

The Effects of Modulation of the L-Arginine-Nitric Oxide Pathway on Myocardial Stunning Following Repetitive Coronary Occlusion in Dogs

Nobuyuki MORISHIMA

The First Department of Internal Medicine, Hiroshima University School of Medicine, 1-2-3 Kasumi, Minami-ku, Hiroshima, 734, Japan

ABSTRACT

In order to determine the role of nitric oxide (NO) in myocardial stunning, the effects of both augmenting and inhibiting NO production on contractile function, following repetitive coronary occlusions, were evaluated in anesthetized dogs. The effect of the experimental protocol on endothelial function was also assessed. The increases in coronary blood flow in response to acetylcholine and nitroglycerin at 30 and 60 min after reperfusion were similar to those before coronary occlusions. Therefore, loss in vasodilator reserve was not observed following the multiple coronary occlusions used in this study. N^G-nitro-L-arginine methyl ester (L-NAM-E) elevated blood pressure slightly, but did not change left ventricular end-diastolic pressure, left ventricular maximum positive dp/dt, and coronary blood flow. Although the degree of systolic bulging and collateral circulation during coronary occlusions was comparable to the control group, contractile function after reperfusion was significantly worse in the presence of L-NAME than in the control. The recovery of contractile function was also considerably delayed with administration of L-arginine. This deleterious effect on contractile function was not observed with its enantiomer D-arginine. Differences in collateral blood flow determined with microspheres and hemodynamic variables did not account for the effects of L-arginine. These results suggest that endogenous NO is important in limiting myocardial stunning following repetitive coronary occlusion. However, NO may be cytotoxic when present in substantial excess.

Key words: *Myocardial stunning, Nitric oxide, Arginine*

Advances in the study of mechanisms of myocardial injury in ischemia and reperfusion have made it possible to decrease myocardial damage by pharmacological interventions^{4,20}. Recently, evidence of the important role of nitric oxide (NO) in ischemia-reperfusion has been presented⁹, and there are attempts to inhibit reperfusion injury by manipulation of NO. However, previous reports have shown inconsistent results on the role of NO in the ischemic insult. NO is synthesized from L-arginine (L-arg) and the latter is converted to L-citrulline by enantiomer-specific NO synthase (NOS)²⁴. Synthesis is blocked by L-arg analogues such as N^G-nitro-L-arginine methyl ester (L-NAME) and this antagonism can be reversed by exogenous L-arg, but not by D-arginine (D-arg).

The importance of endothelial NO release has been demonstrated in previous studies which indicate that administration of NO donor²⁷ or L-arg³¹ reduced endothelial damage and myocardial necrosis. Conversely, NO may also be cytotoxic^{2,9}, as an inhibitor of NOS has been reported to reduce both myocardial re-oxygen-

ation injury²¹ and cerebral reperfusion injury⁶.

Most prior studies have focused attention on NO manipulation and its resultant salutary protective effect against prolonged ischemic insult. However, little is known on the possible consequences of NO modulation on the development of reperfusion injury following brief periods of ischemia insufficient to induce myocardial necrosis (myocardial stunning).

In order to determine the role of NO in the development of myocardial stunning, the influence of administering the substrate and the inhibitor of NOS on contractile dysfunction, following repetitive brief coronary artery occlusions, was evaluated. A multiple-occlusion regimen rather than a single brief one was selected, because recurrent ischemia may commonly be observed in patients with unstable angina pectoris, during coronary artery bypass surgery, and during repeated coronary dilation with transluminal angioplasty.

MATERIALS AND METHODS

Surgical Preparation

Adult mongrel dogs of either sex weighing 13 to 22 kg were sedated with ketamine hydrochloride (100 mg IM) and anesthetized with sodium pentobarbital (30 mg/kg IV). The dogs were then intubated and ventilated with room air by a Harvard ventilator. Ventilatory parameters were adjusted to maintain normal pH and satisfactory oxygenation of the blood. Cannulas were inserted into the right femoral vein for the administration of fluids and into the right femoral artery for the measurement of arterial pressure. Left thoracotomy was then performed at the fifth intercostal space and the heart was suspended in the pericardial cradle. A micromanometer-tipped pressure transducer (SPC 360, Millar Instruments, Houston, TX) was inserted into the left ventricle via a stab wound in the apical dimple (Fig. 1). The left atrium was cannulated for the infusion of drugs and for injection of colored microspheres to measure regional myocardial blood flow. A segment of the left anterior descending coronary artery (LAD) just distal to its first major diagonal branch was isolated for placement of an electromagnetic flow probe (FJ-020T, Nihon Kohden, Tokyo) to measure mean coronary blood flow (CBF). Two pairs of ultrasonic crystals were implanted in the mid-myocardium parallel to the minor axis (10–15 mm apart), one pair within the area perfused by the LAD and the other in the area perfused with the circumflex artery (Cx). Coronary occlusions were performed by occluding the LAD immediately distal to the flow probe using a hydraulic occluder. Full reactive hyperemia was allowed during the reperfusion period. Standard lead II of the electrocardiogram was recorded for heart rate (HR) determination.

