EXPERIMENTAL STUDIES

Attenuation of Angiotensin II-Mediated Coronary Vasoconstriction and Vasodilatory Action of Angiotensin-Converting Enzyme Inhibitor in Pacing-Induced Heart Failure in Dogs

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OBJECTIVES
We investigated the changes in coronary vascular resistance caused by angiotensin II, angiotensin-converting enzyme (ACE) inhibition and angiotensin II type 1 or 2 receptor (AT1R and AT2R, respectively) antagonists in chronic heart failure (CHF).

BACKGROUND
Angiotensin II is an intense vasoconstrictor, and increased angiotensin II in CHF might exert significant vasoconstriction.

METHODS
Eleven dogs were studied. Before and after three and five weeks of rapid pacing, coronary flow dynamics were evaluated by the coronary pressure–flow relationship (PFR) in long diastole, before and after intracoronary injection of angiotensin II, the ACE inhibitor enalaprilat, the AT1R antagonist L158,809 or the AT2R antagonist PD123319.

RESULTS
Before rapid pacing, angiotensin II reduced the slope of PFR (1.16 ± 0.08 to 0.81 ± 0.07 ml/min/100 g left ventricular mass per mm Hg; p < 0.01) and increased the perfusion pressure at which coronary flow ceased (zero-flow pressure [Pf = 0]), whereas enalaprilat did not change either of them. After rapid pacing, angiotensin II did not change the slope or Pf = 0. In contrast, enalaprilat increased the slope (three weeks: 1.20 ± 0.05 to 1.50 ± 0.03; five weeks: 1.25 ± 0.19 to 1.37 ± 0.08; both p < 0.05) and decreased Pf = 0 after three weeks of pacing, but not after five weeks. Pretreatment with the bradykinin antagonist HOE-140 attenuated the enalaprilat-induced increase in coronary blood flow. L158,809 and PD123319 had no effect both before and after rapid pacing.

CONCLUSIONS
This suggests that the coronary vasoconstrictive effect of angiotensin II would disappear and the vasodilatory effect of the ACE inhibitor, partly through bradykinin, would be enhanced in the early stage of CHF. (J Am Coll Cardiol 2001;38:1188–94) © 2001 by the American College of Cardiology

There is considerable evidence of diminished coronary flow reserve in chronic heart failure (CHF) (1–3), which might play a role in its development and progression. With respect to this, not only would increased ventricular filling pressure and elevated coronary venous pressure impede coronary inflow (4–7), but also various neurohumoral factors activated in CHF, such as sympathetic neuron-derived norepinephrine (NE) (8), angiotensin II (9) and endothelin-1 (10), would affect coronary vascular tone. Recently, the clinical benefits of angiotensin-converting enzyme (ACE) inhibition and angiotensin II type 1 receptor (AT1R) antagonism in the treatment of CHF have been reported (11–13). Angiotensin II has been shown to exert a direct vasoconstrictive effect on coronary arteries in normal subjects (14), and in CHF, it increases not only in circulating blood but also in myocardial tissue. However, it is not known how increased angiotensin II exerts a coronary vasoconstrictive action in CHF. It has been reported recently that AT1Rs are selectively downregulated, and angiotensin II type 2 receptors (AT2Rs) are relatively increased in chronically failing ventricular myocardium (15). If these changes also occur in the coronary arteries, it is possible that they might lead to attenuation of angiotensin II-induced vasoconstriction. In contrast, ACE inhibition increases coronary blood flow in patients with dilated cardiomyopathy (16). However, it is not clear how such vasodilatory action is brought about in CHF after administration of the ACE inhibitor.

The purpose of this study was to investigate whether increased angiotensin II exerts a strong coronary vasoconstrictive action in CHF in a dose-dependent manner, as in the normal heart, and whether the vasodilatory action of the ACE inhibitor is observed, and, if it is, whether it is related to an increase in bradykinin. We investigated the effects of angiotensin II, ACE inhibition and AT1R antagonism on the coronary circulation before and after pacing-induced heart failure in dogs (17–19). In addition, the effect of AT2R antagonist was also examined, because the role of the AT2R-mediated pathway on coronary vascular tone has not been fully defined.
were given intramuscular injections of ketamine hydrochloride, and the Japanese Government Animal Protection and Management Guidelines on Animal Experiments at Fukushima Medical University School of Medicine (approval no. 980010) and in accordance with the University School of Medicine (approval no. 980010) and the Japanese Government Animal Protection and Management Law (no. 115).

