Inhibition of Endothelium-Derived Relaxing Factor Enhances Myocardial Stunning in Conscious Dogs

Naoyuki Hasebe, MD; You-Tang Shen, MD; Stephen F. Vatner, MD

**Background.** Impaired endothelial-dependent vascular responses after coronary artery occlusion (CAO) and reperfusion (CAR) have been investigated extensively. However, it is not known whether impaired endogenous endothelium-derived relaxing factor production affects postischemic myocardial dysfunction, ie, myocardial stunning.

**Methods and Results.** Eight dogs were instrumented with an intracoronary catheter and a hydraulic occluder on the left circumflex coronary artery. The effects of a 10-minute CAO randomized with and without intracoronary administration of L-arginine (L-NA), a nitric oxide (NO) synthesis inhibitor, were compared in the same conscious dogs. Postischemic regional contractile dysfunction in subendocardial and subepicardial as well as transmural wall thickening was measured with ultrasonic dimension crystals, and myocardial blood flow was measured with radioactive microspheres. Intracoronary infusion of L-NA did not affect systemic hemodynamics, and transmural myocardial blood flow was reduced slightly (~8%), but significantly, only in the left circumflex territory. The recovery of wall thickening was significantly delayed in the presence of L-NA compared with the absence of L-NA, eg, at 30-minute CAR, not only in the subendocardium (~7% versus -49±9%) but also in the subepicardium (~52±8% versus -29±7%). During CAO, blood flow was decreased identically in both conditions, and during CAR, the differences in blood flow were minor (7%).

**Conclusions.** Inhibition of NO synthesis enhanced myocardial stunning transmurally in conscious dogs, potentially independent of its effects on blood flow. (Circulation. 1993;88:2862-2871.)

**Key Words** • myocardium • relaxing factors • coronary artery • occlusion • flow • L-arginine

The endothelium plays a significant role in modulating coronary vasomotor tone and myocardial contraction via the synthesis and metabolism of vasodilating and vasoconstricting agents.1-3 Several studies have demonstrated impaired endothelium-dependent vascular responses after coronary artery occlusion (CAO) and its relief by reperfusion (CAR) both in vitro4-6 and in vivo.7,8 Most prior studies on the role of the endothelium in myocardial ischemia have focused on either altered endothelial control of vascular function4-8 or the development of myocardial necrosis.9-11 However, the extent to which endothelial function modulates the recovery of regional myocardial function following a brief period of CAO, insufficient to induce myocardial necrosis, ie, myocardial stunning,12,13 is not known.

Accordingly, the primary goal of the current investigation was to determine whether inhibition of endogenous endothelium-derived relaxing factor (EDRF), eg, nitric oxide (NO),14 enhances postischemic myocardial dysfunction. A second goal was to determine if the alterations in myocardial stunning, by the inhibition of endogenous EDRF, was nonuniform transmurally since it has been suggested that endothelium-dependent vasodilation is most prominent in the subendocardium15 and is selectively attenuated in the subependocardium after ischemia and reperfusion.16 Since systemic administration of NO synthesis inhibitors increases arterial and left ventricular pressures, ie, afterload, and potentially preload, which would, by itself, affect myocardial stunning,17,18 it was thought to be critical to avoid these complicating issues by administering the NO synthesis inhibitor locally. To accomplish this, an infusion of L-arginine (L-NA) was administered intracoronary to inhibit the NO synthesis pathway selectively in the territory of the left circumflex coronary artery, which was destined to become ischemic, relatively sparing the nonischemic zone and systemic circulation. L-NA was selected since it is more potent than L-arginine (L-NMMA) as an inhibitor of NO synthesis19,20 and does not have a muscarinic receptor antagonistic action.21 A 10-minute period of CAO was selected for this study to avoid the potential problem that myocardial necrosis might develop with longer periods of ischemia.22 However, the results from the initial 10-minute period of CAO were discarded since prior studies have shown that the response to an initial CAO differ from those from a subsequent second and...
third CAO.23 The conscious dog was studied to avoid the complicating influence of recent surgery and anesthesia,24 and the size of its heart was sufficiently large to permit measurement of regional subendocardial and subepicardial wall thickness,25,26 thereby allowing examination of the effects of ischemia and subsequent myocardial dysfunction on regional as well as transmural myocardial function in the same dogs on different days.

