

Although there are studies suggesting that nitric oxide may participate in the regulation of coronary circulation, in vivo studies of the effects of inhibition of nitric oxide synthesis on coronary blood flow yield discrepant results, as this inhibition may reduce (Chu et al., 1991; Quyyumi et al., 1995; Kaneko et al., 1996) or may not affect (Parent et al., 1992; Davis et al., 1998) resting coronary blood flow. Kirkebøen et al. (1994) consider that it is difficult to

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conclude whether inhibition of nitric oxide synthesis or the associated hemodynamic systemic changes are responsible for the reduction of coronary blood flow after administration of L-arginine analogues. In order to analyze whether nitric oxide is producing or not a basal vasodilator tone in the coronary circulation, we performed a group of experiments comparing the effects of i.v. and intracoronary administration of the inhibitor of nitric oxide synthesis, N^{w}-nitro-L-arginine methyl ester (L-NAME), on resting coronary blood flow.

With regard to the role of nitric oxide in mediating the coronary vasodilator responses to vasoactive stimuli, most studies showed that it is involved in the coronary vasodilatation in response to acetylcholine (Chu et al., 1991; Parent et al., 1992; Kaneko et al., 1996). Acetylcholine, the archetype of receptor-operated endothelium-dependent agents, is one of the most powerful stimulators of nitric oxide production in various vascular beds, including the coronary vasculature (Moncada et al., 1991; Bassenge, 1995). Recently, it has been reported that the coronary vasodilatation in response to acetylcholine is mediated not only by endothelial nitric oxide but also by an endothelium-dependent hyperpolarizing factor (EDHF) (Feletou and Vanhoutte, 1988; Ming et al., 1997) and that EDHF may be a cytochrome *P*-450 metabolite of arachidonic acid (Ming et al., 1997). Still unclear is the role of nitric oxide in the coronary vascular effects in response to β-adrenoceptor stimulation (Rubanyi and Vanhoutte, 1985; Parent et al., 1993; Béa et al., 1994; Kaneko et al., 1996; Crystal et al., 1998) and that of adenosine (Nees et al., 1990; Ishizaka et al., 1991; Parent et al., 1992; Canty and Schwartz, 1994; Quyyumi et al., 1997; Davis et al., 1998) as it has been reported that the effects of these two types of vasoactive stimuli may be dependent on, or independent of, nitric oxide. As this issue could be of interest, the present study was also performed to examine the role of nitric oxide in the coronary vasodilatation produced by isoproterenol, a β-adrenoceptor agonist, and by adenosine, by recording their effects and those of acetylcholine on the coronary circulation under control conditions, after i.v. administration of L-NAME, and after i.v. administration of L-NAME plus L-arginine, the substrate for nitric oxide formation. The experiments were performed in open-chest, anesthetized goats where blood flow through the left circumflex coronary artery was electromagnetically measured, and the coronary vasodilators were injected directly into this coronary artery. The goat is an animal species that has a coronary circulation similar to that of humans, which is an extensive collateral circulation (Brown et al., 1991). We also have previously reported that nitric oxide may produce a basal vasodilator tone in the goat coronary circulation (García et al., 1992). The coronary vasodilatation produced by isoproterenol may be mediated by β_1- and β_2-adrenoceptors (Feigl, 1983), and that produced by adenosine may be mediated by adenosine A_2 receptors (Olsson and Pearson, 1990).

2. Material and methods

2.1. Experimental preparation

The present experiments were performed in 12 anesthetized adult, female goats (35–53 kg). Anesthesia was induced with an intramuscular injection of 10 mg kg^{-1} ketamine hydrochloride and i.v. administration of 2% sodium thiopental; supplementary doses were given as necessary for maintenance. After orotracheal intubation, artificial respiration with room air was instituted with a Harvard respirator.