Baseline study. Seven days after the operation, the animals were given intramuscular injections of ketamine hydrochloride (10 mg/kg), and they were then intubated and artificially ventilated as described previously. Eight-French sheath introducers (Termo Co., Ltd., Tokyo, Japan) were inserted into the left carotid artery and external jugular vein for monitoring arterial and central venous pressure, respectively, using pressure transducers and amplifiers (AP-641G, Nihon Koden, Tokyo, Japan). Then, a bolus of 100 IU/kg heparin sodium was injected, followed by 50 IU/kg per h throughout the experiment.

BLOOD SAMPLING. Fifteen milliliters of arterial blood were drawn to measure NE, angiotensin II, atrial natriuretic peptide (ANP) and endothelin-1 concentrations. The blood was collected into tubes containing EDTA and placed on ice immediately. After centrifugation for 15 min at 3,000 rpm at 4°C, the plasma was separated and stored at −20°C. Plasma NE levels were determined by high performance liquid chromatography (21), and ANP, endothelin-1 and angiotensin II concentrations were measured by radioimmunoassay (22–24).

HEMODYNAMIC MEASUREMENTS. Left ventricular pressure, left ventricular end-diastolic pressure, maximal positive and negative left ventricular pressure derivatives (+dP/dt and −dP/dt, respectively) and aortic pressure were measured with pressure amplifiers using a transducer-tipped pressure monitoring catheter (4F Camino, San Diego, California), which was inserted from the left carotid artery into the left ventricle. Cardiac output and pressures in the right side of the heart were measured using a Swan-Ganz catheter (7F, model 93-121A, American Edwards Laboratories, Santa Ana, California).

EVALUATION OF THE CORONARY PRESSURE-FLOW RELATIONSHIP (PFR). A 5F hand-crafted right Judkins-type catheter was inserted through the sheath introducer into and through the left carotid artery for drug administration into the left coronary artery. The pressure through the sheath introducer inserted into the left carotid artery was regarded as the coronary perfusion pressure. During transient stopping of the pacing, coronary perfusion pressure and coronary blood flow were measured continuously under the following conditions at baseline (n = 11) and after three (n = 11) and five weeks (n = 5) of rapid pacing: 1) before drug administration; 2) 10 s after intracoronary infusion of angiotensin II (0.1, 1.0 and 10 ng/kg) for 30 s; 3) 2 min after bolus injection of the ACE inhibitor enalaprilat (0.2 mg/kg); 4) 2 min after bolus injection of the selective AT1R antagonist L158,809 (0.1 mg/kg) (25); 5) 2 min after bolus injection of the selective AT2R antagonist PD123319 (0.2 mg/kg) (26); and 6) 10 s after intracoronary injection of angiotensin II (10 ng/kg) after blockade of AT1R or AT2R. Then, the effect of enalaprilat was assessed after inhibition of the bradykinin type 2 receptor by intracoronary administration of 0.5 mg of HOE-140 (27) to 4 of the 11 dogs after three weeks of rapid pacing.
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Table 1. Hemodynamic Data and Neurohumoral Variables at 100 Beats/Min Pacing Rate

<table>
<thead>
<tr>
<th></th>
<th>Baseline (n = 11)</th>
<th>Three Weeks of Pacing (n = 11)</th>
<th>Five Weeks of Pacing (n = 5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP (mm Hg)</td>
<td>78.6 ± 2.4</td>
<td>67.4 ± 4.3*</td>
<td>72 ± 1.3*</td>
</tr>
<tr>
<td>RAP (mm Hg)</td>
<td>2.8 ± 0.9</td>
<td>9.6 ± 0.9†</td>
<td>9.0 ± 0.9†</td>
</tr>
<tr>
<td>LVEDP (mm Hg)</td>
<td>8.4 ± 0.7</td>
<td>22.6 ± 1.4†</td>
<td>22.5 ± 2.4†</td>
</tr>
<tr>
<td>+dP/dt (mm Hg/s)</td>
<td>1.64 ± 0.4</td>
<td>866 ± 7†</td>
<td>1,043 ± 46†</td>
</tr>
<tr>
<td>−dP/dt (mm Hg/s)</td>
<td>1,328 ± 72</td>
<td>995 ± 74*</td>
<td>1,124 ± 90*</td>
</tr>
<tr>
<td>CO (liters/min)</td>
<td>1.52 ± 0.08</td>
<td>0.75 ± 0.04‡</td>
<td>0.74 ± 0.08‡</td>
</tr>
<tr>
<td>LVEF (%)</td>
<td>45.2 ± 3.2</td>
<td>25.2 ± 2.0†</td>
<td>22.2 ± 2.2†</td>
</tr>
<tr>
<td>NE (ng/ml)</td>
<td>0.10 ± 0.05</td>
<td>0.75 ± 0.24*</td>
<td>0.89 ± 0.19*</td>
</tr>
<tr>
<td>ANP (pg/ml)</td>
<td>56 ± 10</td>
<td>255 ± 17*</td>
<td>381 ± 44‡</td>
</tr>
<tr>
<td>ET-1 (pg/ml)</td>
<td>4.8 ± 0.3</td>
<td>8.6 ± 1.0</td>
<td>10.8 ± 1.0†</td>
</tr>
<tr>
<td>Angiotensin II (pg/ml)</td>
<td>24 ± 6</td>
<td>283 ± 98*</td>
<td>363 ± 88*</td>
</tr>
<tr>
<td>Coronary flow (ml/min per 100 g)</td>
<td>56.3 ± 4.6</td>
<td>51.4 ± 4.4</td>
<td>54.3 ± 3.1</td>
</tr>
<tr>
<td>LV mass of LAD perfusion area (g)</td>
<td>25.6 ± 3.1</td>
<td>29.8 ± 3.2</td>
<td>30.5 ± 2.7</td>
</tr>
</tbody>
</table>

