—Report on Clinics and Experiments—

Does inhibition of coronary nitric oxide synthesis alter coronary vascular tone in normal dogs?

Takao Endo¹⁾, Haruo Kaneko¹⁾, Kaname Kiuchi¹⁾,
Shinsuke Fujita¹⁾, Takeshi Yamamoto¹⁾, Gen Takagi¹⁾,
Naoto Takahashi¹⁾, Kuniya Asai¹⁾, Ikuyo Suzuki¹⁾,
Jun Najima¹⁾, Yoshihiro Suzuki²⁾
and Hirokazu Hayakawa¹⁾

¹⁾First Department of Internal Medicine, Nippon Medical School

²⁾Radionuclear Laboratory Research Center, Nippon Medical School

Summary

To examine whether endothelial nitric oxide formation contributes to the vascular tone of resistance coronary vessels in vivo, we administered N^G-nitro-L-arginine methyl ester (L-NAME) (10 and 100 μ g/kg/min), a nitric oxide synthase inhibitor, as well as D-enantiomer into the left circumflex artery in normal dogs. Intracoronary L-NAME, which was associated with dose-related reductions in acetylcholine-induced coronary vasodilation, significantly reduced the baseline left circumflex blood flow by 6% and increased coronary vascular resistance of the left circumflex artery by 6%. D-enantiomer was ineffective in altering baseline coronary blood flow and vascular resistance of the left circumflex artery. These results indicate that continuous nitric oxide formation in the vasculature is important in the regulation of the coronary vascular tone of resistance vessels in vivo, and serves to maintain the vessels in a dilated state.

Key words: nitric oxide, coronary blood flow, coronary vascular resistance, L-NAME, D-NAME

Introduction

It is now acknowledged that nitric oxide (NO), formed enzymatically by the action of NO synthase from L-arginine, is a physiologic regulator molecule in various tissues. NO synthase is known to exist in at least three distinct isoforms, i.e., inducible, endothelial, and neuronal^{1~4)}. Endothelium-derived NO is a major physiologic regulator of vascular tone in many organs^{1,2,4)}. In vitro studies performed in rabbits or rat aortic rings or in isolated perfused rabbit or guinea pig hearts have shown that inhibition of endogenous NO synthesis causes vasoconstriction in these preparations, and that NO is continuously produced in the endothelium^{5~9)}. However, whether NO continuously produced in the endothelium of resis-

tance coronary vessels regulates basal coronary blood flow (CBF) and coronary vascular resistance (CVR) in vivo remains unsettled¹⁰⁻¹⁶.

The goal of this study was to determine whether coronary NO synthesis regulates basal CBF and CVR as well as their responses to acetylcholine in intact canine hearts.

Materials and Methods

1. *Experimental preparations*

Thirteen healthy adult mongrel dogs of both sexes (16.7 ± 1.3 kg) were anesthetized with pentobarbital sodium (30 mg/kg IV), and the lungs were artificially ventilated with room air. The heart was exposed by a left thoracotomy in the fifth intercostal space and suspended in a pericardial cradle. A polyethylene catheter was placed in the left carotid artery for monitoring of arterial pressure. A micromanometer-tipped catheter was inserted into the left ventricle (LV) through an apical stab wound to measure LV pressure and to obtain the first derivative of LV pressure over time, LV dP/dt. Transonic flowmeters (Transonic Systems Inc, Ithaca, NY) were implanted around each coronary artery, 2~3 cm downstream from the bifurcation of the main left coronary artery to measure left circumflex and left anterior descending CBFs. A polyethylene catheter with an external diameter of 0.6 mm was placed in the left circumflex artery proximal to the flow probe, and its tip was positioned in the lumen 1~2 cm downstream from the bifurcation of the main left coronary artery for the intracoronary injections of the drugs. Hemodynamic variables and electrocardiographic lead II were recorded continuously throughout the experiments.