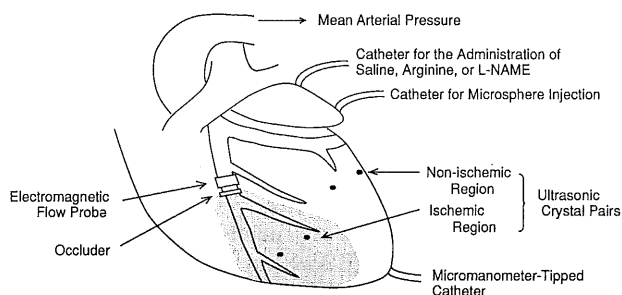


Fig. 1. Schematic representation of the surgical preparation. Repetitive occlusions of the left anterior descending coronary artery were produced by tightening the occluder. Two pairs of ultrasonic crystals implanted within ischemic and non-ischemic regions were used to measure contractile function. L-NAME, N^G nitro-L-arginine methyl ester.

Experimental protocol

The experimental protocol is summarized in Fig. 2. After a 30 min stabilization period, baseline systemic and coronary hemodynamics were obtained. Dogs were randomized into four groups ($n = 9$ for each group): control, L-arg, D-arg, and L-NAME groups. The first colored microsphere injection was performed prior to the randomization. All dogs were subjected to four 5 min episodes of left anterior descending coronary artery occlusion alternating with 5 min of reperfusion followed by a 60 min reperfusion period. The control group received an infusion of 0.9% saline (0.1 ml/kg/min) via the left atrium, and the treatment groups received L-arg (10 mg/kg/min), or its enantiomer D-arg (10 mg/kg/min) beginning 5 min before the first occlusion and continuing through 5 min of the final reperfusion period. The dose of arginine was based on the previous report by Girerd et al¹³. The L-NAME group received a slow infusion of the drug (1 mg/kg) to avoid acute hemodynamic changes beginning 5 min before the first coronary occlusion. A second microsphere injection was performed during the first coronary occlusion period. Systemic and coronary hemodynamics were measured after 3 min during each occlusion or reperfusion period. The third microsphere injection was performed at 60 min of reperfusion following the last coronary occlusion. Of 36 dogs entered in the protocol, five dogs that died from ventricular fibrillation, four dogs that had high collateral flow or lack of epicardial cyanosis during coronary artery occlusion, and one additional dog had small risk area (<10%) were excluded. Thus, a total of 26 dogs (control group 7, L-arg 7, D-arg 6, and L-NAME 6) were included in the final analysis.

Whether the repetitive brief coronary artery

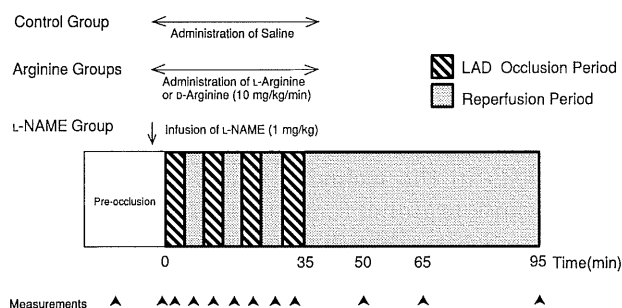


Fig. 2. Time-course of coronary occlusions and reperfusions. Arrow heads indicate the time of measuring hemodynamic parameters, coronary flow, and regional contractile functions. Measured hemodynamic parameters are as follows: heart rate, mean arterial pressure, left ventricular end-diastolic pressure and left ventricular dp/dt. L-NAME, N^G nitro-L-arginine methyl ester.

occlusions used in this study would impair endothelial function to the degree of prolonged ischemia^{15,26,28)} was evaluated by measuring coronary vasodilator reserve at baseline, and 30 and 60 min after the final occlusion in the 6 dogs (body weight 13–15 kg). These dogs had been cannulated in the jugular vein and acetylcholine (0.01 mg) and nitroglycerin (0.05 mg) were injected in randomized order over 10 sec into the jugular vein. The hemodynamic parameters and coronary blood flow were measured. One dog was excluded from this vasodilator reserve trial because epicardial cyanosis was not observed during coronary occlusion. Thus, data presented are for the remaining 5 dogs.

Data analysis

At each measurement point, HR, mean arterial pressure (MAP), peak left ventricular pressure, left ventricular end-diastolic pressure (LVEDP), mean coronary blood flow (CBF), left ventricular (LV) dp/dt and segment lengths were recorded. Data from a representative cycle of 3 to 5 consecutive cardiac cycles were taken for each point and averaged. Regional segment lengths were determined at end-diastole and end-systole. The end-diastolic length (EDL) was measured at the onset of the positive deflection in the dp/dt signal. The end-systolic length (ESL) was measured at peak negative dp/dt. Myocardial segment shortening (SS), an index of regional contractile function, was defined as $(EDL - ESL) / EDL \times 100$. The values were normalized and expressed as a percentage of their pre-occlusion baseline values.