A polyethylene catheter was placed in one temporal artery to measure systemic arterial blood pressure (Statham transducer), and to obtain blood samples for gas and pH analysis (Radiometer ABL, Copenhagen, Denmark).

A thoracotomy was performed through the fourth left intercostal space, and the pericardium was opened to gain access to the left aspect of the heart. The proximal segment of the left circumflex coronary artery was dissected, and an electromagnetic flow transducer (Biotronex) was placed on this artery to measure blood flow. A snare type occluder was also placed around this artery, just distal to the flow probe, to obtain zero flow baseline.

Coronary blood flow, systemic arterial pressure and heart rate were recorded on a Grass recorder (Model 7 polygraph) continuously throughout the experiments.

2.2. Experimental protocol

In four goats, the hemodynamic effects of intracoronary infusion of L-NAME were recorded. L-NAME was dissolved in physiological saline (1 mg ml^{-1}) and was administered at a rate of 30–40 μg min^{-1} kg^{-1} body weight for 15–20 min.

In another eight goats, the hemodynamic effects of i.v. administration of L-NAME were also recorded. In this case, L-NAME was dissolved in physiological saline (10 mg ml^{-1}) and injected i.v. in eight animals: first, a bolus of 35 mg kg^{-1} within 15 min, and then, an infusion of 0.05–0.08 mg min^{-1} kg^{-1} over 70–80 min; in total, each animal received 40 mg kg^{-1} of L-NAME. After the experiments with L-NAME alone ended, six of the eight animals also received L-arginine, dissolved in physiological saline (5 mg ml^{-1}), i.v. over 10–20 min at 200–300 mg kg^{-1}.

In the animals treated with i.v. L-NAME, the hemodynamic effects of isoproterenol, adenosine and acetylcholine were tested. Isoproterenol (10, 30 and 100 ng, eight animals), adenosine (0.3, 1, 3 and 10 μg, seven animals) and acetylcholine (3, 10, 30 and 100 ng, five animals) were dissolved in physiological saline and injected in a volume of 0.3 ml directly into the coronary artery where blood flow was measured. This was performed under control conditions, during the i.v. infusion of L-NAME and after i.v. administration of L-NAME plus L-arginine.

Table 1
Blood flow in the left circumflex coronary artery (LCC), mean systemic arterial pressure (MAP), coronary vascular conductance (CVC), HR and arterial pCO_2, pO_2 and pH obtained under control conditions (eight goats), after i.v. L-NAME, (eight goats) and after i.v. L-NAME plus L-arginine (six goats)
Values are mean ± SEM.

	Control	L-NAME	L-NAME + L-arginine
LCC blood flow (ml min^{-1})	39 ± 4	32 ± 4[a]	37 ± 4
MAP (mm Hg)	99 ± 5	115 ± 4[b]	95 ± 6
CVC (ml min^{-1} mm Hg^{-1})	0.39 ± 0.04	0.27 ± 0.03[a]	0.40 ± 0.05
HR (beats min^{-1})	93 ± 4	81 ± 3[a]	88 ± 4
pCO_2	31 ± 3	30 ± 3	30 ± 3
pO_2	87 ± 4	88 ± 4	86 ± 4
pH	7.40 ± 0.02	7.40 ± 0.02	7.39 ± 0.02

[a] Value statistically different from the control at $P < 0.05$.
[b] Value statistically different from the control at $P < 0.01$.

The effects of intracoronary and i.v. administration of L-NAME, as well as those of L-arginine on the coronary circulation were evaluated as changes in coronary blood flow. The effects of isoproterenol, adenosine and acetylcholine on the coronary circulation under the different conditions tested were evaluated as changes in coronary vascular conductance. At the end of the experiments, the animals were killed with an i.v. injection of an overdose of sodium thiopental and a saturated solution of KCl. Coronary vascular conductance was calculated by dividing coronary blood flow in ml min^{-1} by mean systemic arterial pressure in mm Hg.