2. *Experimental protocol*

Heart rate, phasic and mean arterial blood pressures, LV pressure, LV dP/dt, mean left circumflex and left anterior descending CBFs were recorded continuously. The intracoronary bolus doses of acetylcholine chloride (Daiichi Pharmaceutical Co., Ltd, Tokyo) ($0.003 \mu\text{g/kg}$) and nitroglycerin (Nippon Kayaku Co., Ltd, Tokyo) ($0.175 \mu\text{g/kg}$) were injected into the left circumflex artery. Intracoronary administration of the same amount of saline used to dissolve acetylcholine and nitroglycerin was examined to verify that there were no changes in left circumflex CBF. In eight dogs, after baseline hemodynamics had been achieved, we infused 10 and $100 \mu\text{g/kg/min}$ N^G-nitro-L-arginine methyl ester (L-NAME) (Wako Pure Chemical Industries, Ltd, Tokyo), a specific inhibitor of NO synthesis, for 10 minutes into the left circumflex artery and repeated the same procedure. Ten minutes were allowed after the end of L-NAME administration before the drug injections. These doses of L-NAME also resulted in stable inhibition of acetylcholine-induced coronary vasodilation for the duration of the experiments. We infused the drugs directly into the coronary artery to avoid the confounding hemodynamic influences associated with systemic administration. To verify that the alterations with L-NAME were due to specific inhibition of NO synthesis, an identical experimental procedure was performed with N^G-nitro-D-arginine methyl ester (D-NAME) (Wako Pure Chemical Industries, Ltd, Tokyo), an inactive enantiomer, in the same low and high doses in the remaining five dogs.

Myocardial work was estimated by the triple product, the product of heart rate, LV systolic pressure and peak LV dP/dt. CVR was calculated as the ratio of mean arterial pressure to mean CBF.

3. Statistical analysis

All data are mean $\pm$ SEM. Comparisons of baseline levels of coronary vascular and systemic variables as well as responses of CBF and CVR to acetylcholine and nitroglycerin before and after L-NAME or D-NAME in a low and high dose were performed with Student's t test for paired data. A value of $p < 0.05$ was considered indicative of a significant difference.

Results

Experiments with L-NAME were conducted in eight dogs. In one dog, the intracoronary bolus dose of acetylcholine was not examined because marked bradycardia developed during intracoronary acetylcholine. Experiments with D-NAME were initiated in five dogs. In one of the five dogs, CBF was not measured for technical reasons.

1. Effects on coronary vascular and systemic variables

Intracoronary L-NAME in a low dose ($10 \mu\text{g/kg/min}$ for 10 min; $n=8$) significantly decreased left circumflex CBF by $6 \pm 1\%$ from a baseline of $34.1 \pm 5.6 \text{ ml/min}$ and

Fig. 1 Effects of L-NAME (left) and D-NAME (right) in a low (open circles; $10 \mu\text{g/kg/min}$) and high dose (closed circles; $100 \mu\text{g/kg/min}$) on left circumflex coronary blood flow (CBF-LCX), left circumflex coronary vascular resistance (CVR-LCX) and mean arterial pressure (MAP). All values are mean $\pm$ SEM. * $p < 0.05$ as compared with values before the drug in a low and high dose.

increased left circumflex CVR by $6 \pm 1\%$ from 3.68 ± 0.67 mmHg/ml/min, demonstrating tonic basal release of NO from the coronary microvessels (**Fig. 1; left**). L-NAME in a high dose ($100 \mu\text{g/kg/min}$ for 10 min; $n=8$) did not further alter left circumflex CBF and CVR. Mean arterial pressure did not change after L-NAME in a low and high dose. Heart rate decreased by $2 \pm 1\%$ from 143 ± 8 beats/min at the low dose, and LV systolic pressure increased by $7 \pm 2\%$ from 114 ± 7 mmHg, and LV end-diastolic pressure increased by $6 \pm 2\%$ from 6 ± 1 mmHg at the high dose of L-NAME, while the triple product remained unchanged. CBF and CVR of the left anterior descending artery did not change after L-NAME in a low or high dose. D-NAME in low and a high dose ($n=5$) had no effects on coronary vascular and systemic variables (**Fig. 1; right**).

2. Effects on coronary vascular responses to acetylcholine (**Fig. 2; top**)

Intracoronary bolus injection of acetylcholine ($n=7$) increased left circumflex CBF by $94 \pm 16\%$ from a baseline of 31.9 ± 6.7 ml/min and reduced CVR by $47 \pm 5\%$ from 4.32 ± 0.75 mmHg/ml/min. Because baseline CBF and CVR were significantly altered after L-NAME, their changes with acetylcholine and nitroglycerin were expressed as percent change from baseline and compared before and after L-NAME in low and a high dose. Thus, the acetylcholine-induced increase in peak left circumflex CBF was $48 \pm 3\%$ ($p<0.05$) after a low dose and $47 \pm 16\%$ ($p<0.05$) after a high dose of L-NAME. The acetylcholine-induced reduction in CVR was $36 \pm 5\%$ at the low dose and $30 \pm 5\%$ at the high dose of L-NAME.

Fig. 2 Effects of increasing doses of L-NAME (open circles) and D-NAME (closed circles) on the percent changes in the left circumflex coronary blood flow (CBF-LCX) with intra-coronary acetylcholine (top) or nitroglycerin (bottom). All values are mean $\pm$ SEM. * $p<0.05$ as compared with control.