At the end of each experiment, the perfusion territory of the LAD was visualized by intracoronary injection of methylene blue immediately before killing the dogs by injection of potassium chloride. All hearts were cut into 5 to 7 transverse slices, parallel to the atrioventricular groove. Correct placement of the ultrasonic crystals within the middle of the myocardium was visually verified. Epicardial and endocardial contours of the basal surfaces of the heart slices and margins of the area at risk were traced onto ace-

tate sheets. Heart slices were weighed after right ventricular tissue was trimmed off. The extent of the area at risk in each heart slice was quantified by computed planimetry and corrected for the weight of the tissue slice. Total weight of the area at risk was then calculated for each heart and expressed as a percentage of the total left ventricular weight.

Measurement of regional myocardial blood flow

Regional myocardial blood flow (RMBF) was measured using 15 μ m colored microspheres (Dye-Trak, Triton Technologies Inc. San Diego, CA.). For each measurement, approximately 7×10^6 microspheres suspended in 6 ml of saline (containing 0.02% Tween 80) were injected into the left atrium. Each microsphere injection was followed by a flush of 6 ml of saline. The withdrawal of arterial reference blood samples was started 10 s before injection of the microspheres and continued for 100 s at a rate of 6.3 ml/min (CVF-3100, Nihon Kohden, Tokyo). After completion of the study, the heart was subdivided into subepicardial, mid-myocardial, and subendocardial segments, and the tissue samples were weighed. Colored microspheres were extracted from the tissue samples and the reference blood by digestion with potassium hydroxide (4 M) and subsequent microfiltration. The dyes were removed from the microspheres by adding 0.1 ml dimethylformamide, and the photometric absorption was determined by spectrophotometer (UV-1200, Shimadzu, Kyoto). The composite spectrum of each dye solution was resolved into the spectra of the single constituents using a preprogrammed IBM computer (Miss, Triton Technologies Inc. San Diego, CA.). RMBF was calculated from the equation $Q_m = Q_r \times A_m / A_r$ where Q_m is the RMBF, Q_r is the rate of withdrawal of the reference blood flow samples (6.3 ml / min), A_r is the specific absorbance of reference blood flow sample, and A_m is that of tissue sample. Transmural blood flow was calculated as the weighted average of all the samples in the respected

Table 1. Changes of coronary blood flow and mean arterial pressure in response to intravenous injections of acetylcholine and nitroglycerin

	Before drug injection		Maximum response to 0.01 mg of acetylcholine		Maximum response to 0.05 mg of nitroglycerin	
	CBF (ml/min)	MAP (mmHg)	CBF (ml/min)	MAP (mmHg)	CBF (ml/min)	MAP (mmHg)
Pre-occlusion	17.5 $\pm$ 6.8	107 $\pm$ 16	32.9 $\pm$ 11.9*	83 $\pm$ 8*	25.8 $\pm$ 8.9*	89 $\pm$ 12*
30 min after R	21.1 $\pm$ 9.3	105 $\pm$ 16	34.0 $\pm$ 12.5*	89 $\pm$ 15*	28.9 $\pm$ 8.8*	86 $\pm$ 16*
60 min after R	20.3 $\pm$ 8.2	101 $\pm$ 11	34.5 $\pm$ 11.4*	82 $\pm$ 4*	30.2 $\pm$ 9.2*	81 $\pm$ 9*

CBF, coronary blood flow ; MAP, mean arterial pressure ; R, reperfusion

*p < 0.05 vs corresponding values before drug injection

Table 2. Hemodynamic Variables in the control, L-arginine, D-arginine, and N^Gnitro-L-arginine methyl ester groups

	Baseline	After L-NAME	Occlusion 1	Reperfusion 1	Occlusion 4	15 min	30 min	60 min
HR, beats/min								
Control (7)	131 ± 12		137 ± 11	138 ± 14	138 ± 14	137 ± 18	138 ± 20	140 ± 19
L-arginine (7)	141 ± 17		145 ± 14	144 ± 14	146 ± 11	148 ± 11	147 ± 12	139 ± 15
D-arginine (6)	148 ± 19		145 ± 16	143 ± 16	144 ± 18	138 ± 17	136 ± 13	139 ± 14
L-NAME (6)	142 ± 15	138 ± 11	141 ± 11	144 ± 13	141 ± 13	140 ± 13	139 ± 20	145 ± 24
MAP, mmHg								
Control (7)	96 ± 11		92 ± 11	96 ± 11	88 ± 11	95 ± 11	99 ± 17	110 ± 17
L-arginine (7)	108 ± 12		102 ± 13	103 ± 11	100 ± 19	110 ± 13	109 ± 13	111 ± 12
D-arginine (6)	100 ± 15		93 ± 9	99 ± 10	102 ± 10	109 ± 10	108 ± 10	110 ± 10
L-NAME (6)	108 ± 10	120 ± 10*#	117 ± 10*#	127 ± 9*#	119 ± 7*#	122 ± 9*#	123 ± 5*#	118 ± 6
LVEDP, mmHg								
Control (7)	4 ± 1		7 ± 2	6 ± 2	8 ± 4	6 ± 3	6 ± 3	6 ± 3
L-arginine (7)	5 ± 1		7 ± 1	5 ± 2	6 ± 2	5 ± 1	5 ± 1	6 ± 1
D-arginine (6)	3 ± 1		6 ± 3	4 ± 3	6 ± 2	5 ± 2	5 ± 1	5 ± 1
L-NAME (6)	4 ± 1	4 ± 1	7 ± 1	5 ± 2	8 ± 1	6 ± 1	5 ± 1	5 ± 1
LV dp/dt, mmHg/s/100								
Control (7)	2.0 ± 0.2		1.8 ± 0.1	2.1 ± 0.3	1.9 ± 0.4	1.9 ± 0.4	1.9 ± 0.4	2.0 ± 0.5
L-arginine (7)	1.9 ± 0.3		1.8 ± 0.4	1.9 ± 0.3	1.9 ± 0.6	1.9 ± 0.4	1.9 ± 0.4	1.8 ± 0.4
D-arginine (6)	2.2 ± 0.5		1.9 ± 0.5	1.9 ± 0.3	1.8 ± 0.3	1.7 ± 0.3	1.8 ± 0.4	1.8 ± 0.3
L-NAME (6)	2.0 ± 0.1	2.0 ± 0.2	2.1 ± 0.2	2.1 ± 0.1	2.2 ± 0.3	1.9 ± 0.1	1.8 ± 0.1	1.8 ± 0.2
CBF, ml/min								
Control (7)	16 ± 8			61 ± 36		19 ± 8	19 ± 7	19 ± 7
L-arginine (7)	11 ± 2			31 ± 15		14 ± 2	14 ± 2	15 ± 4
D-arginine (6)	12 ± 8			39 ± 35		17 ± 10	14 ± 10	16 ± 10
L-NAME (6)	16 ± 5	16 ± 5		58 ± 24		16 ± 6	16 ± 5	15 ± 6

