Role of Nitric Oxide in Regulation of Coronary Blood Flow in Response to Increased Metabolic Demand in Dogs With Pacing-Induced Heart Failure

Hideo Tada, MD; Kensuke Egashira, MD; Mitsutaka Yamamoto, MD; Makoto Usui, MD; Yukinori Arai, PhD; Yousuke Katsuda, MD; Hiroaki Shimokawa, MD; Akira Takeshita, MD

The role of endothelium-derived nitric oxide (NO) in the metabolic control of coronary blood flow (CBF) in heart failure (HF) is poorly understood, so the present study investigated the effects of inhibitors of NO synthesis on the response of CBF to changes in myocardial oxygen consumption (MVO_2) in dogs with HF produced by rapid ventricular pacing and in control dogs. The CBF, MVO_2, and other hemodynamic parameters were measured in anesthetized animals. Before infusion of N^ω-nitro-L-arginine methyl ester (L-NAME), the increases in CBF and MVO_2 during pacing tachycardia were not significantly different between the control and HF dogs. Intracoronary infusion of L-NAME did not alter the responses of CBF or MVO_2 to pacing tachycardia in the control dogs, but in the HF dogs, it reduced the CBF response to pacing tachycardia without altering the tachycardia-induced changes in MVO_2. Intracoronary infusion of L-arginine reversed the effect of L-NAME. These results suggest that in HF dogs NO contributes to the regulation of CBF in response to an increased metabolic demand. (Jpn Circ J 2001; 65: 827–833)

Key Words: Circulation; Endothelium-derived factors; Heart failure; Nitric oxide; Vasodilation

C hronic heart failure (HF) is a clinical syndrome with a poor prognosis in which because of cardiac dysfunction the oxygen supply to peripheral tissues is not adequate to meet the demand.1–2 Because oxygen extraction is near maximal at rest, coronary blood flow (CBF) changes rapidly in response to any changes in myocardial oxygen demand under normal conditions to maintain the balance between myocardial demand and supply.3 If the CBF response to an increased metabolic demand is impaired by the presence of HF, myocardial function would deteriorate because of the inadequate CBF to the failing myocardium. Abnormalities in CBF regulation despite the absence of coronary artery disease have been demonstrated in patients with HF caused by dilated cardiomyopathy (DCM) and by pressure-overload hypertrophy.4–7

The effects of left ventricular (LV) hypertrophy on the regulation of CBF have been extensively studied and most studies have shown that the progression of compensated LV hypertrophy to HF leads to decreases in the coronary vasodilator capacity and in subendocardial blood flow associated with myocyte necrosis and fibrosis.5–7 These abnormalities in myocardial perfusion appear to be the consequence of the hypertrophic process and not the HF process per se. A reduced coronary vasodilator reserve in response to dipyridamole and adenosine has been observed in animal and human models of dilated cardiomyopathy5–7,10–12 but the response of CBF to pacing tachycardia is controversial. Pasterncak et al reported that there was no difference in the increase in CBF in response to pacing tachycardia between patients with DCM and control subjects, and that pacing did not alter the coronary venous oxygen and lactate concentrations in either group.4 The patients with DCM had a reduced CBF per unit of myocardial mass at rest and during pacing, probably resulting from reduced myocardial oxygen consumption (MVO_2). Weiss et al reported that CBF was closely related to the determinants of the myocardial metabolic demand in patients with DCM and recent studies5,11,12 have shown that CBF is impaired at rest and during pacing tachycardia in animals and human models of HF compared with controls. However, none of those studies directly evaluated MVO_2 and thus it is difficult determine whether the impaired CBF response was related to the myocardial metabolic state.

There is increasing evidence that endothelium-derived nitric oxide (NO) contributes to the regulation of CBF in physiologic and pathophysiologic conditions.13–15 Endothelium-dependent/NO-mediated vasodilation is impaired in both animal and human models of HF.16–20 and recent evidence suggests that impaired endothelial production of NO may reduce peripheral perfusion in patients with HF.21 However, little is known about the role of NO in the regulation of CBF in response to metabolic demands in the presence of HF.

