Nitric oxide modulates cardiac contractility and oxygen consumption without changing contractile efficiency

NAOYUKI SUTO,1 ATSUSHI MIKUNIYA,1 TOMOYUKI OKUBO,1 HIROYUKI HANADA,1 NOBUYO SHINOZAKI,1 AND KEN OKUMURA1

1Second Department of Internal Medicine, Hirosaki University School of Medicine and 2Department of Radiological Technology, School of Allied Medical Sciences, Hirosaki University, Hirosaki 036, Japan

Suto, Naoyuki, Atsushi Mikuniya, Tomoyuki Okubo, Hiroyuki Hanada, Nobuyo Shinozaki, and Ken Okumura. Nitric oxide modulates cardiac contractility and oxygen consumption without changing contractile efficiency. Am. J. Physiol. 275 (Heart Circ. Physiol. 44): H41–H49, 1998.—Nitric oxide (NO) affects myocardial contractility and myocardial oxygen consumption (MVO2) in vitro. In α-chloralose-anesthetized dogs instrumented for the measurements of left ventricular (LV) pressure, LV volume using a conductance catheter, coronary blood flow, and coronary venous oxygen saturation (ScvO2) using a fiber-optic catheter, LV end-systolic pressure-volume relationships (ESPVR) and the relationship between MVO2 and LV pressure-volume area (PVA) were analyzed before and after intravenous infusions of the NO synthase inhibitor Nω-monomethyl-L-arginine acetate (L-NMMA; 5 mg/kg, 8 dogs) and the NO substrate L-arginine (600 mg/kg, 7 dogs). L-NMMA increased the slope of the ESPVR (Emax (P < 0.05) without changing contractile efficiency indicated by the inverse of the slope of the MVO2-PVA line. L-NMMA also increased unloaded MVO2, indicated by the y-axis intercept of the MVO2-PVA line (P < 0.05). In contrast, L-arginine decreased Emax (P < 0.05) while decreasing MVO2 (P < 0.05), and without changing contractile efficiency. The basal oxygen metabolism was not affected by L-NMMA and L-arginine. These data imply that endogenous NO spares MVO2 by reducing oxygen use in excitation-contraction coupling and attenuates cardiac contractility without changing contractile efficiency.

myocardial oxygen consumption; pressure-volume area; excitation-contraction coupling

NITRIC OXIDE (NO) is produced in the vascular endothelium and shows a potent vasodilator effect (23, 26). Although NO is produced and released in the coronary artery system both in the basal condition and in response to diverse stimuli, its effect on cardiac contractility and myocardial oxygen consumption (MVO2) is not well characterized. Previous studies have shown that NO attenuates cardiac myocyte contraction (3, 4, 9), mediates vagal inhibition of the cardiac inotropic response to β-adrenergic stimulation (12), and regulates oxygen consumption (10, 32, 33, 45). In patients with left ventricular (LV) dysfunction, NO was reported to attenuate the positive inotropic response to β-adrenergic stimulation (13). Several isoforms of NO synthase (NOS) responsible for the conversion of L-arginine to L-citrulline plus NO have been identified (27, 29). Under physiological conditions, a constitutive form of NO synthase (cNOS) is associated with low levels of NO formation, which are continuously generated in the vascular endothelium. Another form of NOS, known as inducible NOS (iNOS), can be activated in endotoxin shock, heart failure, and ischemia (7, 8, 19, 21, 24, 30, 41). Most of the previous studies examined the role of NO produced by iNOS in cardiac contractility and MVO2 and only a few studies examined the role of NO produced by cNOS in a physiological condition (12).

The purpose of the present study was to investigate the effect of NO on cardiac contractility and MVO2 in hearts in situ. Using an in vivo canine heart, we evaluated 1) the slope (Emax) of the end-systolic pressure-volume relationship (ESPVR), 2) MVO2, and 3) the pressure-volume area (PVA). Emax allows estimation of cardiac contractility irrespectively of the changes in preload and afterload (38, 39). With the MVO2-PVA relationship, it is possible to partition total energy output into mechanical and nonmechanical components: 1) the excess MVO2 above the MVO2-axis intercept (unloaded MVO2) and 2) MVO2 at PVA = 0 (16, 34). In addition, the MVO2 for nonmechanical energy utilization can be divided into that for excitation-contraction coupling and that for basal metabolism. Thus cardiac contractile efficiency, an expression of the efficiency of chemomechanical energy transduction by the contractile proteins when both MVO2 and PVA are expressed in standard energy, can be evaluated from the inverse of the slope of the linear MVO2-PVA relation. We investigated the effects of the NOS inhibitor Nω-monomethyl-L-arginine acetate (L-NMMA) and of L-arginine, a substrate of NO, on these parameters in order to clarify the role of NO in cardiac contractility and MVO2.