HR, heart rate; MAP, mean arterial pressure; LVEDP, left ventricular end-diastolic pressure; CBF, coronary blood flow; L-NAME, N^Gnitro-L-arginine methyl ester.

#p<0.05 vs before L-NAME *p<0.05 vs Control Numbers in the parenthesis indicate the number of dogs examined in the experiment

region. The flow deprivation (FD) during the first coronary occlusion period of the mid-myocardial layer where the ultrasonic crystals were located was calculated as follows:

FD = 1 - mid-myocardial blood flow in LAD / mid-myocardial blood flow in Cx

Drugs

Acetylcholine, L-arg, D-arg, L-NAME, dimethylformamide, and Tween 80 were purchased from Sigma Chemical Co., St. Louis, MO. Nitroglycerin was obtained from Nihon Kayaku, Tokyo.

Statistical Analysis

Data are expressed as a mean $\pm$ SD. Groups were compared using an analysis of variance with Scheffe's F test. Student's t tests for paired data were used to compare the measured variables before and after L-NAME infusion and for the response to vasodilator substances before and after coronary occlusion. CBF and vasodilatory responses before coronary occlusions vs 30 and 60 min after the reperfusion period were assessed by paired t tests, respectively. A probability level of < 0.05 was considered significant.

RESULTS

Vasodilator reserve trial

In vasodilator reserve trial, MAP was 107 ± 16 mmHg at baseline and remained unchanged 30 and 60 min after reperfusion. Injection of acetylcholine and nitroglycerin decreased MAP. There was a trend toward a modest increase in CBF after coronary occlusion but there was no statistical significance. The increases in CBF in response to acetylcholine at 30 and 60 min after reperfusion were similar to that before coronary occlusions. The response to nitroglycerin also remained constant during the experiment. Thus, loss in vasodilator reserve was not observed after repeated coronary occlusions (Table 1).

Hemodynamics

Values of all hemodynamic variables measured before the first coronary occlusion were comparable among the four groups. As anticipated, L-NAME elevated MAP and statistical significance was observed until 30 min into the final reperfusion period. MAP did not significantly change among the control, L-arg, and D-arg groups during experimental measurements. HR, LVEDP, LV dp/dt max, and CBF were not significantly different among the four groups throughout the experimental protocol (Table 2).

Segment shortening (SS)

SS data for the ischemic-reperfused area are illustrated in Figures 3–5. Paradoxical systolic bulging was observed during each ischemic episode and the severity of this dysfunction was

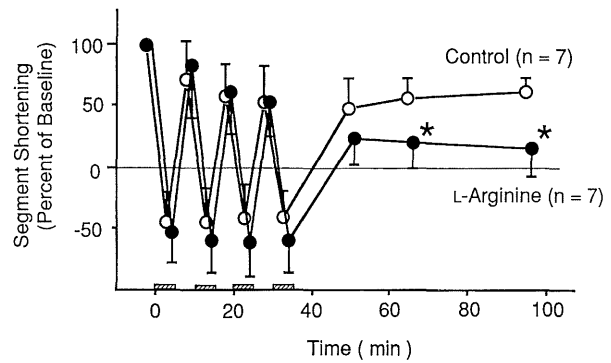


Fig. 3. Segment shortening in the ischemic region expressed as a percentage of baseline values for the control and L-arginine groups. Hatched bars indicate the periods of left anterior descending coronary artery occlusion. *Denotes a significant difference from the control group.