**Methods**

**Implantation of Instrumentation**

Eight mongrel dogs of either sex weighing 30-51 kg were premedicated with xylazine (Rompun 0.2 mg/kg IM) (Fig 1). General anesthesia was induced with sodium thiopental (12.5 mg/kg) and was maintained with halothane (1 to 1.5 vol%). With the use of sterile technique, an incision was made in the fifth left intercostal space. Tygon catheters (Norton Elastic and Synthetic Division, Akron, Ohio) were implanted in the descending thoracic aorta and left atrium. The left circumflex coronary artery was isolated 3 to 5 cm distal from its origin, and an ultrasonic Doppler flow transducer was implanted around the vessel and later used to verify complete CAO and CAR and inhibition of the coronary vasodilator response to acetylcholine. An hydraulic occluder, made of polyethylene tubing, was implanted distal to the flow probe. A Silastic catheter was implanted proximal to the flow probe with the tip in the lumen of the coronary artery with a diameter of 0.6 mm. To measure regional myocardial wall thickening, an ultrasonic crystal was implanted on the epicardium, while the endocardial ultrasonic crystal was implanted through a stab wound in the epicardium and advanced obliquely to, but not through, the endocardium. In addition, a piezoelectric ultrasonic dimension crystal was advanced obliquely to the midwall of the myocardium between the endocardial and epicardial crystals. Crystal placement was aided by monitoring the signal on an oscilloscope. The procedure of oblique implantation of crystals minimizes injury to the myocardium between the ultrasonic transducers, where wall thickening is measured. Two adjacent sets of full and regional wall thickness crystals were placed in the central ischemic area, and another set of full wall thickness crystals was placed in the nonischemic area in each animal. A solid-state miniature pressure transducer (model P
Konigsberg Instruments, Pasadena, Calif) was implanted into the left ventricle through the apex for measurement of left ventricular (LV) pressure and LV dP/dt. The thoracotomy incision was closed in layers, and the animals were allowed to recover for more than 1 week before the study. The animals used in this study were maintained in accordance with the guidelines of the Committee on Animals of the Harvard Medical School and the “Guide for the Care and Use of Laboratory Animals” of the Institute of Laboratory Animal Resources, National Council (Department of Health and Human Services publication No. [NIH] 85-23, revised 1985).

**Experimental Measurements**

Statham strain-gauge manometers (model P23-XL, Spectra-Med, Inc, Oxford, Calif) connected to the chronically implanted catheters were calibrated with a mercury manometer and used to measure aortic and left atrial pressures. LV pressure was measured with a solid-state miniature pressure gauge, which was calibrated in vitro with a mercury manometer and in vivo by comparing the measurement of LV systolic pressure with that of systolic arterial pressure, which was measured with a Statham strain-gauge manometer. LV full wall thickness and subendocardial and subepicardial wall thicknesses were measured with an ultrasonic transit-time dimension gauge. The dimension gauge generates a voltage linearly proportional to the transit time of the ultrasonic impulses traveling at the velocity of 1.58×10^6 mm/s between the 5-MHz crystals. The frequency response of the dimension gauge is flat to 60 Hz. At constant room temperature, the thermal drift of the instrument is minimal (ie, less than 0.02 mm in 6 hours). Any drift in the measurement system was eliminated during the experiment by periodic calibration accomplished by substituting impulses of known duration from a pulse generator. The position of all catheters and crystals was confirmed at autopsy.

**Blood Flow Measurement**

Regional myocardial blood flow was measured with radioactive labeled microspheres (15±2 μm in diameter, New England Nuclear, Boston, Mass). The radioactive label of the microspheres (141Ce, 113Sn, 114In, 51Cr, 103Ru, 98Nb, 88Sr, or 46Sc) was chosen randomly. The microspheres were suspended in 0.01% Tween 80 solution (10% dextran) agitated by direct application of the microspheres and placed in an ultrasonic bath for at least 30 minutes before injection. Before injection of microspheres, 1 mL Tween 80 dextran solution (without microspheres) was injected to determine if the diluent for the microsphere suspension would have an adverse effect on measurement of cardiac or systemic hemodynamics. From 1 to 2 million microspheres suspended in 10% dextran were injected through the left atrial catheter for determination of blood flow. A reference sample of arterial blood was withdrawn (7.75 mL/min) from the catheter in the descending aorta. Reference sample withdrawal was initiated 15 seconds before microsphere injection and continued for a total of 120 seconds.