MATERIALS AND METHODS

Surgical Preparation

Experiments were carried out in 31 mongrel dogs with a mean body weight of 23.0 ± 0.9 (mean ± SE) kg. All of the experiments were performed in accordance with the guidelines established for animal experiments in Hirosaki University. The dogs were premedicated with a subcutaneous injection of xylazine (4 mg/kg) and atropine sulfate (0.05 mg/kg) and were anesthetized with α-chloralose (100 mg/kg) and pancuronium bromide (0.7 mg/kg). A small supplemental dose of α-chloralose (20 mg·kg–1·h–1) was continuously infused throughout the experiment. Under the anesthesia, dogs were intubated and ventilated by a fixed-volume positive-pressure respirator (model 613, Harvard Apparatus, South Natick, MA) with room air supplemented by oxygen. Arterial blood gases were measured every hour, and arterial Po2 (PaO2) and pH were corrected when necessary by adjustments of tidal volume, oxygen concentration, and administration of sodium bicarbonate. The arterial blood oxygen saturation especially was kept to be constant at a level ≥98% throughout the experiment.
the experiment. Blood volume loss resulting from the surgery was supplemented with 6% dextran and/or normal saline before the experimental protocol was initiated.

With the animal in a supine position, the right femoral artery was isolated and a 6-Fr pig-tail catheter (Cordis, Miami, FL) was inserted into the descending aorta to measure aortic pressure with a pressure transducer (model TP-400T, Nihon Kohden, Tokyo, Japan). A midline cervical incision was performed to expose the bilateral carotid arteries, and a 7-Fr catheter-tip micromanometer (model PC 780 N, Millar, Houston, TX) was advanced through the right carotid artery for the measurement of LV pressure. A 6-Fr eight-electrode conductance catheter (model 2SB, Transonic Systems, Ithaca, NY) was snugly applied around the artery for the measurement of coronary blood flow (CBF). For the continuous evaluation of rapid changes in coronary venous oxygen saturation (ScvO₂, %), a proximal portion of the coronary vein running along with the LAD was dissected and connected to the left femoral vein with a 7-Fr Teflon tube (Kawasumi, Tokyo, Japan) under the controlled perfusion using a pump (model AP-7000, Atto, Tokyo, Japan), and a 3-Fr fiber-optic catheter (Opticath, Abbott Lab, North Chicago, IL) was inserted into the bypass tube. A snare occluder was attached to the inferior vena cava (IVC) for a brief occlusion to measure E,max. To avoid undesirable reflexes, we isolated each stellate ganglion and ligated it tightly at its junction with the ansa subclavia. Each cervical vagus nerve was also crushed with a ligature.

Measurements

LV volume. The conductance catheter method was used for the measurement of LV volume (1, 2). Briefly, the method is based on the measurement of the time-varying electrical conductance of blood at five segments of the LV, estimating LV volume from the blood conductivity. An alternating current (0.07-mA root mean square at 20 kHz) was applied between the neighboring two electrodes of the conductance catheter. Five time-varying segmental conductances, Gᵢ(t), were measured and converted to total LV volume, V(t), with the use of the formula

\[ V(t) = \frac{(1/\alpha)(L^2/\sigma r)[G(t) - G₀]}{\rho} \]

where \( \sigma \) represents blood conductivity, \( \alpha \) is the slope constant, \( L \) is the electrode distance, \( G(t) \) is the sum of the five segmental conductances, and \( G₀ \) represented the parallel conductance formed by the tissue surrounding the LV cavity (myocardium, right ventricle contents, etc.). \( G₀ \) was determined in each experiment by the injection of 12 ml of hypertonic saline (6 mol/l) through the pulmonary artery into the LV. Current generation, conductance measurement, and analog computations to obtain the desired volume variable \( G(t) \) were performed with the use of a model Sigma 5 signal-conditioner processor (Cardio-Dynamics, Rijnsburg, The Netherlands).