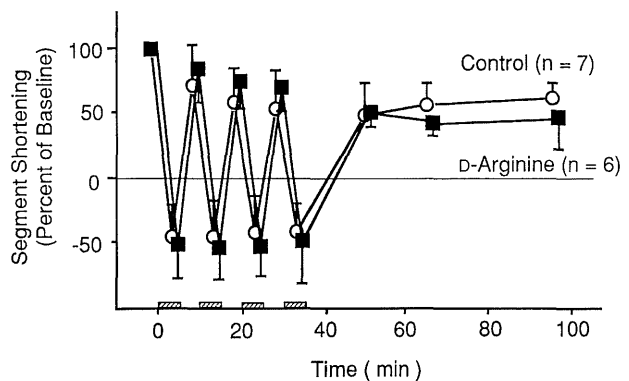


Fig. 4. Segment shortening in the ischemic region expressed as a percentage of baseline values for the control and D-arginine groups. Hatched bars indicate the periods of left anterior descending coronary artery occlusion. No significant difference was observed between the groups.

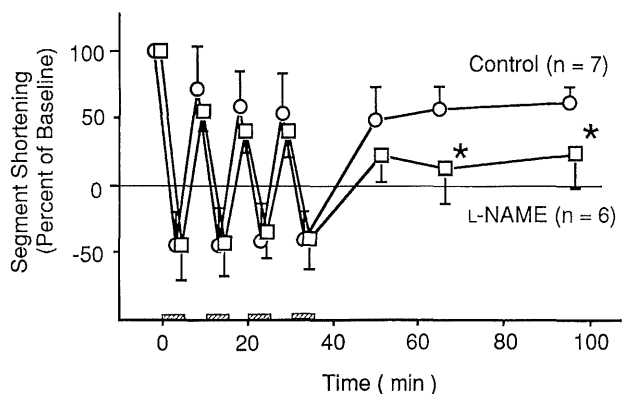


Fig. 5. Segment shortening in the ischemic region expressed as a percentage of baseline values for the control and N^G nitro-L-arginine methyl ester (L-NAME) groups. Hatched bars indicate the periods of left anterior descending coronary artery occlusion. *Denotes a significant difference from the control group.

Table 3. Transmural ischemic/non-ischemic blood flow ratios and flow deprivation in the mid-myocardium by coronary occlusion

	Ischemic/non-ischemic blood flow ratio			Flow deprivation
	Pre-occlusion	Occlusion	Reperfusion	
Control	1.014 ± 0.122	0.078 ± 0.041	0.987 ± 0.260	0.911 ± 0.078
L-Arginine	0.950 ± 0.106	0.061 ± 0.038	1.081 ± 0.290	0.947 ± 0.055
D-Arginine	0.933 ± 0.164	0.083 ± 0.036	0.863 ± 0.163	0.910 ± 0.089
L-NAME	0.956 ± 0.160	0.119 ± 0.048	1.014 ± 0.426	0.805 ± 0.258

L-NAME, N^G nitro-L-arginine methyl ester

comparable among the four groups. There were no significant differences in SS during the alternating brief reperfusion periods. However, SS in the ischemic zone of the L-arg (Fig. 3) and L-NAME (Fig. 5) groups remained significantly depressed compared with the control group 30 and 60 min after the final reperfusion ($p < 0.05$). The recovery of contractile function in the D-arg group was delayed slightly compared with the control, but was not significant (Fig. 4). The SS in the non-ischemic area (data not shown) was not different and remained close to the preocclusion level throughout the final reperfusion period in all groups. L-NAME by itself had no significant influence on SS in the LAD territory before the first coronary occlusion (data not shown).

Regional myocardial blood flow (RMBF)

The data on RMBF are summarized in Table 3. No significant changes were observed in the non-ischemic left circumflex region in any of the groups throughout the experimental period. The flow deprivation of the mid-myocardial region where the piezoelectric crystals were located was similar during coronary occlusion in all series. These data indicate that during the first coronary occlusion, all four groups were subjected to a similar degree of ischemia. The transmural ischemic/non-ischemic blood flow ratio, which provides an index of the oxygen supply to demand ratio in the ischemic region, decreased to near 0.1 during the coronary occlusion and recovered to pre-occlusion levels 60 min after reperfusion in all groups. Thus, in the region perfused by the LAD, occlusion produced nearly identical reductions in flow in all four groups.

Area at risk

The areas at risk were similar among the control, L-Arg, D-Arg, and L-NAME groups (25.1 ± 5.3 , 22.6 ± 6.1 , 22.8 ± 4.8 , and 24.4 ± 6.3 % of the left ventricle, respectively).

DISCUSSION

Endothelial function after repetitive coronary occlusion-reperfusion

Recent studies have demonstrated that prolonged coronary occlusion followed by reperfusion impairs the response to endothelium-dependent vasodilator agents in coronary epicardial conduit arteries²⁹⁾ as well as in microvessels²²⁾. Although the precise mechanism has not been determined, impaired NO synthesis or impaired release of NO may be responsible for this endothelial dysfunction. It has been reported that damage to the endothelium activates neutrophils and eventually progresses to myocardial damage¹⁸⁾. To evaluate whether the endothelium would be impaired in the repetitive ischemic regimen used in this study, vasodilator reserve was measured before and after ischemia-reperfusion in 5 dogs. There was no change in the response to acetylcholine and nitroglycerin 30 and 60 minutes after reperfusion when compared with baseline, suggesting that endothelial function in microvessels was not impaired. Although vascular function in large conduit arteries was not evaluated in this study, endothelial function would likely be preserved, since microvessels are more vulnerable to the ischemic insult than conduit arteries²⁵⁾.