A canine model of pacing-induced HF has hemodynamic and neurohumoral characteristics that closely resemble the clinical manifestations of HF caused by DCM in humans, so we used this model to investigate the role of NO in the regulation of CBF in response to metabolic demands.
Methods

This study was approved by the Committee on the Ethics of Animal Experiments, Faculty of Medicine, Kyushu University, and was conducted according to the Guidelines for Animal Experiments of the Faculty of Medicine, Kyushu University, and Law (No. 105) and Notification (No. 6) of the Japanese Government.

Drugs

N\textsuperscript{ω}-nitro-L-arginine methyl ester (L-NAME), N\textsuperscript{ω}-nitro-L-arginine (L-NA), L-arginine, adenosine, and sodium nitroprusside were obtained from Sigma Co (St Louis, MO, USA), acetylcholine from Daiichi Pharmaceutical Co and UL-FS 49 from Nippon Boehringer Ingelheim Co. All drugs were dissolved in normal saline.

Induction of Pacing-Induced Heart Failure

Experiments were performed in mongrel dogs weighing 21.3±0.7 kg. Under general anesthesia, a bipolar pacing lead (1236T, Pace Setter Inc, Sylmer, CA, USA) was introduced into the right external jugular vein and advanced to the apical area of the right ventricle. A 7F catheter was inserted into the superior vena cava through the left external jugular vein under fluoroscopic guidance. The dogs were then allowed to recover from the surgery.

The lead was connected to an external pulse generator (Nikon-Kohden, Tokyo) and HF was induced by continuous rapid ventricular pacing at 220–260 beats/min for 4 weeks\textsuperscript{10–12}. This HF model is characterized by decreased myocardial contractility associated with LV dilation, resting hypotension and tachycardia, impaired coronary vasodilatory capacity in the control and HF dogs in a preliminary study (unpublished data). Thus, it is unlikely that UL-FS 49 had significant effects on myocardial function and coronary vasodilatory capacity. The criteria for inclusion in the present experiments were (1) an Hb concentration >9 g/dl; (2) an arterial pH of 7.35–7.45, a PO\textsubscript{2} of 100–200 mmHg, and a PCO\textsubscript{2} of 25–45 mmHg; and (3) coronary venous PO\textsubscript{2} <30 mmHg. These variations in the baseline conditions were similar between the 2 groups, and under these conditions the intrinsic coronary vasodilatory capacity is not compromised\textsuperscript{24,25}.

Experimental Protocols

After stabilization of hemodynamic parameters at a HR of 160 beats/min, 0.25 mg/kg of UL-FS 49 (a selective bradycardia agent) was administered intravenously to reduce the basal HR to less than 100 beats/min, as we had found in previous studies that UL-FS 49 reduced HR in normal dogs, but had no significant effects on myocardial contractility, metabolism, or the coronary vasodilatory capacity in response to intracoronary administration of adenosine, acetylcholine, and sodium nitroprusside\textsuperscript{24,25}. We obtained the same findings in HF dogs in a preliminary study (unpublished data). Thus, it is unlikely that UL-FS 49 had significant effects on myocardial function and coronary vasodilatory capacity in the control and HF dogs in the present study.

The criteria for inclusion in the present experiments were (1) an Hb concentration >9 g/dl; (2) a PBO2 of 100–200 mmHg, a PCO2 of 25–45 mmHg; and (3) coronary venous PO2 <30 mmHg. These variations in the baseline conditions were similar between the 2 groups, and under these conditions the intrinsic coronary vasodilatory capacity is not compromised\textsuperscript{24,25}.