CBF. The LAD blood flow was continuously measured with an ultrasound transit time flowmeter (model T206, Transonic Systems) and was corrected with the weight of the LAD perfusion territory.

Perfusion territory. The perfusion territory of the LAD was defined at the conclusion of the experiment. A 7-Fr Teflon tube (Kawasumi, Tokyo, Japan) was inserted into the proximal segment of the LAD, and indocyanine green solution was injected through the tube keeping a similar perfusion pressure to that during the experiment with the use of a tonometer (Nihon Kohden). The weight of the area stained with indocyanine green was measured.

\[ \text{ScvO}_2 \text{, Using a fiber-optic catheter indwelled into the bypass tube between the coronary vein and femoral vein, we continuously measured ScvO}_2 \text{ with the oximeter (Oximetrix 3, Abbott Labs). This oximeter system displays successively an average of oxygen saturation measured during the latest 5-s period. Furthermore, ScvO}_2 \text{ was measured in the bypass tube and not in the intraluminal or pericardial coronary vein, so that there seemed to be a time lag between the changes in the coronary venous oxygen saturation and ScvO}_2 \text{ measured in this study. Therefore, in the estimation of ScvO}_2 \text{, we corrected its time course so as to make the nadir of ScvO}_2 \text{ during IVC occlusion coincide with that of the LV systolic pressure.} \]

Plasma norepinephrine concentration. Coronary venous blood samples (5 ml) were collected in prechilled tubes containing 5 mg of Na₂EDTA. The plasma was separated by centrifugation (4°C, 3,000 rpm, 15 min) and stored at −20°C until the assay. The plasma norepinephrine concentration was determined with an automatic fluorescence analyzer (model HLC-725CA, Tosoh, Tokyo, Japan).

Throughout the experiment, electrocardiographic (ECG) lead II, LV volume, aortic pressure, LV pressure, LAD flow, ScvO₂, and LV pressure-volume loops were displayed on-line using a storage oscilloscope. Data were displayed on an eight-channel heat-stylus recorder (model WS-681G, Nihon Kohden) and also stored on a magnetic tape with the use of an analog tape recorder (model A614, Sony, Tokyo, Japan) for subsequent analysis.

Experimental Protocol

The following experiments were performed in a condition with stabilized hemodynamics lasting for >30 min. Validation of IVC-occlusion method in estimating the MV0₂-PVA relationship (n = 3). In the original reports (16, 35), MV0₂-PVA relationship was analyzed in a steady-state loading condition using an isolated, cross-circulated canine heart. Thus both MV0₂ and PVA were measured in several conditions consisting of different end-diastolic volumes and different systolic pressures. For the analysis of the effect of each drug on the MV0₂-PVA relationship in an in vivo model with intact circulation, we measured beat-to-beat changes in MV0₂ and PVA during IVC occlusion before and after the drug. To validate this IVC-occlusion method in estimating the MV0₂-PVA relationship, we performed transient IVC occlusion and subsequent volume loading, and the ESPVR and MV0₂-PVA relationship obtained from these two methods were compared. After E,max and the MV0₂-PVA relationship were measured by transient IVC occlusion, the blood in the left atrium was removed to the reservoir through a catheter inserted to the left atrial appendage so as to obtain systolic pressures almost equal to that at the end of IVC occlusion. The removed blood supplemented with normal saline (500 ml) was then injected into the left atrium in a stepwise fashion to obtain systolic pressures of 90, 110, and 130 mmHg. At each volume-loading state, at least 1 min was allowed for stabilization, and E,max and MV0₂ were measured while both end-diastolic volume and systolic pressure were in steady states.
Effect of L-NMMA (n = 8). After the measurements of ECG, LV volume, aortic pressure, LV pressure, LAD coronary blood flow, and ScvO₂, IVC was occluded transiently for 10 s to determine the ESPVR (baseline 1). After the hemodynamic parameters recovered to the baseline values, IVC was occluded in the same way to observe the reproducibility of the ESPVR (baseline 2). L-NMMA (5 mg/kg; Wako, Osaka, Japan) diluted with 10 ml of normal saline was then administered intravenously. Fifteen minutes after injection of L-NMMA at which the hemodynamic parameters were stabilized, a transient IVC occlusion with the measurements of hemodynamics and oxygen saturation was repeated. Blood samples for the measurement of the plasma norepinephrine concentration were collected from the coronary vein before and after administration of L-NMMA. At the conclusion of the experiment, the dogs were euthanized with injection of potassium chloride (0.04 M) and the basal oxygen consumption was measured immediately to examine the effect of L-NMMA on it.