Total left circumflex coronary blood flow was measured using a Doppler ultrasonic flowmeter in seven dogs. In one dog, the signal was not technically acceptable.

**Experimental Protocol**

At 1 to 2 weeks after surgery, when the animals were healthy and had recovered from surgery and trained to lie quietly on their right sides, the effects of an initial 10-minute CAO were examined and discarded. Preliminary data from our laboratory suggested that myocardial stunning following an initial CAO was enhanced significantly compared with that observed in the same conscious dogs following subsequent second and third periods of CAO, but there was no significant difference between the myocardial stunning observed following the second and the third CAO. Therefore, in this study we discarded the results from the first CAO and randomized the second and the third CAO with and without EDRF inhibition in the same eight dogs, ie, four dogs underwent the experiment with L-NA before the saline control, whereas the other four dogs experienced the reverse sequence. Aortic pressure; LV pressure; rate of change of LV pressure (dP/dt); LV full, subendocardial, and subepicardial wall thicknesses; heart rate; and lead II ECG were monitored continuously throughout the study. All animals received an injection of morphine sulfate (0.3 to 0.5 mg/kg IM) before CAO. After control measurements were recorded, including the first injection of microspheres in five dogs, CAO was accomplished by inflating the hydraulic occluder. In three dogs, microspheres were not given because of technical difficulties. At 5 minutes after CAO, the second injection of microspheres was performed. Ventricular premature contractions were prevented and treated with bolus left atrial injections of 2% lidocaine, and the identical total amounts of lidocaine were used in the experiments with and without L-NA. Reperfusion was carried out after 10 minutes of CAO. The coronary artery occluder was released slowly over 30 seconds. The third injection of microspheres was given at 30 minutes after CAR. In five dogs, L-NA was infused at a rate of 30 μg·kg⁻¹·min⁻¹ IC for 12 minutes in 0.5 mL/min saline before CAO, and at a maintenance dose of L-NA (6 μg·kg⁻¹·min⁻¹ delivered in 0.1 mL/min of saline) starting simultaneously with CAR and continuing up to 1 hour after CAR. In the other three dogs, twice the dose of L-NA was administered intracoronary. Ten minutes were allowed after the completion of initial loading of L-NA before any intervention. In the control CAO experiment, the same volumes of saline were administered during the same periods. Hemodynamics were monitored continuously for 6 hours and then intermittently until full recovery of regional myocardial function was confirmed at 24 hours after CAR. At least 48 hours elapsed between each 10-minute CAO and 24-hour CAR.

L-NA was dissolved in saline following 20 to 30 minutes of sonication as described by Moore et al. No precipitation in the L-NA solution was observed. The pH of the L-NA solution (6.0±0.3) was similar to that of saline (6.2±0.4). The L-NA solution was prepared fresh before use each day. The effectiveness of L-NA in inhibition of the coronary blood flow increase to acetylcholine was tested in five dogs. In these experiments, 5 ng/kg acetylcholine (dissolved in 0.3±0.02 mL saline) was administered intracoronarily in a bolus and flushed by an infusion pump with a constant speed (0.06 mL/s) before L-NA infusion and at 30 to 40 minutes after the initial loading dose (30 μg·kg⁻¹·min⁻¹ IC for 12 minutes) of L-NA. At this time, the peak response of
coronary blood flow to acetylcholine was diminished by 37±6% ($P<.01$), and the total coronary blood flow response (the area of increased mean coronary blood flow determined by planimetry) to acetylcholine was diminished by 58±4% ($P<.01$). In contrast, the peak coronary blood flow response to nitroglycerine 0.2 μg/kg IC (dissolved in 0.3±0.02 mL of saline) was not affected (2.9±4.5%, NS). The amount of inhibition of the coronary blood flow response to acetylcholine we observed was similar to the results by Parent et al. with intracoronary L-NAME in conscious dogs.