Effects of NO inhibition

Inhibition of NOS significantly augmented contractile dysfunction following repeated brief coronary occlusions. This result is consistent with the report by Hasebe et al¹⁵⁾ in a single brief coronary occlusion-reperfusion model. There are some aspects that must be considered when evaluating the effect of NO inhibition in this study. First, inhibition of NOS may augment the degree of ischemia during coronary occlusions and affect the recovery of contractile function. However, L-NAME did not alter CBF in the LAD region and the FDs of the mid-myocardium where the piezoelectric crystals were located were not significantly different between the control and L-NAME groups. These data suggest that during coronary occlusion the two groups were subjected to a similar degree of ischemia. Second, L-NAME by itself

elevated blood pressure. Whereas elevation in blood pressure can exert the beneficial effect of increased coronary blood flow³³, it may change the systolic loading condition of reperfused myocardium, which would indirectly affect contractile function. However, the persistence of contractile dysfunction after 60 min of reperfusion (when the MAP was not different from the control group) suggests that the deleterious effect of L-NAME would not be caused by its effect on hemodynamics.

It is becoming evident that NO may also influence the interaction between the endothelium and circulating cells such as platelets¹²) and neutrophils¹⁷). Neutrophils may contribute to myocardial stunning by the release of toxic oxidants and proteases, as well as by the production of oxygen radicals. Furthermore, activated neutrophils could adhere to endothelial surfaces and result in capillary plugging, leading to heterogeneous regional ischemia, despite normal total myocardial flow from the recruitment of neighboring unoccluded capillary beds. Thus, the inhibition of NO has a potential to contribute significantly to myocardial stunning through the enhanced release of toxic mediators from neutrophils and through interference with microvascular perfusion.

Although precise mechanisms have not been elucidated in this study, it seems likely that endogenously synthesized NO plays an important role in limiting reperfusion injury in repetitively occluded-reperfused myocardium.

Effects of arginine on stunned myocardium

NO released from the endothelium participates in the regulation of smooth muscle tone in arterioles and thereby is a modulator of blood supply in tissues. Supplementation of L-arg has been reported to augment vasodilation under certain conditions^{1,14}). This vasodilating action of L-arg may be salutary when blood flow has been compromised^{23,31}). However, in this study, L-arg produced a deterioration of the regional contractile function in the previously ischemic region. Because this deleterious effect was not observed with D-arg, increased NO might be, at least in part, responsible for the aggravation of myocardial stunning.

There are several possible mechanisms by which L-arg could produce deleterious effects on reperfusion based on the conversion of this semi-essential amino acid to NO. First, administration of L-arg could reduce blood pressure via augmented synthesis or release of NO¹⁶). A reduction in blood pressure would decrease coronary perfusion pressure and adversely affect myocardial contractile function. However, blood pressure and CBF were comparable to both control and D-arg groups in the present study. Second, NO can

increase myocardial cGMP and attenuate myocardial contraction^{5,11}). However, this was not likely because contractile dysfunction did not recover after the discontinuation of the L-arg infusion. Third, NO and superoxide rapidly react to form the stable peroxynitrite anion^{2,26}). Peroxynitrite decomposition generates a strong oxidant with reactivity similar to a hydroxyl radical. Recently, Matheis et al²¹) have demonstrated that re-oxygenation with a high intra-arterial oxygen tension following hypoxia results in an elevation of NO in coronary venous blood; maintaining NO at the pre-hypoxic level using NOS inhibitor afforded nearly complete protection against myocardial re-oxygenation injury. Because oxygen-derived free radicals are important mediators of reperfusion injury³), an increase in the NO level during reperfusion may further aggravate myocardial stunning. Fourth, NO has been reported to inactivate mitochondrial iron-sulfur enzymes¹⁰). This action of NO may become apparent when energy production in mitochondria is compromised as postischemic contractile dysfunction⁸).

In this study, L-arg was administered in order to evaluate the influence of increased NO levels on myocardial stunning. However, the effect of L-arg independent from NO pathway must be considered. Supra-physiologic levels of arginine stimulate insulin secretion from pancreatic cells³⁰). Insulin has been reported to increase myocardial metabolic demand¹⁹), which could affect the recovery of contractile function in previously ischemic myocardium. Girerd et al. have demonstrated that L- and D-arg induces comparable increases in serum insulin in rabbits¹³). Thus, this is an unlikely mechanism of the deleterious effect observed in the present study. Additionally, L-arg increases the secretion of growth hormone. However, growth hormone augments myocardial contractility³²). Since the release of this positive inotropic substance should result in enhanced contractility, this is unlikely to explain the results of this study. Therefore, the myocardial stunning enhanced by L-arg seems to be mediated through augmented NO production.