Protocol 1

After baseline hemodynamics were recorded
for 2 min. 8 HF dogs and 8 control dogs underwent 3 trials of atrial pacing before and after intracoronary administration of L-NAME and after intracoronary administration of L-arginine. The CBF at the LAD and the AoP, HR, LVP, and LVEDP/dt were recorded during pacing. Before infusion of L-NAME, HR was increased in a stepwise fashion from 100 to 160 and then to 200 beats/min and at each stage CBF and other hemodynamic variables were allowed to stabilize for at least 2 min before the pacing rate was increased to the next level. MVO₂ was determined as in Protocol 1. We previously reported that there were no time-related changes in hemodynamic responses to pacing before and after drugs or between groups, 2-way ANOVA followed by Bonferroni’s multiple comparison test was used. A p value of less than 0.05 was considered statistically significant.

Results

There were no significant differences between the control and HF dogs in the arterial and coronary venous blood gas data, venous Hb concentration, and the plasma concentrations of C-reactive protein, creatinine, blood urea nitrogen, sodium, and potassium (Table 1). Plasma renin activity and norepinephrine concentrations were increased in the HF dogs (Table 1).

Effects of L-NAME and L-Arginine on Pacing-Induced Coronary Vasodilation (Protocol 1)

Intravenous administration of UL-FS 49 reduced HR, CBF and MVO₂ in both groups, but did not affect the other hemodynamic parameters (Tables 2, 3). The UL-FS 49-induced decreases in CBF were proportional to the decreases in MVO₂.

After administration of UL-FS 49, HR was maintained at 100 beats/min by atrial pacing in both control and HF dogs. The AoP, LVP, and LVEDP/dt were significantly lower and the LVEDP was significantly higher in HF dogs than in control dogs. There were no significant differences in CBF, MVO₂, or the oxygen metabolism between groups. Before administration of L-NAME, pacing-induced increases in HR (from 100 to 160 and then to 200 beats/min) increased CBF, with no significant changes in AoP, LVEDP, or LVEDP/dt, andHF dogs (Tables 2, 3). The pacing-induced increases in CBF were associated with corresponding increases in MVO₂ in the 2 groups (Fig 1).
Intracoronary administration of L-NAME did not alter CBF, MV\textsubscript{O}2, LV\textsubscript{EDP}, or the LVdp/dt at a HR of 100 beats/min, but significantly increased the AoP in the control dogs only (Tables 2, 3). L-NAME did not alter the pacing-induced increases in CBF, MV\textsubscript{O}2, or other hemodynamic parameters in the control dogs. In contrast, L-NAME significantly reduced the pacing-induced increase in CBF in the HF dogs but did not alter the pacing-induced increase in MV\textsubscript{O}2 (Fig 1). L-NAME reduced SvO\textsubscript{2} during rapid pacing at 160 and 200 beats/min.

Subsequent intracoronary administration of L-arginine had no effect on hemodynamic parameters at a HR of 100 beats/min in the control dogs, other than reversing the pressor effect of L-NAME on AoP in the control dogs. L-arginine did not alter the CBF response to pacing tachycardia in the control dogs, but reversed the effect of L-NAME on the CBF response to pacing tachycardia in the HF dogs (Tables 2, 3; Fig 1).

Reproducibility of Pacing-Induced Coronary Vasodilation (Protocol 2)

The responses of CBF and the other variables to pacing-induced tachycardia were similar in the first, second, and third experiments (data not shown).

Coronary Vasodilatory Responses to Acetylcholine and Sodium Nitroprusside (Protocol 3)

Before administration of L-NAME, the acetylcholine-induced increase in CBF was less in the HF dogs than in the control dogs (p<0.01) (Fig 2). L-NAME reduced the acetylcholine-induced increase in CBF in both groups. There was no significant difference in the percent increase