2.3. Data analysis

Hemodynamic measurements before (control), after intracoronary and i.v. treatment with L-NAME and after i.v. treatment with L-NAME plus L-arginine were compared using the animal as its own control. The hemodynamic effects of L-NAME and L-NAME plus L-arginine were analyzed with an analysis of variance for repeated measures, followed by a Dunnett test to compare the hemodynamic data before and after these two treatments. The increases in coronary vascular conductance produced by isoproterenol, adenosine and acetylcholine before and after i.v. administration of L-NAME alone and L-NAME plus L-arginine were also evaluated by an analysis of variance for repeated measures, followed by a Dunnett test. $P < 0.05$ was considered statistically significant.

2.4. Chemicals

L-NAME, isoproterenol hydrochloride, and acetylcholine chloride from Sigma, and adenosine from Carlo Erba.

3. Results

3.1. Effects of L-NAME

In four goats, intracoronary infusion of L-NAME (30–40 μg min^{-1} kg^{-1} during 15–20 min) reduced the resting coronary blood flow by 14 ± 3% (coronary blood flow was 26 ± 4 vs. 31 ± 4 ml min^{-1}) ($P < 0.05$), without significantly changing either systemic arterial pressure or heart rate.

In another eight goats, i.v. administration of L-NAME reduced the resting coronary blood flow by 19 ± 4% ($P < 0.05$), increased the mean systemic arterial pressure by 22 ± 3% ($P < 0.01$), and decreased heart rate by 10 ± 2% ($P < 0.05$), without significantly changing either blood gases or pH. These effects of L-NAME were partially, but significantly, reversed after L-arginine (six goats). Table 1 summarizes the hemodynamic values and the values for blood gases and pH in anesthetized goats under control conditions and after treatment with i.v. administration of L-NAME and L-NAME plus L-arginine.

3.2. Effects of isoproterenol, adenosine and acetylcholine

Fig. 1 shows actual recordings of the effects of isoproterenol, adenosine and acetylcholine on coronary blood

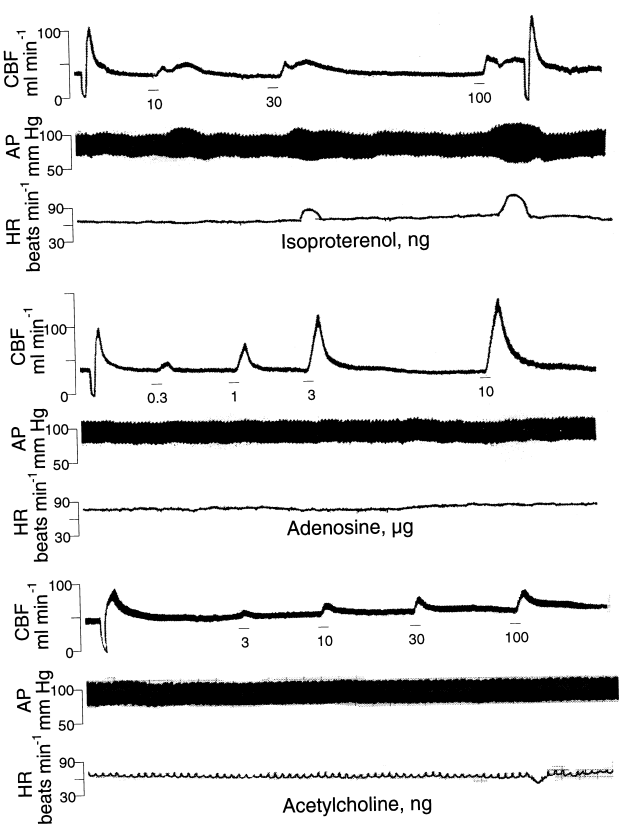

Fig. 1. Recordings showing the effects of isoproterenol (Top), adenosine (Middle) and acetylcholine (Bottom) on coronary blood flow (CBF), systemic arterial pressure (AP) and heart rate (HR) obtained in one anesthetized goat under control conditions.

flow, systemic arterial pressure and heart rate in one anesthetized goat under control conditions. The effects of these substances on coronary vascular conductance under the different conditions tested are summarized in Fig. 2.