Effect of L-arginine (n = 5). As in the study of the effect of L-NMMA, the baseline measurements of hemodynamics, oxygen saturation, and ESPVR were carried out twice. L-Arginine (600 mg/kg; Sigma, St. Louis, MO) diluted with 10 ml of normal saline was then administered intravenously, and the same measurements were repeated. Blood samples for the measurement of plasma norepinephrine concentration were collected from the coronary vein before and after administration of L-arginine. At the conclusion of the experiment, the basal oxygen consumption was measured to examine the effect of L-arginine on it.

Effects of sequential administration of L-NMMA and L-arginine (n = 5). To examine whether the effect of L-NMMA, especially on cardiac contractility, was reversed by the administration of L-arginine, we performed the measurements of hemodynamics and ESPVR at baseline, after intravenous L-NMMA (5 mg/kg), and after intravenous L-arginine (600 mg/kg). The time interval between L-NMMA and L-arginine administration was 20 min. Venous blood was taken before and after L-NMMA and L-arginine, and the plasma NOx (nitrate and nitrite) level was examined with the use of a Griess method (Cayman Chemical, Ann Arbor, MI). In this protocol of experiments, to observe the influence of frequency of contraction, the measurements were done during sinus rhythm and atrial pacing at rates of 130 and 150 beats/min at each of the conditions at baseline, after L-NMMA, and after L-arginine.

Basal oxygen consumption in control dogs. In eight dogs with the experimental instruments attached in the same way as in the other experiments and without treatment of any L-NMMA and L-arginine, the basal oxygen consumption was measured immediately after euthanasia with potassium chloride. It was then compared with that obtained from dogs treated with L-NMMA and L-arginine.

Data Analysis

All data stored on a magnetic tape were digitized. The sample frequency for analog-to-digital conversion was 200 Hz at 12-bit accuracy. Data of systemic hemodynamics were analyzed by the acquisition-archive system (Po-Ne-Mah, Storrs, CT) and of LV pressure-volume loops by the pressure-volume analysis program (Cardio-Dynamics).

ESPVR and PVA. ESPVR was determined by linear regression of the individual end-systolic points in the combined LV pressure-volume loops. The slope of the ESPVR (E_max) during a transient IVC occlusion was then calculated. PVA was obtained as the specific area in the pressure-volume diagram circumscribed by the ESPVR line, the end-diastolic pressure volume curve, and the systolic segment of the pressure-volume trajectory.

MV O₂-PVA relationships. With values of hemoglobin (Hb; g/dl), coronary arterial oxygen saturation (ScvO₂, %) and ScvO₂, LAD flow (ml·min⁻¹·100 g⁻¹), and heart rate (HR; beats/min), we calculated coronary arteriovenous oxygen content difference (a-vO₂; ml O₂/100 ml) and MV O₂ per beat (ml O₂·beat⁻¹·100 g⁻¹) with the following formulas, respectively

\[
a-vO₂ = (ScvO₂ - ScvO₂) \times 10^{-2} \times 1.34 \times Hb(g/dl)
\]

\[
MV O₂ per beat = (a-vO₂) \times 10^{-2} \times (LAD flow) / HR
\]

The MV O₂-PVA relationship was constructed by MV O₂ per beat against PVA.

Contractile efficiency. It is known that 1 mmHg·ml of PVA is equal to 1.33 × 10⁻² J on a physical basis and that 1 ml of oxygen consumed by myocardium is approximately equivalent to 20 J under normal aerobic conditions. Accordingly, MV O₂ (in ml O₂·beat⁻¹·100 g⁻¹) and PVA (in mmHg·ml·beat⁻¹·100 g⁻¹) can each be converted to the same unit of energy (J·beat⁻¹·100 g⁻¹). We obtained contractile efficiency (%) from the ratio of PVA to MV O₂ (with both in J·beat⁻¹·100 g⁻¹).