*p < 0.05 and †p < 0.01 versus corresponding value before pacing. Data are presented as the mean value ± SEM.


correlation coefficient of linear regression was \( r = 0.99 \pm 0.01 \), the use of linear regression analysis seemed to be reasonable. Then, because the zero-flow pressure \( (P_f = 0) \) value was sometimes more difficult to determine accurately in actual tracings, we obtained \( P_f = 0 \) by extrapolation of the regression line in each PFR. There were no large differences between extrapolated the \( P_f = 0 \) value and \( P_f = 0 \) determined from the flowmeter curves.

Statistical analyses. Data are expressed as the mean value ± SEM. Multiple comparisons were performed using analysis of variance followed by the Fisher post hoc comparisons test. For comparison of paired data, the Student t test was used. A p value <0.05 was considered statistically significant.

RESULTS

Changes in hemodynamic and neurohumoral variables. Table 1 shows the hemodynamic and neurohumoral data obtained with 100 beats/min of right ventricular pacing before and after three and five weeks of rapid pacing. At three weeks of rapid pacing, the mean right atrial pressure and left ventricular end-diastolic pressure increased significantly, whereas the mean aortic pressure, +dP/dt and −dP/dt and cardiac output decreased significantly. Plasma NE, ANP, endothelin-1 and angiotensin II increased significantly after rapid pacing. However, from three to five weeks of rapid pacing, significant changes were not observed.

Coronary PFR before and after three and five weeks of rapid pacing. After rapid pacing, the coronary blood flows with respect to each corresponding perfusion pressure did not change, as compared with the baseline values (i.e., the slope of PFR did not change significantly—baseline: 1.16 ± 0.08; three weeks: 1.20 ± 0.05; five weeks: 1.29 ± 0.08 ml/min/100 g left ventricular mass per mm Hg; \( p = \) NS for three and five weeks vs. baseline), whereas \( P_f = 0 \)

LEFT VENTRICULOGRAPHY. Fluoroscopic images were obtained with a Toshiba X-ray system (model KXO-15D). Left ventriculography was performed through a 6F pigtail catheter, which was inserted through the sheath introducer into the left carotid artery. Left ventriculograms were analyzed using a video projector (Ikegami Picture Monitor PM 14-3H, Ikegami Tsushinki, Co., Ltd., Tokyo, Japan). The end-diastolic and end-systolic frames in the end-expiratory phase were selected for volumetric analysis.

Correction of coronary blood flow with respect to myocardial weight. To delineate the perfusion area of the left anterior descending coronary artery, the catheter was advanced just distal to the flow probe, and Evans blue dye (10 mg/ml, Wako Co. Ltd.) was injected through the catheter, which was inserted through the sheath introducer. After sacrificing the dogs, the wet weight of the cardiac weight.