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Table 2  Hemodynamic Parameters During Pacing-Induced Tachycardia in Control Dogs (n=8) (Protocol 1)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Before UL-FS 49 at pacing rate of 160 beats/min</th>
<th>Before L-NAME (after UL-FS 49)</th>
<th>Pacing rate (beats/min)</th>
<th>After L-NAME</th>
<th>Pacing rate (beats/min)</th>
<th>After L-NAME + L-arginine</th>
<th>Pacing rate (beats/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CBF (ml/min per 100 g)</td>
<td>107±7</td>
<td>97±5</td>
<td>200</td>
<td>92±5</td>
<td>113±2†††</td>
<td>141±9†††</td>
<td>172±9†††</td>
</tr>
<tr>
<td>Mean AoP (mmHg)</td>
<td>106±2</td>
<td>107±8</td>
<td>200</td>
<td>110±7†</td>
<td>124±9††</td>
<td>96±2</td>
<td>111±3</td>
</tr>
<tr>
<td>LVP (mmHg)</td>
<td>126±1</td>
<td>125±9</td>
<td>120±8</td>
<td>110±4</td>
<td>123±11†††</td>
<td>111±3</td>
<td>113±2</td>
</tr>
<tr>
<td>LVEDP (mmHg)</td>
<td>6±1</td>
<td>6±1</td>
<td>4±1</td>
<td>5±1</td>
<td>6±1</td>
<td>5±1</td>
<td>5±1</td>
</tr>
<tr>
<td>Positive LVdp/dt (mmHg/s)</td>
<td>2,200±200</td>
<td>2,100±254</td>
<td>2,257±218</td>
<td>2,167±233</td>
<td>2,124±256</td>
<td>2,124±255</td>
<td>2,124±255</td>
</tr>
<tr>
<td>SvO\textsubscript{2} (%)</td>
<td>47±5</td>
<td>48±4</td>
<td>46±4</td>
<td>38±2</td>
<td>38±2</td>
<td>38±2</td>
<td>38±2</td>
</tr>
<tr>
<td>PrO\textsubscript{2} (mmHg)</td>
<td>24±1</td>
<td>25±2</td>
<td>23±1</td>
<td>22±1</td>
<td>22±1</td>
<td>22±1</td>
<td>22±1</td>
</tr>
<tr>
<td>MV\textsubscript{O} (ml/min per 100 g)</td>
<td>7.3±0.5</td>
<td>7.2±0.4</td>
<td>7.5±0.7</td>
<td>8.4±0.6</td>
<td>9.9±0.4*</td>
<td>10.2±0.7††</td>
<td>10.2±0.7††</td>
</tr>
</tbody>
</table>

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Table 3  Hemodynamic Parameters During Pacing-Induced Tachycardia in HF Dogs (n=8) (Protocol 1)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Before UL-FS 49 at pacing rate of 160 beats/min</th>
<th>Before L-NAME (after UL-FS 49)</th>
<th>Pacing rate (beats/min)</th>
<th>After L-NAME</th>
<th>Pacing rate (beats/min)</th>
<th>After L-NAME + L-arginine</th>
<th>Pacing rate (beats/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CBF (ml/min per 100 g)</td>
<td>104±8</td>
<td>111±6††</td>
<td>200</td>
<td>107±10*</td>
<td>131±2†††</td>
<td>147±2†††</td>
<td>160†††</td>
</tr>
<tr>
<td>Mean AoP (mmHg)</td>
<td>86±5</td>
<td>84±3</td>
<td>200</td>
<td>90±10</td>
<td>124±9*</td>
<td>96±2</td>
<td>111±3</td>
</tr>
<tr>
<td>LVP (mmHg)</td>
<td>100±5</td>
<td>95±4</td>
<td>120±8</td>
<td>110±4</td>
<td>123±11†††</td>
<td>111±3</td>
<td>113±2</td>
</tr>
<tr>
<td>LVEDP (mmHg)</td>
<td>18±2</td>
<td>24±2</td>
<td>19±1</td>
<td>5±1</td>
<td>6±1</td>
<td>5±1</td>
<td>5±1</td>
</tr>
<tr>
<td>Positive LVdp/dt (mmHg/s)</td>
<td>3,160±122</td>
<td>1,274±117</td>
<td>1,254±124</td>
<td>1,123±113</td>
<td>1,052±105</td>
<td>1,052±105</td>
<td>1,052±105</td>
</tr>
<tr>
<td>SvO\textsubscript{2} (%)</td>
<td>39±3</td>
<td>42±3</td>
<td>39±2</td>
<td>41±1</td>
<td>35±10*</td>
<td>35±10*</td>
<td>35±10*</td>
</tr>
<tr>
<td>PrO\textsubscript{2} (mmHg)</td>
<td>23±1</td>
<td>25±1</td>
<td>24±1</td>
<td>24±1</td>
<td>23±1</td>
<td>23±1</td>
<td>23±1</td>
</tr>
<tr>
<td>MV\textsubscript{O} (ml/min per 100 g)</td>
<td>7.7±0.8</td>
<td>5.2±0.3</td>
<td>7.5±0.6</td>
<td>10.2±0.7††</td>
<td>10.2±0.7††</td>
<td>10.2±0.7††</td>
<td>10.2±0.7††</td>
</tr>
</tbody>
</table>