Statistics

All data are shown as means ± SE. The hemodynamic parameters before and after each of the drugs (L-NMMA and L-arginine) were compared by paired t-test. ESPVR, MV O₂-PVA relationships, and plasma norepinephrine concentrations at baseline 1, at baseline 2, and after L-NMMA or L-arginine, and the plasma NOx levels before and after L-NMMA and L-arginine, were compared with a repeated-measures ANOVA. The effects of L-NMMA and L-arginine sequentially administered were also analyzed with ANOVA for repeated measures. The basal oxygen consumption in dogs with treatment of L-NMMA and L-arginine and without any treatment was compared by one-way ANOVA. The level of significance was P < 0.05.

RESULTS

Comparison of E_max and MV O₂-PVA Relationships Obtained by Transient IVC-Occlusion and Volume-Loading Methods

Representative analog recordings of ECG, LV volume, aortic pressure, LAD flow, LV pressure, and ScvO₂ during transient IVC occlusion and volume loading in one dog are shown in Fig. 1, left. Figure 1, middle, shows the pressure-volume loops obtained from these two methods, and Fig. 1, right, shows the MV O₂-PVA relationships. It is noted that E_max values obtained from the two methods are almost equal and that MV O₂ is linearly correlated with PVA in both methods. A similar observation was made in the other two dogs, and thus the MV O₂ change during IVC occlusion is linearly related to the change in PVA.

Changes in Systemic and Coronary Hemodynamics After L-NMMA and L-Arginine

Table 1 summarizes changes in systemic and coronary hemodynamics after L-NMMA and L-arginine. There were no significant changes in HR, LV end-diastolic pressure (LVEDP), LV end-diastolic volume...
(LVEDV), and LV end-systolic volume (LVESV) after L-NMMA. However, L-NMMA increased LV end-systolic pressure (LVESP) (P < 0.05) and decreased LAD flow (P < 0.05). There were no significant changes in HR, LVEDP, and LVESV after L-arginine. There were trends toward a decrease in LVESP (P = 0.08) and increases in LVEDV (P = 0.08) and LAD flow (P = 0.14) after L-arginine.

**Fig. 1.** Myocardial oxygen consumption (MV\(\dot{O}_2\))-pressure-volume area (PVA) relationships measured by transient inferior vena caval (IVC)-occlusion method (A) and by steady-state, volume-loading method (B). A and B show representative analog recordings of electrocardiogram (ECG), left ventricular (LV) volume (LVV), aortic pressure (AoP), coronary blood flow (CBF), LV pressure (LVP), and coronary venous oxygen saturation (Scv\(_{O_2}\)) (left); end-systolic pressure-volume relationship (ESPVR) (middle); and MV\(\dot{O}_2\)-PVA relationship (right). See text for discussion.

**Table 1.** Systemic and coronary hemodynamics before and after L-NMMA and L-arginine

<table>
<thead>
<tr>
<th></th>
<th>HR, beat/min</th>
<th>LVESP, mmHg</th>
<th>LVEDP, mmHg</th>
<th>LVEDV, ml</th>
<th>LVESV, ml</th>
<th>LAD Flow, ml·min(^{-1})·100 g(^{-1})</th>
<th>MV(\dot{O}_2), ml O(_2)·min(^{-1})·100 g(^{-1})</th>
</tr>
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<tbody>
<tr>
<td>Before L-NMMA</td>
<td>105 ± 5</td>
<td>103 ± 3</td>
<td>10 ± 2</td>
<td>40 ± 4</td>
<td>27 ± 6</td>
<td>54.0 ± 2.8</td>
<td>6.07 ± 0.52</td>
</tr>
<tr>
<td>After L-NMMA</td>
<td>100 ± 4</td>
<td>116 ± 4*</td>
<td>13 ± 1</td>
<td>41 ± 5</td>
<td>27 ± 9</td>
<td>49.1 ± 2.4*</td>
<td>6.75 ± 0.53*</td>
</tr>
<tr>
<td>Before L-arginine</td>
<td>104 ± 4</td>
<td>108 ± 4</td>
<td>12 ± 2</td>
<td>48 ± 4</td>
<td>32 ± 4</td>
<td>54.1 ± 2.1</td>
<td>6.09 ± 0.53</td>
</tr>
<tr>
<td>After L-arginine</td>
<td>108 ± 3</td>
<td>100 ± 4</td>
<td>10 ± 1</td>
<td>57 ± 5</td>
<td>38 ± 4</td>
<td>58.3 ± 3.2</td>
<td>4.96 ± 0.69*</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 8 dogs for N\(^\circ\)-monomethyl-L-arginine acetate (L-NMMA) and n = 7 dogs for L-arginine. HR, heart rate; LAD, left anterior descending artery; LVEDP, left ventricular (LV) end-diastolic pressure; LVEDV, LV end-diastolic volume; LVESV, LV end-systolic volume; LAD, left anterior descending coronary artery; MV\(\dot{O}_2\), myocardial oxygen consumption. *P < 0.05 vs. before treatment.
Effects of L-NMMA on Cardiac Contractility and MV\textsubscript{O2}