The possible deleterious effect of excessive NO may be important during the early phase of reperfusion under both experimental and clinical conditions. Abrupt release of coronary occlusion produces a transient marked reactive hyperemia, and the resultant increased shear stress to the endothelium can stimulate NO release. It has been demonstrated that staged reperfusion accelerates improvement in cardiac function when compared with sudden reperfusion³⁴). Although the mechanism of deleterious effect of sudden reperfusion remains to be elucidated, an increased NO level during reperfusion might interfere with the recovery of contractile function.

Conclusion

The effects of both augmentation and inhibition of NO production on post-ischemic contractile dysfunction, that was not accompanied by endothelial dysfunction, was evaluated serially in this study. Although it seems likely that endogenous NO plays an important protective role, it may be cytotoxic when NO is produced in excess in this model of myocardial stunning.

ACKNOWLEDGMENTS

I am grateful to Dr. Goro Kajiyama, Professor of our laboratory, for his helpful discussion. I thank Dr. Hideo Matsuura and my colleagues in the laboratory for their advice. I would also like to thank Nihon Kayaku for generously providing me with nitroglycerin.

This study was in part presented at the 43rd Annual Scientific Session of the American College of Cardiology, Atlanta, GA., 1994, and published in abstract form (1994, *J. Am. Coll. Cardiol.* 23 [suppl A]: 263A).

(Received August 11, 1995)

(Accepted November 16, 1995)

REFERENCES

1. Aisaka, K., Gross, S.S., Griffith, O.W. and Levi, R. 1989. L-arginine availability determines the duration of acetylcholine-induced systemic vasodilatation in vivo. *Biochem. Biophys. Res. Commun.* **163**: 710–717.
2. Beckman, J.S., Beckman, T.W., Chen, J., Marshall, P.A. and Freeman, B.A. 1990. Apparent hydroxyl radical production by peroxynitrite: Implications for endothelial injury from nitric oxide and superoxide. *Proc. Natl. Acad. Sci. USA.* **87**: 1620–1624.
3. Bolli, R., Jeroudi, M.O., Patel, B.S., DuBose, C.M., Lai, E.K., Roberts, R. and McCay, P.B. 1989. Direct evidence that oxygen-derived free radicals contribute to postischemic myocardial dysfunction in the intact dogs. *Proc. Natl. Acad. Sci. USA.* **86**: 4695–4699.
4. Bolli, R., Patel, B.S., Zhu, W.-X., O'Nelli, P.G., Hartley, C.J., Charlat, M.L. and Roberts, R. 1987. The iron chelator desferrioxamine attenuates postischemic ventricular dysfunction. *Am. J. Physiol.* **253**: H1372–H1380.
5. Brady, A.J.B., Warren, J.B., Poole-Wilson, P.A., Williams, T.J. and Harding, S.E. 1993. Nitric oxide attenuates cardiac myocyte contraction. *Am. J. Physiol.* **265**: H176–H182.
6. Buisson, A., Plotkine, M. and Boulou, R.G. 1992. The neuroprotective effect of a nitric oxide inhibitor in a rat model of focal cerebral ischemia. *Br. J. Pharmacol.* **106**: 766–767.
7. Cooke, J.P., Rossitch, E.Jr., Andon, N.A., Loscalzo, J. and Dzau, V.J. 1991. Flow activates endothelial potassium channel to release an endogenous nitrovasodilator. *J. Clin. Invest.* **88**: 1663–1671.
8. DeBoer, L.W.V., Ingwall, J.S., Kloner, R.A. and Brawnwald, E. 1980. Prolonged derangements of canine myocardial purine metabolism after a brief coronary occlusion not associated with anatomic evidence of necrosis. *Proc. Natl. Acad. Sci. USA.* **77**: 5471–5475.
9. Dinerman, J.L., Lowenstein, C.J. and Snyder, S.H. 1993. Molecular mechanisms of nitric oxide regulation. Potential relevance to cardiovascular disease. *Circ. Res.* **73**: 217–222.
10. Drapier, J.-C. and Hibbs, J.B.Jr. 1988. Differentiation of murine macrophages to express nonspecific cytotoxicity for tumor cells results in L-arginine dependent inhibition of mitochondrial iron-sulfur enzymes in the macrophage effector cells. *J. Immunol.* **140**: 2829–2838.
11. Finkel, M.S., Oddis, C.V., Jacob, T.D., Watkins, S.C., Hattler, B.G. and Simmons, R.L. 1992. Negative inotropic effects of cytokines on the heart mediated by nitric oxide. *Science* **257**: 387–389.
12. Furlong, B., Henderson, A.H., Lewis, M.J. and Smith, J.A. 1987. Endothelium-derived relaxing factor inhibits in vitro platelet aggregation. *Br. J. Pharmacol.* **90**: 687–692.
13. Girerd, X.J., Hirsch, A.T., Cooke, J.P., Dzau, V.J. and Creager, M.A. 1990. L-arginine augments endothelium-dependent vasodilation in cholesterol-fed rabbits. *Circ. Res.* **67**: 1301–1308.
14. Gold, M.E., Bush, P.A. and Ignarro, L.J. 1989. Depletion of arterial L-arginine causes reversible tolerance to endothelium-dependent relaxation. *Biochem. Biophys. Res. Commun.* **164**: 714–721.
15. Hasebe, N., Shen, Y. and Vatner, S.F. 1993. Inhibition of endothelium-derived relaxing factor enhances myocardial stunning in conscious dogs. *Circulation* **88**: 2862–2871.
16. Higashi, Y., Oshima, T., Ozono, R., Watanabe, M., Matsuura, H. and Kajiyama, G. 1995. Effects of L-arginine infusion on renal hemodynamics in patients with mild essential hypertension. *Hypertension* **25**[part 2]: 898–902.
17. Kubes, P., Suzuki, M. and Granger, D.N. 1991. Nitric oxide: An endogenous modulator of leukocyte adhesion. *Proc. Natl. Acad. Sci. USA.* **88**: 4651–4655.
18. Lefer, A.M., Tsao, P.S., Lefer, D.J. and Ma, X.-I. 1991. Role of endothelial dysfunction in the pathogenesis of reperfusion injury after myocardial ischemia. *FASEB J.* **5**: 2029–2034.
19. Liang, C.-S., Doherty, J.U., Faillace, R., Maekawa, K., Arnold, S., Gavras, H. and Hood, W.B.Jr. 1982. Insulin infusion in conscious dogs: Effects on systemic and coronary hemodynamics, regional blood flow, and plasma catecholamines. *J. Clin. Invest.* **69**: 1321–1336.
20. Ma, X.-I., Johnson III, G. and Lefer, A.M. 1992. Low dose of superoxide dismutase and a stable prostacyclin analogue protect in myocardial ischemia and reperfusion. *J. Am. Coll. Cardiol.* **19**: 197–204.
21. Matheis, G., Sherman, M.P., Buckberg, G.D., Haybron, D.M., Young, H.H. and Ignarro, L.J. 1992. Role of L-arginine-nitric oxide pathway in myocardial reoxygenation injury. *Am. J. Physiol.* **262**: H616–H620.