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CBF, coronary blood flow; CPP, coronary perfusion pressure; AoP, aortic pressure; LVP, left ventricular pressure; LV\textsubscript{EDP}, left ventricular end-diastolic pressure; LVdp/dt, first derivative of left ventricular pressure; SaO\textsubscript{2}, O\textsubscript{2} saturation of arterial blood; SvO\textsubscript{2}, O\textsubscript{2} saturation of coronary venous blood; PrO\textsubscript{2}, O\textsubscript{2} tension of coronary venous blood; MV\textsubscript{O}2, myocardial oxygen consumption. *p<0.05 vs before L-NAME, †p<0.05, ††p<0.01 vs pacing rate of 100 beats/min.

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in CBF induced by acetylcholine after administration of L-NAME between the control and HF dogs. Subsequent administration of L-arginine reversed the CBF response to acetylcholine in the control and HF dogs.

The increases in CBF induced by sodium nitroprusside were not significantly different between the groups and were similar before and after administration of L-NAME and after administration of L-arginine (Fig 2).

Effects of L-NA on Pacing-Induced Coronary Vasodilation (Protocol 4)

The effect of L-NNA was the same as the effect of L-NAME in Protocol 1 (data not shown). Briefly, L-NA did not alter pacing-induced changes in CBF, M\O_2, or other hemodynamic parameters in the control dogs. In the HF dogs, L-NA did not alter the basal hemodynamic variables, but reduced the pacing-induced increase in CBF with no changes in M\O_2 or the other parameters.

Discussion

The role of endothelium-derived NO in the regulation of CBF in response to increased metabolic demand in the presence of HF is poorly understood. In the present study, before administration of L-NAME, the CBF and M\O_2 per unit of myocardial mass at a pacing rate of 100 beats/min or the increases in CBF in response to the increases in M\O_2 induced by pacing tachycardia did not differ between the 2 study groups. We examined the change in CBF in relationship to M\O_2, which is important for studies on the regulation of CBF in HF, because there can be significant differences in the hemodynamic parameters between normal conditions and HF. The CBF response in the presence of HF can be underestimated because coronary perfusion pressure in HF is less than normal.

The important finding of the present study is that inhibition of NO synthesis with L-NAME reduced the tachycardia-induced increase in CBF in HF dogs. Evidence suggests that NO may reduce M\O_2 but it is unlikely that the reduced CBF response to pacing after L-NAME resulted from decreases in M\O_2 because the reduced CBF response to pacing tachycardia was compensated for by increased myocardial oxygen extraction and thus the tachycardia-induced increase in M\O_2 was similar in both groups. The results of the time-control study indicate that the effects of L-NAME were not time related. It is also unlikely that L-NAME had nonspecific effects because L-arginine restored the effects of L-NAME on the CBF response to pacing tachycardia. The coronary vasodilatory response to sodium nitroprusside was similar before and after administration of L-NAME and after subsequent administration of L-arginine. The data suggest that during pacing tachycardia in dogs with HF, the coronary vascular L-arginine–NO pathway contributes to the regulation of CBF in response to increased metabolic demand.