The responses of left circumflex CBF and CVR to acetylcholine were not attenuated after D-NAME (n=4).

3. *Effects on coronary vascular responses to nitroglycerin (Fig. 2; bottom)*

Intracoronary bolus injection of nitroglycerin (n=8) increased the peak left circumflex CBF by $79 \pm 7\%$ from a baseline of 33.9 ± 6.5 ml/min and reduced CVR by $44 \pm 2\%$ from 4.10 ± 0.80 mmHg/ml/min. L-NAME in a low and high dose did not change their responses to nitroglycerin. Thus, at the low dose of L-NAME, the increase in CBF was $85 \pm 9\%$ and the decrease in CVR was $46 \pm 3\%$. At the high dose, the CBF increase was $96 \pm 19\%$ and the CVR reduction was $47 \pm 5\%$. D-NAME in a low and high dose (n=4) did not alter their responses to nitroglycerin.

Discussion

1. *Effects of inhibition of NO synthesis*

Our results showed that intracoronary L-NAME, which was associated with dose-related reductions in acetylcholine-induced coronary vasodilation, significantly reduced baseline CBF and increased CVR. The specificity of blockade of NO formation with L-NAME was demonstrated by the findings that D-NAME was ineffective in altering baseline CBF as well as CVR. This indicates that tonic release of NO from the microvasculature under resting conditions contributes to basal coronary microvascular tone, and that at least 6% of resting coronary microvascular tone is due to NO.

2. *Methodologic considerations*

The finding that the flow response to acetylcholine was not completely abolished may be due to incomplete inhibition of NO formation by the dose of L-NAME used in this study, or may suggest that other vasodilating factors are involved in the response to acetylcholine. The increase in CBF with nitroglycerin, an endothelium-independent vasodilator, before and after L-NAME in a low and high dose was similar, suggesting that the effect of L-NAME on the coronary microvessels is specific for inhibiting the endothelium-dependent effects of acetylcholine. We did not administer L-arginine to reverse the inhibition of NO synthesis with L-NAME, because a high dose of L-arginine has been reported to produce nonspecific vasodilation¹⁶⁾. Instead, D-NAME was used to demonstrate the specificity of inhibition of NO synthesis by L-NAME in this study.

3. *Comparison with previous studies*

Although Chu and co-workers showed in intact awake dogs that NO modulated local epicardial coronary vasomotor tone¹⁴⁾, the question of whether continuous NO production in the endothelium of resistance coronary vessels regulates basal CBF in vivo remains unsettled¹⁰⁻¹⁶⁾. Most of the studies conducted in anesthetized or conscious dogs showed that baseline CBF was not altered after intracoronary administration of NO synthase inhibitors¹⁰⁻¹³⁾. In the present study, intracoronary L-NAME in a low dose significantly

decreased baseline CBF and increased CVR, whereas systemic hemodynamic alterations were minimal, myocardial work estimated by the triple product remained unchanged, and compressive determinants of diastolic coronary perfusion decreased. Although CBF remained reduced after L-NAME in a high dose, its effects on CBF were difficult to address because of changes in systemic hemodynamics and compressive determinants of coronary flow. A study by Kirkebøen and colleagues in anesthetized pigs provided support for our conclusion, showing that NO blockade by intracoronary infusion of NO synthase inhibitors reduced basal CBF by 16%¹¹⁾. Recently, Quyyumi and associates showed that NO contributed importantly to resting epicardial and coronary microvascular tone in the human coronary circulation¹⁷⁾. Although the reason for the disparity between our results and those reported previously regarding the effect of the inhibition of coronary NO synthesis on CBF is not known, it is likely to be a result of different levels of inhibition of endogenous NO production due to differences in the doses and the arginine analogues used¹⁵⁾. Thus, we conclude that continuous NO formation is important in the regulation of baseline coronary vascular tone of resistance vessels *in vivo*, and serves to maintain the vessels in a dilated state.

4. Summary

The present study has shown that constitutive release of NO from resistance coronary vessels is an important mechanism responsible for regulating myocardial perfusion under normal conditions. Acetylcholine-induced coronary vasodilation is largely due to the release of NO from coronary microvessels. Abnormalities in the endothelial NO system have been shown in cardiovascular diseases, including atherosclerosis and hypertension^{1,2,4)}. Thus, our findings indicate that impaired NO-mediated coronary resistance adjustments under resting as well as stimulated states can lower the ischemic threshold in these conditions.

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