After the final experiments were completed, the dogs were anesthetized with an overdose of pentobarbital (50 mg/kg IV) to retrieve the heart for microsphere blood flow analysis. Four to six transmural specimens (1 to 2 g) were obtained from both the left circumflex and the left anterior descending coronary artery territories of the left ventricle, and two or three transmural specimens were obtained from the right ventricular free wall. To avoid admixture of ischemic and nonischemic tissue, specimens from the left circumflex territory were obtained at least 1 cm inside the boundaries of the occluded bed. Each specimen from the left ventricle was divided into four samples, reflecting subendocardial, midmyocardial (endodimymocardium [Mid3] epimyocardium [Mid2]), and subepicardial layers. Each specimen from the right ventricle was divided into subendocardial and subepicardial halves. Each sample was weighed and placed in a gamma counter (Canberra Industries, Meriden, Conn) with appropriately selected energy windows. The raw counts were corrected for background and crossover and compared with the reference blood sample to obtain flow expressed in milliliters per minute per gram of tissue.

Data Analysis

The hemodynamic data were recorded on a multichannel tape recorder (model 101, Honeywell, Denver, Colo) and played back on a direct-writing oscillograph (Mark 200, Gould-Brush, Cleveland, Ohio). A cardiotachometer (model 9857B, Beckman Instruments, Fullerton, Calif), triggered by the LV pressure pulse, provided instantaneous and continuous recordings of heart rate. Continuous recordings of LV dP/dt were derived from the LV pressure measurement by use of an operational amplifier connected as a differentiator and with a frequency response of 700 Hz. A triangular wave signal was substituted for the pressure signals to directly calibrate the differentiator. LV end diastole was defined as the point immediately before the onset of LV contraction, indicated by the initial increase in LV dP/dt. LV end systole was defined as the point of maximum negative LV dP/dt. LV systolic wall thickening was defined as the maximal systolic increase in wall thickness from the end-diastolic value. Paradoxical systolic thinning was defined as the maximal systolic decrease in thickness from the end-diastolic value. Coronary blood flow debt, excess flow during reactive hyperemia, and repayment of flow debt were calculated as described by Olsson and Gregg.28 Areas of flow debt and reactive hyperemic flow were computed by a planimetry of the mean coronary blood flow measurement.

Statistical Analysis

The data were stored and analyzed using an IBM computer, and all values are reported as mean±SE.

Differences between baseline measurements and subsequent values were assessed by repeated-measures ANOVA. If an overall difference was found, comparisons were performed using the analysis of contrasts. The data for reactive hyperemia were analyzed by means of paired $t$ tests. Data were considered to be significantly different at $P<.05$.

Results

Systemic Hemodynamics

There were no significant differences in baseline systemic hemodynamics with and without intracoronary L-NA, verifying that intracoronary administration of L-NA in the present study did not affect systemic hemodynamics (Table 1). During CAO (just before CAR), heart rate, LV end-diastolic pressure, LV systolic pressure, and mean aortic pressure increased significantly and similarly in both conditions. Furthermore, there were no significant differences in systemic hemodynamics during CAO between the two conditions. After CAR, all measurements of systemic hemodynamics returned to baseline levels by 30 minutes, and there were no significant differences between the two conditions. No significant differences were observed in the three dogs with twice the dose of L-NA compared with the other five dogs studied.

Regional Myocardial Function

During CAO, systolic wall thickening was replaced by wall thinning, which was more prominent subendocardially than subepicardially (Table 2 and Figs 2 through 4). The extent of wall thinning during CAO tended to be greater in the presence of L-NA but did not reach statistical significance. In the nonischemic zone, there were also no significant differences in transmural systolic wall thickening between the two conditions. There were no significant differences in nonischemic zone wall thickening between the two conditions throughout the entire CAR period. In contrast, the recovery of systolic wall thickening in the ischemic zone was significantly delayed in the presence of L-NA (Figs 2 through 4). The recovery of subendocardial systolic wall thickening was delayed more than in the subepicardium under both conditions. The extent of postischemic dysfunction (myocardial stunning) was more prominent ($P<.05$) in the presence of L-NA than in the absence of L-NA, not only in the subendocardium but also in the subepicardium and transmurally (Figs 2 through 4).