Isoproterenol (10–100 ng, eight goats) produced dose-dependent increases in coronary vascular conductance, and also frequently caused increases in systolic arterial pressure and decreases in diastolic arterial pressure without changing significantly the mean systemic arterial pressure; the doses of 30 and 100 ng also increased heart rate by 15% ($P < 0.05$) and 45% ($P < 0.01$), respectively. The increases in coronary blood flow caused by isoproterenol were usually biphasic: an immediate, brief increase, followed by another increase, more sustained than the immediate one. This second increase in coronary blood flow after isoproterenol mostly coincided with the changes in systolic and diastolic arterial pressure and heart rate (Fig. 1). In this study, only the immediate increase in coronary blood flow after isoproterenol was considered, as it was mostly independent of systemic effects. This increase in coronary vascular conductance under control conditions ranged from $32 \pm 5\%$ (10 ng) to $82 \pm 12\%$ (100 ng) for eight animals.

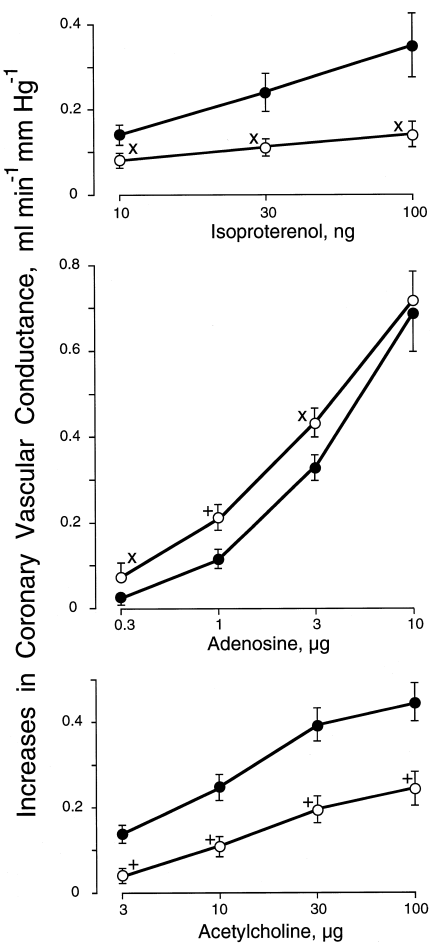

Fig. 2. Summary of the effects produced by isoproterenol (eight goats), adenosine (seven goats) and acetylcholine (five goats) on coronary vascular conductance of anesthetized goats under control conditions (●—●), and after i.v. administration of L-NAME (○—○). $^{+}P < 0.05$ and $^{X}P < 0.01$ compared with its corresponding control.

During the effects of L-NAME (eight animals) the increases in coronary vascular conductance (the immediate increases) caused by isoproterenol, as both percentages and absolute values (Fig. 2), were significantly lower than those under control conditions. In six of these goats, treated with L-NAME plus L-arginine, the effects of isoproterenol on coronary vascular conductance were not significantly different from those found under control conditions (not shown).

The effects on heart rate induced by isoproterenol after L-NAME and L-NAME plus L-arginine were not significantly different from those recorded under control conditions ($P > 0.05$).

Adenosine (0.3–10 μg, seven animals) produced dose-dependent increases in coronary vascular conductance which ranged from $6 \pm 2\%$ (0.3 μg) to $174 \pm 22\%$ (10 μg), and it did not significantly affect either systemic arterial pressure or heart rate. The effects of 0.3–3 μg, but not those of 10 μg of adenosine on coronary vascular conductance after L-NAME (seven animals) were significantly greater than those under control conditions, when expressed as both percentage and absolute values (Fig. 2). In five of these animals treated with L-NAME plus L-arginine, the coronary effects of adenosine were not significantly different from those recorded under control conditions (not shown).