The corresponding weight of the perfusion area was measured, and catheter. After sacrificing the dogs, the wet weight of the myocardium in the perfusion area was measured, and coronary blood flow was normalized to flow per 100 g of left ventricular mass. The corresponding weight of the perfusion area before rapid pacing was estimated by employing the ventricular mass. The corresponding weight of the perfusion area was measured, and catheter. After sacrificing the dogs, the wet weight of the myocardium in the perfusion area was measured, and coronary blood flow was normalized to flow per 100 g of left ventricular mass. The corresponding weight of the perfusion area before rapid pacing was estimated by employing the following equation using the left ventriculogram: left ventricular mass = \( \pi/6 \times (L'D'^2 - L'D^2) \) CF^3. Thus, left ventricular mass before pacing equals left ventricular mass measured after sacrifice \( \times (L'D'^2 - L'D^2) \) before pacing/ \( [L'D'^2 - L'D^2] \) after pacing), where L and D are the longest lengths of the long and short axes, respectively, in the ventricular chamber; L' and D' are the corresponding lengths of the pericardial silhouette; and CF^3 is the volume correction factor proposed by Kennedy et al. (28).

Slope and zero-flow pressure of PFR. After stopping the pacing, coronary blood flow was measured at every 5 mm Hg of coronary perfusion pressure after the incisure of the last arterial pressure curve before stopping the pacing until reaching zero flow. The slope of the regression line calculated by using all data points, excluding the point of zero flow, was defined as the slope of PFR. As the
increased slightly at three weeks (baseline: 31.1 ± 1.2; three weeks: 33.9 ± 0.9 [p < 0.05 vs. baseline]; five weeks: 31.7 ± 2.2 mm Hg).

**Effect of angiotensin II.** Before rapid pacing (n = 11), angiotensin II decreased coronary blood flow in a dose-dependent manner (Fig. 1a). After administration of 10 ng/kg of angiotensin II, the slope of PFR decreased significantly from 1.16 ± 0.08 to 0.81 ± 0.07 ml/min/100 g left ventricular mass per mm Hg (p < 0.01), and P_f = 0 increased significantly from 31.1 ± 1.2 to 33.7 ± 1.3 mm Hg (p < 0.05). Coronary blood flow decreased by 44.2 ± 10.9% at 40 mm Hg, by 50.3 ± 4.9% at 50 mm Hg and by 40.3 ± 3.0% at 60 mm Hg of perfusion pressure. In contrast, angiotensin II changed neither the slope of PFR (three weeks: 1.20 ± 0.05 to 1.18 ± 0.04; five weeks: 1.29 ± 0.08 to 1.27 ± 0.18 ml/min/100 g left ventricular mass per mm Hg; p = NS) nor the P_f = 0 (three weeks: 33.9 ± 0.9 to 33.7 ± 0.9; five weeks: 31.7 ± 2.2 to 31.5 ± 2.2 mm Hg; p = NS) after both three (n = 11) and five weeks (n = 5) of rapid pacing (Fig. 1b, c).

**Effect of ACE inhibition.** The slope of PFR and the P_f = 0 did not alter after intracoronary injection of enalaprilat before rapid pacing (n = 11) (Fig. 2a). However, the slope of PFR was increased significantly by enalaprilat after three (n = 11) and five weeks (n = 5) of rapid pacing (three weeks: 1.20 ± 0.05 to 1.50 ± 0.03; five weeks: 1.25 ± 0.19 to 1.37 ± 0.08 ml/min/100 g left ventricular mass per mm Hg; p < 0.05 for after vs. before enalaprilat at three and five weeks), and P_f = 0 decreased significantly after three and five weeks of rapid pacing (three weeks: 33.9 ± 0.9 to 33.7 ± 0.9; five weeks: 31.7 ± 2.2 to 31.5 ± 2.2 mm Hg; p = NS) after both three (n = 11) and five weeks (n = 5) of rapid pacing (Fig. 1b, c).
32.3 ± 1.7 to 27.0 ± 1.3 mm Hg; five weeks: 31.4 ± 0.9 to 28.0 ± 0.9 mm Hg; p < 0.05 vs. before enalaprilat at three and five weeks) (Fig. 2b, c). Thus, the vasodilatory effect of enalaprilat appeared in the failing state.

**Effect of bradykinin antagonist on coronary vasodilation induced by ACE inhibition.** After infusion of HOE-140, coronary blood flow did not change before and after rapid pacing (data not shown). However, after three weeks of pacing, the enalaprilat-induced increase in the slope of PFR (control state: 1.21 ± 0.04; enalaprilat: 1.45 ± 0.05 [p < 0.05 vs. control state]; pretreatment with HOE-140: 1.28 ± 0.04 ml/min/100 g left ventricular mass per mm Hg), as well as the decrease in P_f = 0 (control state: 32.3 ± 1.7; enalaprilat: 27.0 ± 1.3 [p < 0.05 vs. control state]; pretreatment with HOE-140: 31.8 ± 0.9 mm Hg) disappeared after pretreatment with HOE-140.