As shown in Table 2 and Fig. 2, neither ESPVR (E\textsubscript{max}) nor the MV\textsubscript{O2}-PVA relationship differs between the measurements at baseline 1 and baseline 2, indicating the reproducibility of the measurements with the IVC-occlusion method. After L-NMMA administration, E\textsubscript{max} was significantly increased (P < 0.05), and the y-axis intercept of the MV\textsubscript{O2}-PVA relationship (unloaded MV\textsubscript{O2}) was also significantly increased (P < 0.05). The slope of the MV\textsubscript{O2}-PVA relationship (the inverse of the contractile efficiency) remained unchanged after L-NMMA. Contractile efficiency (%), calculated by the ratio of PVA to MV\textsubscript{O2}, was 48.4 ± 1.5% at baseline and 47.3 ± 1.7% after L-NMMA (P = not significant (NS)). An example of the change in MV\textsubscript{O2}-PVA relationship after L-NMMA is shown in Fig. 3A. L-NMMA shifts the line upward, with an increase of the y-axis intercept and without changing the slope, indicating that L-NMMA increased unloaded MV\textsubscript{O2} without changing contractile efficiency.

Effects of L-Arginine on Cardiac Contractility and MV\textsubscript{O2}

As shown in Table 2 and Fig. 2, E\textsubscript{max} was significantly decreased after L-arginine (P < 0.05). The y-axis intercept of the MV\textsubscript{O2}-PVA relationship was significantly decreased after L-arginine (P < 0.05). The slope of the MV\textsubscript{O2}-PVA relationship remained unchanged after L-arginine. Contractile efficiency was 44.8 ± 1.1% at baseline and 45.5 ± 0.9% after L-arginine (P = NS). As shown in Fig. 3B, L-arginine shifts the line downward, with a decrease of the y-axis intercept and without changing the slope, indicating that L-arginine decreased unloaded MV\textsubscript{O2} without changing contractile efficiency.

Effects of Sequential Administration of L-NMMA and L-Arginine

Figure 4 shows E\textsubscript{max} measured at baseline, after L-NMMA, and after L-arginine that was administered following L-NMMA. The measurement was done during sinus rhythm (110 ± 5 beats/min at baseline, 106 ± 4 beats/min after L-NMMA, and 110 ± 5 beats/min after L-arginine; P = NS among the 3 conditions) and during atrial pacing at rates of 130 and 150 beats/min. At all HRs, E\textsubscript{max} was significantly increased after L-NMMA (all P < 0.05) and then decreased after L-arginine (all P < 0.05). When the changes in E\textsubscript{max} after L-NMMA and L-arginine from baseline were compared among experiments during sinus rhythm and atrial pacing, no significant difference was noted.

The plasma NO\textsubscript{X} levels at baseline and after L-NMMA and after L-arginine were 2.3 ± 0.7, 2.8 ± 0.6, and 2.7 ± 0.6 µM, respectively. There was no statistical difference among them.

Table 2. ESPVR, MV\textsubscript{O2}-PVA relationship and plasma norepinephrine concentration before and after L-NMMA and L-arginine