Adenosine did not affect heart rate after L-NAME and after L-NAME plus L-arginine as occurred under control conditions ($P > 0.05$).

Acetylcholine (3–100 ng, five animals) produced dose-dependent increases in coronary vascular conductance ranging from $39 \pm 5\%$ (3 ng) to $145 \pm 15\%$ (100 ng), and it did not significantly change arterial pressure; the doses of 30 and 100 ng also decreased heart rate significantly. During L-NAME (five animals), the increases in coronary vascular conductance, in absolute values, induced by acetylcholine were significantly lower than those under control conditions (Fig. 2). In these five animals treated with L-NAME plus L-arginine the effects of acetylcholine on coronary vascular conductance were not significantly different from those under control conditions (not shown).

The effects of acetylcholine on heart rate after L-NAME and after L-NAME plus L-arginine were comparable to those found under control conditions ($P > 0.05$).

4. Discussion

The endothelium of coronary vessels can release not only nitric oxide but also prostacyclin and EDHF. And these substances, by producing vasodilatation, may modulate coronary vascular tone in large and small coronary arteries. Experimental evidences suggest both that nitric

oxide produces a basal vasodilator tone, thus playing a role in regulating coronary blood flow under resting conditions, and that nitric oxide may be involved in the coronary vasodilatation induced by a number of vasoactive stimuli (Bassenge, 1995). Prostacyclin may play a minor role in the regulation of resting coronary blood flow, but it may be involved in the coronary vasodilatation produced by some vasoactive factors (Bassenge, 1995). EDHF, which may produce coronary vasododilatation, likely by activating potassium channels, may not be an important determinant of resting coronary vascular tone (Bauersachs et al., 1994; Ming et al., 1997) but may mediate the coronary vasodilatation in response to acetylcholine (Feletou and Vanhoutte, 1988; Ming et al., 1997).

An accepted experimental approach for studying the role of nitric oxide in the regulation of coronary circulation under basal and stimulated conditions is the analysis of the effects of some L-arginine analogues on resting coronary blood flow and on the coronary effects of various vasoactive stimuli. The present results show that the L-arginine analogue, L-NAME, administered i.v., decreased resting coronary blood flow, increased systemic arterial pressure and decreased heart rate. Also, these effects were partially reversed by i.v. administration of L-arginine, confirming previous observations from our laboratory (García et al., 1992). Results regarding the effects of inhibition of nitric oxide synthesis on coronary blood flow are unconclusive (Chu et al., 1991; Parent et al., 1992; Quyyumi et al., 1995; Kaneko et al., 1996; Davis et al., 1998), because it is difficult to decide whether inhibition of nitric oxide synthesis or the associated hemodynamic alterations are responsible for the reduction of coronary blood flow after administration of L-arginine analogues (Kirkebøen et al., 1994). This difficulty can be circumvented by injecting intracoronarily the inhibitor of nitric oxide production at relatively low doses, which may affect coronary blood flow without changing systemic arterial pressure and heart rate. We found that intracoronary infusion of L-NAME reduced the resting coronary blood flow and did not alter systemic arterial pressure and heart rate. This was also found by Kirkebøen et al. (1994) in anesthetized pigs after intracoronary administration of another inhibitor of nitric oxide synthesis. Therefore, the reduction of resting coronary blood flow after i.v. administration of L-NAME may result from the inhibition of nitric oxide formation rather than from the associated hemodynamic changes, this being consistent with the idea that nitric oxide may produce a basal vasodilator tone in the coronary circulation (Chu et al., 1991; Quyyumi et al., 1995; Kaneko et al., 1996).