The average data at 30 minutes after CAR for all eight dogs studied are summarized in Table 2. It is important to note that there were no major differences in responses in the three dogs receiving twice the dose of L-NA compared with responses in the other five dogs. For example, at 30 minutes after CAR, ischemic zone full wall thickness was depressed more in the presence of L-NA ($−54.9±7.3\%$) than in the absence of L-NA ($−34.0±6.7\%$) in the group of five dogs. Similarly, ischemic zone full wall thickness in the three dogs with twice the dose of L-NA was also depressed more at 30 minutes after CAR ($−63.1±12.8\%$) than in the respective control experiments ($−35.7±15.6\%$). Responses of subendocardial and subepicardial wall thickenings were also similar in the two subgroups of five and three dogs, respectively.
TABLE 1. Effects of Coronary Artery Occlusion and Reperfusion on Hemodynamics With and Without L-NA in Eight Conscious Dogs

<table>
<thead>
<tr>
<th>Condition</th>
<th>Baseline</th>
<th>CAO 10 min</th>
<th>CAR 30 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate, bpm</td>
<td>L-NA(-) 62±4</td>
<td>+42±9*</td>
<td>+14±7</td>
</tr>
<tr>
<td></td>
<td>(+) 62±5</td>
<td>+49±14*</td>
<td>+15±8</td>
</tr>
<tr>
<td>Mean arterial pressure, mm Hg</td>
<td>L-NA(-) 95±5</td>
<td>+17±5*</td>
<td>+2±3</td>
</tr>
<tr>
<td></td>
<td>(+) 96±5</td>
<td>+21±6*</td>
<td>+4±4</td>
</tr>
<tr>
<td>LV systolic pressure, mm Hg</td>
<td>L-NA(-) 115±4</td>
<td>+10±5*</td>
<td>-1±2</td>
</tr>
<tr>
<td></td>
<td>(+) 116±4</td>
<td>+15±5*</td>
<td>+2±3</td>
</tr>
<tr>
<td>LV end-diastolic pressure, mm Hg</td>
<td>L-NA(-) 6.2±1.0</td>
<td>+4.0±1.1*</td>
<td>-0.3±0.2</td>
</tr>
<tr>
<td></td>
<td>(+) 6.1±0.9</td>
<td>+4.7±1.3*</td>
<td>-0.2±0.2</td>
</tr>
<tr>
<td>LV dP/dt, mm Hg/s</td>
<td>L-NA(-) 2918±101</td>
<td>+43±177</td>
<td>-33±37</td>
</tr>
<tr>
<td></td>
<td>(+) 2806±72</td>
<td>+128±98</td>
<td>-153±95</td>
</tr>
</tbody>
</table>

L-NA indicates N0-nitro-L-arginine; CAO, coronary artery occlusion; L-NA(-), CAO without L-NA; L-NA(+), CAO with L-NA; and CAR, coronary artery reperfusion.

*P<.05 compared with baseline values.

Regional Myocardial Blood Flow

Myocardial blood flow in the distribution of the left circumflex territory, which received the L-NA, tended to decrease with L-NA before CAO, ie, transmural myocardial blood flow was decreased slightly but significantly (−8.3±1.7%, P<.05) (Table 3 and Figs 5 and 6). However, there were no significant differences in the left anterior descending territory and the right ventricular territory (Fig 5). The endo/epi ratios were not significantly different.

During CAO, endocardial blood flow was significantly lower than epicardial blood flow under both conditions,

TABLE 2. Effects of Coronary Artery Occlusion and Reperfusion on Regional Myocardial Function With and Without L-NA in Eight Conscious Dogs