<table>
<thead>
<tr>
<th>Condition</th>
<th>E\textsubscript{max} mmHg ml\textsuperscript{-1} 100 g\textsuperscript{-1}</th>
<th>Slope, 0.1 ml O\textsubscript{2} mmHg ml\textsuperscript{-1}</th>
<th>y-Axis intercept, 0.1 ml O\textsubscript{2} beat\textsuperscript{-1} 100 g\textsuperscript{-1}</th>
<th>Norepinephrine, pg/ml</th>
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</thead>
<tbody>
<tr>
<td>Before L-NMMA (baseline 1)</td>
<td>3.30 ± 0.08</td>
<td>1.38 ± 0.04</td>
<td>2.43 ± 0.10</td>
<td>46.7 ± 5.5</td>
</tr>
<tr>
<td>Before L-NMMA (baseline 2)</td>
<td>3.29 ± 0.10</td>
<td>1.40 ± 0.05</td>
<td>2.44 ± 0.11</td>
<td>51.2 ± 6.2</td>
</tr>
<tr>
<td>After L-NMMA</td>
<td>4.07 ± 0.09*</td>
<td>1.42 ± 0.05</td>
<td>3.09 ± 0.10*</td>
<td>52.3 ± 7.3</td>
</tr>
<tr>
<td>Before L-arginine (baseline 1)</td>
<td>3.20 ± 0.05</td>
<td>1.49 ± 0.04</td>
<td>2.44 ± 0.04</td>
<td>44.0 ± 6.6</td>
</tr>
<tr>
<td>Before L-arginine (baseline 2)</td>
<td>3.20 ± 0.05</td>
<td>1.48 ± 0.06</td>
<td>2.43 ± 0.05</td>
<td>43.5 ± 5.8</td>
</tr>
<tr>
<td>After L-arginine</td>
<td>2.15 ± 0.10*</td>
<td>1.47 ± 0.03</td>
<td>1.47 ± 0.06*</td>
<td>44.0 ± 5.6</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 8 dogs for L-NMMA and 7 dogs for L-arginine. E\textsubscript{max}, slope of end-systolic pressure-volume relationship (ESPVR); Norepinephrine, plasma norepinephrine concentration; PVA, pressure-volume area. *P < 0.05 vs. baseline.

DISCUSSION

The present study showed that blockade of NOS with L-NMMA resulted in the increases in E\textsubscript{max} (the slope of ESPVR), MV\textsubscript{O2}, and unloaded MV\textsubscript{O2} (the y-axis intercept of the MV\textsubscript{O2}-PVA line) without changing contractile efficiency (the inverse of the slope of the MV\textsubscript{O2}-PVA line). In contrast, L-arginine, a substrate of NO, decreased E\textsubscript{max} and unloaded MV\textsubscript{O2} without changing contractile efficiency. Furthermore, the increasing effect of L-NMMA on E\textsubscript{max} was reversed by the additional administration of L-arginine. These indicate that endogenous NO reduces cardiac contractility and MV\textsubscript{O2} without affecting contractile efficiency.

Previous studies in isolated myocytes have shown that NO reduces cardiac contractility and MV\textsubscript{O2} (3, 4, 9, 45). Most of the experiments were done in the settings of endotoxic shock, heart failure, and ischemia (7, 9, 19, 21, 24, 30, 41). In these pathological conditions, NO was most likely to be produced via an iNOS pathway and not a cNOS one. On the other hand, a positive inotropic
effect (18, 22) or no detectable effect of NO (44) was also reported. Only a few studies have examined the role of NO in cardiac contractility and $\dot{M}VO_2$ in vivo (12). Thus it remains to be elucidated whether endogenous NO is involved in the regulation of cardiac contractility and $\dot{M}VO_2$.

In the present study, we used in vivo canine hearts with intact circulation and evaluated $E_{max}$ as an index of cardiac contractility and the $\dot{M}VO_2$-PVA relationship from the point of view of cardiac energetics. Suga et al. (36) reported that PVA, an expression of the total mechanical energy output of ventricular contraction on the basis of the time-varying elastance model (35), is linearly related to $\dot{M}VO_2$ per beat (37). In their original reports, the PVA and $\dot{M}VO_2$ relationship was determined in a steady-state, isovolumic contraction model. Thus the relationship was determined in several states of ventricular contraction from different end-diastolic volumes and against different systolic pressures using excised, cross-circulated canine hearts. In order to examine the effects of NOS inhibition and NO substrate supplement in vivo, we evaluated $E_{max}$ and the $\dot{M}VO_2$-PVA relationship with a transient IVC-occlusion method, and not with a steady-state, volume-loading method, because the latter method was considered not to be appropriate for the evaluation of the effects of drugs in an in vivo model. Previous studies with in vivo experiments (20, 25, 28) showed that $\dot{M}VO_2$ and PVA were linearly related during steady-state isovolumic contraction with alternation in loading conditions, whereas they were not during a beat-to-beat alternating condition such as that during transient IVC occlusion. In these previous studies, however, $\dot{a}$-Vo$_2$ was not measured on a beat-by-beat basis. We measured the oxygen saturation in the coronary sinus continuously using a fiber-optic catheter, keeping the oxygen saturation in the arterial blood constant at a level $\approx 98\%$, and analyzed the $\dot{M}VO_2$-PVA relationship on a beat-to-beat basis. The present IVC-occlusion method in estimating the $\dot{M}VO_2$-PVA relationship was validated because $E_{max}$ obtained from the IVC-occlusion and steady-state volume-loading methods are almost equal and, furthermore, $\dot{M}VO_2$ was linearly correlated with PVA in both methods.