The present results with isoproterenol, adenosine and acetylcholine were analyzed using changes in coronary vascular conductance because these values probably better reflect the in vivo vascular effects, especially when blood flow is the variable mainly affected (Lautt, 1989). Our results with these substances indicate that inhibition of nitric oxide synthesis diminished the coronary vasodilatation in reponse to acetylcholine and to β-adrenoceptor stimulation with isoproterenol, and that it potentiated the coronary vasodilatation in response to adenosine. Also, the results showed that these effects were reversed by L-arginine. The present results with acetylcholine add to previous results from our laboratory (García et al., 1992) and agree with the idea that the coronary vasodilatation caused by this neurotransmitter is mediated in part by the release of nitric oxide (Chu et al., 1991; Parent et al., 1992; Kaneko et al., 1996). There are many studies showing that acetylcholine, and vagal stimulation, produce coronary vasodilatation (Feigl, 1983), and it has also been reported that acetylcholine is one of the most powerful stimulators of nitric oxide production in several vascular beds, including coronary vessels (Moncada et al., 1991; Bassenge, 1995). Although epicardial vasoconstriction in response to acetylcholine has been reported (Hodgson and Marshall, 1989; Van Winkle and Feigl, 1989), an accurate assessment of the effects of acetylcholine on the coronary circulation requires determination of coronary blood flow, as the net effect of acetylcholine may depend on the interplay between direct vasoconstriction and endothelium-dependent vasodilatation (Hodgson and Marshall, 1989). Therefore, although in our experiments, acetylcholine may have crossed the endothelium to reach the smooth muscle and may have caused constriction of epicardial arteries, the net effect of the doses of acetylcholine we used was vasodilatation, as indicated by the increases in coronary blood flow. As what occurred with acetylcholine, L-NAME also inhibited the coronary vasodilatation in response to isoproterenol, and this inhibitory effect was reversed by L-arginine. Thus, it is suggested that nitric oxide formation from L-arginine may also participate in the coronary vasodilatation caused by isoproterenol, and that a common pathway may be involved in the coronary vasodilatation induced by both acetylcholine and isoproterenol. This agrees with the report by Parent et al. (1993) and Kaneko et al. (1996) from experiments with conscious dogs. As the coronary effects of acetylcholine and isoproterenol were partially blocked by L-NAME, it is possible that the blockade of nitric oxide synthesis was incomplete, or that other mediators distinct from nitric oxide are also involved in the coronary vasodilatation induced by these two drugs. As acetylcholine may produce coronary vasodilatation by releasing both nitric oxide and EDHF, it is probable that the observed increases in coronary blood flow caused by acetylcholine and resistant to L-NAME treatment may be related at least in part to the release of EDHF. With regard to isoproterenol, it must be considered that the observed increases in coronary blood flow after this drug may also be due in part to the vasodilatation caused by the probable concomitant increase of myocardial metabolism, and this metabolic effect of isoproterenol may not be affected by L-NAME. The probability that the reductions of coronary effects of acetylcholine and isoproterenol after L-NAME are related to blockade of a flow-dependent process involv-

ing nitric oxide release (Kuo et al., 1990) is small as the effects were not observed with adenosine, where a flow-dependent process involving nitric oxide formation should be also present. Therefore, the coronary vasodilatation caused by acetylcholine and isoproterenol is more probably related to the release of nitric oxide through a mechanism coupled directly to activation of muscarinic and β-adrenoceptor receptors, respectively. β-Adrenoceptor stimulation may produce endothelium-dependent relaxation, probably mediated by nitric oxide, in isolated canine coronary arteries (Rubanyi and Vanhoutte, 1985). Based on results in conscious dogs, Parent et al. (1993) suggested that because of similarities of the coronary responses to acetylcholine and to isoproterenol regarding their inhibition by L-NAME, and their reversibility with L-arginine, β-adrenoceptor dilatation of resistance coronary vessels most likely involves endothelium-dependent nitric oxide. The idea that nitric oxide is a mediator of coronary vasodilatation in response to β-adrenoceptor stimulation has been not confirmed in other studies, as it has been reported that the effects of dobutamine on canine coronary blood flow (Crystal et al., 1998) and of isoproterenol on the relaxation of large canine coronary arteries (Béa et al., 1994) were not affected after inhibition of nitric oxide production or after endothelium removal. This discrepancy may be related to differences in experimental preparations and approaches.