22. **Mehta, J.L., Nichols, W.W., Donnelly, W.H., Lawson, D.L. and Saldeen, T.G.P.** 1989. Impaired canine coronary vasodilator response to acetylcholine and bradykinin after occlusion-reperfusion. *Circ. Res.* **64**: 43–54.
23. **Morikawa, E., Huang, Z. and Morskowitz, M.** 1992. L-Arginine decreases infarct size caused by middle cerebral arterial occlusion in SHR. *Am. J. Physiol.* **263**: H1632–H1635.
24. **Palmer, R.M.J., Ashton, D.S. and Moncada, S.** 1988. Vascular endothelial cells synthesize nitric oxide from L-arginine. *Nature Lond.* **333**: 664–666.
25. **Quillen, J.E., Sellke, F.W., Brooks, L.A. and Harrison, D.G.** 1990. Ischemia-reperfusion impairs endothelium-dependent relaxation of coronary microvessels but does not affect large arteries. *Circulation* **82**: 586–594.
26. **Radi, R., Beckman, J.S., Bush, K.M. and Freeman, B.A.** 1991. Peroxynitrite oxidation of sulfhydryls : The cytotoxic potential of superoxide and nitric oxide. *J. Biol. Chem.* **266**: 4244–4250.
27. **Siegfried, M.R., Erhardt, J., Rider, T., Ma, X-L. and Lefer, A.M.** 1992. Cardioprotection and attenuation of endothelial dysfunction by organic nitric oxide donors in myocardial ischemia-reperfusion. *J. Pharmacol. Exp. Ther.* **260**: 668–675.
28. **Swain, J.L., Hines, J.J., Sabina, R.L. and Holmes, F.W.** 1982. Accelerated repletion of ATP and GTP pools in postischemic canine myocardium using a precursor of purine de novo synthesis. *Circ.Res.* **51**: 102–105.
29. **VanBenthuyzen, K.M., McMurtry, I.F. and Horwitz, L.D.** 1987. Reperfusion after acute coronary occlusion in dogs impairs endothelium-dependent relaxation to acetylcholine and augments contractile reactivity in vitro. *J. Clin. Invest.* **79**: 265–274.
30. **Visek, W.J.** 1986. Arginine needs, physiological state and usual diets. A reevaluation. *J. Nutr.* **116**: 36–46.
31. **Weyrich, A.S., Ma, X-I. and Lefer, A.M.** 1992. The role of L-arginine in ameliorating reperfusion injury after myocardial ischemia in the cat. *Circulation* **86**: 279–288.
32. **Williams, G.H. and Brawnwald, E.** 1992. Endocrine and nutritional disorders and heart disease, p. 1827-1855. *In* E. Brawnwald (ed.), *Heart Disease*, W. B. Saunders Company, Philadelphia.
33. **Wyatt, H.L., Protasio, L.L., Waters, D.D., Swan, H.J.C. and Forrester, J.S.** 1977. Contrasting influences of alterations in ventricular preload and afterload upon systemic hemodynamics, function, and metabolism of ischemic myocardium. *Circulation* **55**: 318–324.
34. **Yamazaki, S., Fujibayashi, Y., Rajagopalan, R., Meerbaum, S. and Corday, E.** 1986. Effects of staged versus sudden reperfusion after acute coronary occlusion in the dog. *J. Am. Coll. Cardiol.* **7**: 564–572.