<table>
<thead>
<tr>
<th>Condition</th>
<th>Baseline</th>
<th>CAO 10 min</th>
<th>CAR 30 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>IZ-subendocardium</td>
<td>L-NA(-) 5.94±0.20</td>
<td>-4.2±1.2*</td>
<td>0.3±1.2</td>
</tr>
<tr>
<td></td>
<td>(+) 5.99±0.35</td>
<td>-4.5±1.5*</td>
<td>1.1±1.0</td>
</tr>
<tr>
<td>Wall thickening, mm</td>
<td>L-NA(-) 1.87±0.15</td>
<td>-129.5±12.7*</td>
<td>-49.1±9.2*</td>
</tr>
<tr>
<td></td>
<td>(+) 1.72±0.16</td>
<td>-142.6±16.2*</td>
<td>-76.3±9.2*†</td>
</tr>
<tr>
<td>IZ-subepicardium</td>
<td>L-NA(-) 5.70±0.25</td>
<td>-3.1±1.0*</td>
<td>-0.6±0.9</td>
</tr>
<tr>
<td></td>
<td>(+) 5.62±0.33</td>
<td>-3.8±0.8*</td>
<td>-0.9±1.3</td>
</tr>
<tr>
<td>Wall thickening, mm</td>
<td>L-NA(-) 1.71±0.16</td>
<td>-107.5±7.3*</td>
<td>-29.0±6.5*</td>
</tr>
<tr>
<td></td>
<td>(+) 1.64±0.16</td>
<td>-111.1±9.1*</td>
<td>-51.7±7.8*†</td>
</tr>
<tr>
<td>IZ-full wall</td>
<td>L-NA(-) 11.94±0.42</td>
<td>-3.1±0.8*</td>
<td>-0.5±0.3</td>
</tr>
<tr>
<td></td>
<td>(+) 11.84±0.69</td>
<td>-3.3±1.0</td>
<td>-0.2±0.8</td>
</tr>
<tr>
<td>Wall thickening, mm</td>
<td>L-NA(-) 3.23±0.15</td>
<td>-111.1±6.3*</td>
<td>-34.7±6.5*</td>
</tr>
<tr>
<td></td>
<td>(+) 3.16±0.22</td>
<td>-122.2±9.0*</td>
<td>-58.0±6.2*†</td>
</tr>
<tr>
<td>NZ-full wall</td>
<td>L-NA(-) 11.44±0.68</td>
<td>-1.8±0.9</td>
<td>-0.1±1.3</td>
</tr>
<tr>
<td></td>
<td>(+) 11.58±0.72</td>
<td>-1.3±0.9</td>
<td>-0.5±1.1</td>
</tr>
<tr>
<td>Wall thickening, mm</td>
<td>L-NA(-) 3.49±0.18</td>
<td>1.3±1.2</td>
<td>2.7±1.5</td>
</tr>
<tr>
<td></td>
<td>(+) 3.47±0.21</td>
<td>0.9±1.8</td>
<td>1.5±2.8</td>
</tr>
</tbody>
</table>

*P<.05 compared with baseline values.
†P<.05 significantly different from L-NA(-).
CAO indicates coronary artery occlusion; CAR, coronary artery reperfusion; L-NA, N0-nitro-L-arginine; IZ, ischemic zone; NZ, nonischemic zone; L-NA(-), CAO without L-NA; and L-NA(+), CAO with L-NA.
Fig 2. Representative recordings of the measurements of left ventricular (LV) dP/dt, subendocardial (Endo), subepicardial (Epi), and transmural (Full) wall thicknesses (WT) in the ischemic zone (IZ) are shown at baseline, during coronary artery occlusion (CAO), and 30 minutes and 3 hours after coronary artery reperfusion (CAR). In the presence of N⁶-nitro-L-arginine (L-NA(+), lower panel), the recovery of systolic wall thickening was markedly delayed in all three layers compared with control CAO in the absence of NO inhibition response [L-NA(-), upper panel].

and there were no differences between L-NA(−) CAO and L-NA(+) CAO for either endo/epi ratios (0.24±0.05 versus 0.24±0.06) or transmural blood flow (0.18±0.05 versus 0.18±0.06 mL·min⁻¹·g⁻¹). After 30-minute CAR, blood flow recovered faster in epicardial layers compared with endocardial layers, but the recovery of blood flow was similar in both conditions. At 30 minutes after CAR, the endo/epi ratios were similar under both conditions. Transmural blood flow was depressed only modestly more in the presence of L-NA, by 7.0±1.4%, P<.05. There were also no major differences observed in the one dog that received twice the dose of L-NA (blood flow during CAR was reduced by 8.0% with L-NA) compared with the responses in the other four dogs.

There were also no differences in the presence and absence of L-NA in the pattern of total left circumflex coronary blood flow measured using the Doppler flowmeter (Fig 6). L-NA did not affect baseline coronary blood flow measured by Doppler flow probe not only in the group of five dogs but also in two dogs that received twice the dose of L-NA. After CAR, the amount of reactive hyperemia was similar in the presence and absence of L-NA (Fig 6). The peak hyperemic responses were 389±35% in L-NA(−) CAO and 379±48% in
L-NA(+) CAO. The total debt-repayment ratios were 0.82±0.10 in L-NA(−) CAO and 0.78±0.11 in L-NA(+) CAO. There were no significant differences observed in the two dogs with twice the dose of L-NA from the other five dogs studied.