Adenosine is another important coronary vasodilator which has been involved in metabolic coronary vasodilatation (Feigl, 1983). Previous reports show that larger coronary arteries are less sensitive than smaller coronary arteries to adenosine (Harder et al., 1979) and that endocardial microvessels are more sensitive than epicardial microvessels to this substance (Quillen and Harrison, 1992). It has also been reported that adenosine may produce coronary vasodilatation by activation of adenosine A_2 receptors (Olsson and Pearson, 1990) and that these receptors may be located in the coronary vascular endothelium (Abebe et al., 1994). The role of nitric oxide in the coronary vasodilatation produced by adenosine is controversial (Parent et al., 1992; Quillen and Harrison, 1992; Canty and Schwartz, 1994; Kirkebøen et al., 1994; Quyyumi et al., 1997; Davis et al., 1998; Tayama et al., 1998). Our present data suggest that inhibition of nitric oxide production may potentiate the adenosine-induced coronary vasodilatation, which is consistent with results of studies of the human coronary circulation (Shiode et al., 1996). Shiode et al. (1996) found that infusion of adenosine into the human distal left anterior descending coronary artery produced increases in coronary blood flow, which were greater after inhibition of nitric oxide production than under control conditions. The results of other studies, however, suggest that nitric oxide is not involved in the adenosine-induced coronary vasodilatation of conscious dogs (Canty and Schwartz, 1994), anesthetized pigs (Kirkebøen et al., 1994) or patients (Quyyumi et al., 1997), or that nitric oxide may be a mediator of the coronary vasodilatation to adenosine in anesthetized (Parent et al., 1992; Tayama et al., 1998) and conscious (Davis et al., 1998) dogs. The reason for this discrepancy is not apparent, and it may lie in differences in species and/or in the responses of conductance and resistance vessels (Kirkebøen et al., 1994). Experiments with dogs show that intracoronary administration of L-NAME (Matsunaga et al., 1996) or feeding a diet supplemented with L-NAME (Tayama et al., 1998) increases adenosine production in the coronary circulation. Results of these two studies (Matsunaga et al., 1996; Tayama et al., 1998) suggested that the increased adenosine production may play a compensatory role in the regulation of coronary circulation when the release of nitric oxide is decreased. This compensatory role of adenosine may also involve increased sensitivity of coronary vessels to the nucleoside, as suggested by the present results.

As L-NAME, in addition to inhibiting nitric oxide synthases, may also antagonize muscarinic receptors (Buxton et al., 1993), this antagonism may have interfered with the blocking effects of L-NAME on the response of coronary blood flow to the stimuli now used, specially acetylcholine. This, however, may be less probable, as non-esterified L-arginine analogues, which do not produce muscarinic antagonism (Buxton et al., 1993), also inhibit the vasodilatation in response to acetylcholine (Moncada et al., 1991). In addition, we have observed that, in anesthetized goats, the non-esterified L-arginine analogue, N^{w}-nitro-L-arginine (L-NA), also reduced the effects of acetylcholine and of isoproterenol on coronary blood flow as did L-NAME (unpublished observations).

In conclusion, the present data suggest that: (1) under normal conditions, nitric oxide may produce a basal vasodilator tone in the coronary circulation; (2) nitric oxide may mediate, in part, the coronary vasodilatation induced by β-adrenoceptor stimulation and acetylcholine, and (3) the sensitivity of coronary vessels to adenosine may increase when nitric oxide production is reduced.

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