L-NMMA and L-arginine were administered intravenously in this study. L-NMMA significantly increased systolic blood pressure and decreased CBF, which was consistent with previous observations (6, 14). L-Arginine showed a tendency to decrease systolic blood pressure. The use of $E_{max}$ and the $\dot{M}VO_2$-PVA relationship for the estimation of the effects of L-NMMA and L-arginine on cardiac contractility and $\dot{M}VO_2$ has a distinct advantage because these parameters are not affected by the hemodynamic changes induced by the drugs. L-NMMA increased $E_{max}$ and unloaded $\dot{M}VO_2$, whereas L-arginine decreased $E_{max}$ and unloaded $\dot{M}VO_2$.

Fig. 2. Slope ($E_{max}$) of ESPVR at baseline 1, at baseline 2, and after N$\omega$-monomethyl-L-arginine acetate (L-NMMA) (A; $n = 8$) or L-arginine (B; $n = 7$).

Fig. 3. Example of effects of L-NMMA (A) and L-arginine (B) on $\dot{M}VO_2$-PVA relationship. Each baseline indicated before an administration of drug. A: $y = 1.38 \times 10^{-2}x + 2.43 \times 10^{-2}$ at baseline, and $y = 1.42 \times 10^{-2}x + 3.09 \times 10^{-2}$ after L-NMMA. B: $y = 1.49 \times 10^{-2}x + 2.44 \times 10^{-2}$ at baseline, and $y = 1.47 \times 10^{-2}x + 1.47 \times 10^{-2}$ after L-arginine.
The autonomic nervous system is a major determinant for cardiac contractility and $\text{MV}_{\text{O}_2}$. Norepinephrine augments cardiac contractility and increases $\text{MV}_{\text{O}_2}$. A previous study showed that an NOS inhibitor was reported to enhance the evoked norepinephrine release in isolated rat hearts (31), whereas others showed that norepinephrine release was unaffected in several preparations (5, 34, 40, 42). Thus the relationship between endogenous NO and norepinephrine release remains unclear. We measured plasma norepinephrine concentration before and after L-NMMA and L-arginine and failed to demonstrate any changes after the drugs. Thus the effects of L-NMMA and L-arginine shown in this study were independent of norepinephrine release, and NO per se modulates cardiac performance.

Study Limitations

For the analysis of LV $\text{MV}_{\text{O}_2}$, the measurement of total left coronary artery blood flow may be required. In this study, we only measured the LAD flow and not the circumflex artery flow. Because both L-NMMA and L-arginine were administered intravenously, the changes in circumflex artery flow after these drugs were likely to be similar to those in LAD flow. Thus the coronary flow change per unit mass was likely to be uniform in the LV, and the total left coronary artery blood flow was possibly calculated from the LAD flow.

We measured the plasma NO level but could not find any significant change after administration of L-NMMA and L-arginine. The present study, however, clearly showed that L-NMMA increased cardiac contractility and $\text{MV}_{\text{O}_2}$, which were associated with the increase in systolic blood pressure and the decrease in LAD flow. L-Arginine showed effects opposite to those of L-NMMA. Therefore, the changes demonstrated after L-NMMA and L-arginine were most likely to be caused by the inhibition and augmentation of endogenous NO, respectively. The plasma NO level seems not to be affected by the acute administration of L-NMMA and L-arginine (43).

Address for reprint requests: K. Okumura, The Second Dept. of Internal Medicine, Hirosaki Univ. School of Medicine, Zaifu-cho 5, Hirosaki 036, Japan.

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