Buxton et al reported that L-NAME and other alkyl esters of arginine have nonspecific muscarinic receptor blocking effects which raises the possibility that impairment of the coronary vasodilatory response to pacing following L-NAME was caused in part by blockade of muscarinic receptors. However, this is unlikely because when we examined the effect of L-NA, which lacks muscarinic receptor blocking effects, we found that L-NA impaired the CBF response to pacing tachycardia in HF dogs as did L-NAME.

In the present study, L-NAME did not significantly alter the CBF response to pacing tachycardia in the control dogs, which is in agreement with the results of some previous studies suggesting that NO may not play a major role in the overall increases in CBF during pacing and exercise under normal conditions. However, they contradict the
results of other studies. Although the explanation for this discrepancy is unclear, the absence of significant effects of NO inhibitors under physiological conditions does not necessarily indicate that NO does not contribute to the regulation of the coronary microvascular response to an increased metabolic demand. Jones et al found that constriction of relatively large microvascular segments caused by inhibition of NO synthesis was compensated for by dilation of smaller downstream microvascular segments. The compensatory microvascular dilation in response to inhibition of NO synthesis may result from increased release of adenosine and vasodilator prostaglandin, activation of K+ATP channels or both.

There is increasing evidence that endothelium-dependent (NO-mediated) dilation of the coronary and peripheral vasculatures is impaired in the presence of HF in both animals and humans. In particular, recent studies have demonstrated that the acetylcholine-induced NO-mediated increase in CBF and flow-induced dilation of isolated coronary arterioles are impaired in dogs with pacing-induced HF. The present findings showing that the vasodilatory response to acetylcholine and the pressor response to L-NAME were decreased in HF dogs agree with the results of those previous studies. We found in the present study that administration of L-NAME to HF dogs further inhibited the acetylcholine-induced increase in CBF and subsequently inhibited the tachycardia-induced increase in CBF. It is possible that in the presence of HF, which is associated with activation of several important coronary vasodilatory mechanisms, including NO, adenosine, vasodilator prostaglandin, and K+ATP channels, inhibition of an important vasodilatory mechanism, such as NO, reduces coronary vasodilation in response to increased metabolic demand. In fact, K+ATP channels are already activated at baseline in HF dogs as we have reported before and adenosine release is also increased in HF at baseline (data not shown), hence the compensating vasodilatory system under inhibition of NO, such as K+ATP channels or adenosine, could have less contribution to the metabolic vasodilation in comparison with normal conditions.

It should be noted that we used anesthetized open-chest dogs to determine the role of NO in regulation of metabolic coronary vasodilation in HF. General anesthesia is known to considerably affect local metabolic and autonomic responses of the coronary circulation, so although we carefully determined the effects of NO synthesis inhibitors, caution should be exercised in extrapolating our findings to the clinical setting.

Many patients with HF have one or more risk factors, such as aging, hypertension, hyperlipidemia and diabetes mellitus, all of which may impair NO synthesis by coronary vascular endothelial cells. Reduced vascular NO synthesis may reduce myocardial perfusion as well as induce fibroproliferative changes by stimulating the production of growth-promoting factors and extracellular matrix proteins. If these changes occur in the presence of HF, myocardial dysfunction may be worsened. Recent clinical reports have shown administration of angiotensin-converting enzyme inhibitors to patients with angiographically normal coronary arteries or minor coronary artery disease improves coronary endothelial function, possibly by increasing the NO-generating capacity. The results of the present study suggest that NO contributes to the regulation of CBF in response to the increased metabolic demand in HF.

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