Discussion

Impaired endothelium-dependent vascular responses after CAO and CAR have been demonstrated.4-8 NO synthesis inhibitors have been shown to alter vascular responses to CAO and CAR,30 and NO mechanisms have been shown to affect infarct size.9-11 However, the results of the present investigation are the first to demonstrate a role of endogenous NO synthesis inhibition in myocardial stunning, particularly in the conscious animal. The experimental design used several advantageous features. First, the study of the conscious animal eliminates complicating influences of anesthesia and recent surgery and allowed the study of the same animals with and without NO synthesis inhibition on separate days. Second, regional myocardial function was assessed continuously during CAO and CAR in both the subendocardium and the subepicardium as well as transmurally and were compared with measurements of regional myocardial blood flow. Third, the intracoronary catheter permitted local administration of L-NA selectively to the potential ischemic zone, eliminating complicating effects of L-NA–induced systemic hypertension and increases in afterload, which would affect the extent of myocardial stunning.18 In support of the contention that the L-NA acted only locally, intracoronary administration of L-NA reduced coronary blood flow selectively in the perfusion bed of the left circumflex coronary artery but not in the left anterior descending coronary artery territory or right ventricle (Fig 5), and there were no effects on systemic hemodynamics. Therefore, discrete local inhibition of NO synthesis was achieved. Finally, the present experimental design discarded the effects of the initial 10-minute CAO since myocardial stunning is always most severe during the first CAO, but there is little difference between results of a second and third CAO.23 By discarding the results from the first CAO and randomizing the second and third CAO with and without L-NA, we were able to more accurately assess the effects of inhibition of NO synthesis.

The major finding of the current investigation was that inhibition of endogenous NO synthesis enhances myocardial stunning transmurally and in both subendocardial and subepicardial layers (Figs 3 and 4). Even if EDRF control is more potent in the subendocardium,15 this was not reflected in our results, either during CAO or CAR, or on either transmural myocardial function or blood flow.

Several possible mechanisms were considered to explain the adverse effects of NO synthesis inhibition on
TABLE 3. Effects of Coronary Artery Occlusion and Reperfusion on Regional Myocardial Blood Flow With and Without L-NA in Five Conscious Dogs

<table>
<thead>
<tr>
<th>Condition</th>
<th>Baseline</th>
<th>CAO 10 min</th>
<th>CAR 30 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subendocardium</td>
<td>L-NA(−)</td>
<td>1.11±0.08</td>
<td>0.08±0.02*</td>
</tr>
<tr>
<td></td>
<td>(+)</td>
<td>1.02±0.06</td>
<td>0.07±0.03*</td>
</tr>
<tr>
<td>Endomidmyocardium (Mid3)</td>
<td>L-NA(−)</td>
<td>1.17±0.05</td>
<td>0.16±0.05*</td>
</tr>
<tr>
<td></td>
<td>(+)</td>
<td>1.06±0.07</td>
<td>0.14±0.05*</td>
</tr>
<tr>
<td>Epimidyocardium (Mid2)</td>
<td>L-NA(−)</td>
<td>1.03±0.08</td>
<td>0.22±0.06*</td>
</tr>
<tr>
<td></td>
<td>(+)</td>
<td>0.95±0.03</td>
<td>0.24±0.08*</td>
</tr>
<tr>
<td>Subepicardium</td>
<td>L-NA(−)</td>
<td>0.81±0.06</td>
<td>0.28±0.06*</td>
</tr>
<tr>
<td></td>
<td>(+)</td>
<td>0.73±0.04</td>
<td>0.27±0.07*</td>
</tr>
<tr>
<td>Endo/epi flow ratio</td>
<td>L-NA(−)</td>
<td>1.40±0.13</td>
<td>0.24±0.05*</td>
</tr>
<tr>
<td></td>
<td>(+)</td>
<td>1.41±0.11</td>
<td>0.24±0.06*</td>
</tr>
<tr>
<td>Transmural blood flow</td>
<td>L-NA(−)</td>
<td>1.03±0.05</td>
<td>0.18±0.05*</td>
</tr>
<tr>
<td></td>
<td>(+)</td>
<td>0.94±0.03†</td>
<td>0.18±0.06*</td>
</tr>
</tbody>
</table>

*P<.05 compared with baseline.  †P<.05 significantly different from L-NA(−).
CAO indicates coronary artery occlusion; CAR, coronary artery reperfusion; L-NA, N⁰-nitro-L-arginine; L-NA(−), CAO without L-NA; and L-NA(+), CAO with L-NA.
All values given in mL·min⁻¹·g⁻¹.

recovery of regional myocardial function. First, myocardial loading conditions, particularly afterload, could be altered by NO synthesis inhibition, which, during CAR, would enhance myocardial stunning. However, that was not observed using intracoronary administration of L-NA. LV systolic pressures and mean arterial pressures were almost identical during CAR with and without L-NA. Second, inhibition of NO synthesis might induce more intense ischemia during CAO, which could affect the recovery of regional function. The measurement of myocardial blood flow with microspheres ruled out the possibility that depression in blood flow during CAO differed in the presence and absence of L-NA. However, we cannot exclude the possibility that the mismatch between oxygen demand and supply was not more severe in the presence of L-NA, particularly during CAO, where increased LV systolic pressure and mean arterial pressure tended to be greater in the presence of L-NA treatment, as was the extent of regional myocardial wall thinning. Thus, it is possible that myocardial oxygen demands were augmented in the face of similar levels of perfusion. It has been suggested that more intense ischemia enhances myocardial stunning; however, this concept is based on levels of myocardial dysfunction from partial loss (hypokinesis) to complete loss of function (dyskinesis).
Fig 6. Effects of N\(^\text{O}\)-nitro-L-arginine (L-NA) on total coronary blood flow measured in the left circumflex coronary artery. The time course of changes in total left circumflex coronary blood flow is shown at baseline (Base), during 10 minutes of coronary artery occlusion (CAO), and during coronary artery reperfusion in the absence of L-NA [L-NA(−): open circles] and in the presence of L-NA [L-NA(+): closed circles]. Total left circumflex coronary blood flow responded similarly in the presence and absence of L-NA. Data are mean ± SE.

When wall motion is lost more than 100%, ie, paradoxical thinning evolves, there is no longer a relation among blood flow reduction, decreased wall motion during CAO, and extent of myocardial stunning. Therefore, the enhanced myocardial stunning in the presence of NO synthesis inhibition cannot be related to the more intense myocardial wall thinning during CAO.

This leaves us with the intriguing possibility that the mechanism for the augmented myocardial stunning in the presence of L-NA involves EDRF but is independent of its effects on myocardial blood flow. For example, it is conceivable that augmented myocardial stunning following NO synthesis inhibition is due to other functions of NO, such as its action on oxygen radicals, on platelet aggregation, or on neutrophil aggregation. NO has also been shown to be released on reoxygenation following acute hypoxia. Perhaps during CAR, NO is released, which protects the myocardium independent of blood flow, eg, at the level of excitation-contraction coupling, which is known to be involved in the mechanism of myocardial stunning. It is also conceivable that NO induces a protective effect on the myocardial cell in response to injury or transient ischemia. The inhibition of this protective influence would then result in augmented myocardial stunning. Finally, a direct effect of EDRF on regional myocardial function, independent of blood flow during CAR, must also be considered. Recently, the endothelium has been reported to affect myocardial function. Most findings on this topic have suggested a negative inotropic influence from NO. Since blocking a negative inotropic influence should result in enhanced, not further depressed, contractile function, this could not explain the results of the current study. However, during CAR, enhanced NO release may have other actions, eg, it is known that NO can increase myocardial cGMP, and cGMP, in turn, can inhibit Ca\(^{2+}\) influx. The inhibition of Ca\(^{2+}\) influx by cGMP may be an important protective mechanism to ameliorate Ca\(^{2+}\) overload during CAR, which has been suggested as a major mechanism for myocardial stunning.

It is conceivable that in our experiments blunting this mechanism with L-NA resulted in enhanced myocardial stunning.

In conclusion, intracoronary administration of L-NA provided local suppression of NO synthesis without significant changes in systemic hemodynamics. Inhibition of NO synthesis exerted only modest effects on the responses to CAO but enhanced myocardial stunning transmurally. Interestingly, the mechanism does not appear to relate to altered control of myocardial blood flow but rather to an effect on the myocardium.

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