**Role of AT_1 and AT_2 receptors.** L158,809 and PD123319 did not alter the slope of PFR and P_f = 0 at baseline (data not shown). However, the administration of angiotensin II after the AT_2R antagonist PD123319 decreased the slope of PFR before rapid pacing, indicating that coronary flow reduction after angiotensin II is induced through AT_1R (slope before PD123319, after PD123319 and angiotensin II after PD123319: 1.11 ± 0.09, 1.15 ± 0.08 and 0.90 ± 0.04 [p <
neither L158,809 nor PD123319 changed the slope of PFR or P$_T$ = 0 values; 30.0 ± 1.1, 30.3 ± 1.4 and 32.1 ± 0.9 mm Hg; p = NS). Moreover, neither L158,809 nor PD123319 changed the slope of PFR or P$_T$ = 0, despite intracoronary administration of angiotensin II (10 ng/kg) after rapid pacing (data not shown).

**DISCUSSION**

The major findings of this study may be summarized as follows: first, angiotensin II had a vasoconstrictive effect on the coronary circulation through AT$_1$R in a dose-dependent manner, and endogenous angiotensin II levels would be too low to have this effect in the intact canine heart. Second, the coronary vasoconstrictive effect of angiotensin II through AT$_1$R was greatly attenuated in the failing heart. In other words, the coronary vasculature became insensitive to angiotensin II. Third, a significant vasodilatory effect of ACE inhibition was observed in the failing heart, especially in the relatively early stage of CHF that was partly caused by the accumulation of bradykinin, in addition to the desensitization to an increase in angiotensin II. Fourth, increases in coronary vasomotor tone. However, in this procedure, several issues should be taken into account for evaluating the slope of PFR. First, the coronary perfusion pressure after rapid pacing decreased moderately, leading to underestimation of the P$_T$ = 0 value (29). Second, the effects of vascular compliance in coronary vascular beds on diastolic PFR depend on the speed of decrease in perfusion pressure (5,6). Because there was no significant difference in these procedures within the same group or between the baseline and failing heart groups, the effects on coronary hemodynamic data are likely to be similar in this study. Third, as previously mentioned, linear extrapolation of coronary PFRs was used to determine the P$_T$ = 0 value, leading to overestimation of the P$_T$ = 0 value (5). Fourth, increases in right atrial pressure and left ventricular end-diastolic pressure, which have been reported to affect PFR (4–7), were observed in the failing heart. However, in vivo conditions, PFRs before rapid pacing and three and five weeks after rapid pacing did not differ, probably due to many compensatory factors. Moreover, comparisons of PFRs with and without treatment in the same heart were done using intracoronary injection of drugs; thus, preload changes were negligible. Fifth, we used only single concentrations of enalaprilat, the AT$_1$R antagonist L158,809, the AT$_1$R antagonist PD123319 and HOE-140 for bradykinin type 2 receptor blockade. However, concentrations of enalaprilat (0.2 mg/kg), L158,809 (0.1 mg/kg), PD123319 (0.2 mg/kg) and HOE-140 (0.5 mg/kg) seemed to be sufficient for intracoronary administration (27,30,31).

**Attenuation of the vasoconstrictive effect of angiotensin II in CHF.** In the nonfailing heart, exogenously administered angiotensin II had a significant vasoconstrictive effect on the coronary circulation through the AT$_1$R pathway. It should be noted that this vasoconstrictive change occurred despite the availability of compensatory coronary vasomotor mechanisms, which may have minimized the magnitude of the response to the agent. In contrast, the AT$_1$R or AT$_2$R antagonist alone did not change coronary blood flow, suggesting that the AT$_1$R or AT$_2$R pathways had little effect on coronary vasomotion in the normal heart. In contrast, in the failing heart, the vasoconstrictive effect of exogenously administered angiotensin II was almost gone, suggesting that exogenous, as well as endogenous, angiotensin II may not have a vasoconstrictive effect on the coronary circulation in CHF. With respect to this, Asano et al. (15) reported selective downregulation of AT$_1$R in failing human ventricles. Thus, there is a possibility that an altered response to angiotensin II through desensitization of AT$_1$Rs and/or a decrease of AT$_2$Rs might occur in vascular smooth muscle cells in the coronary arterial wall.

**Vasodilatory effect of ACE inhibition in CHF.** Because AT$_1$R and AT$_2$R antagonists had no effect on the coronary circulation, not only in the baseline state, but also in the heart failure state, probably due to the insensitivity to angiotensin II, the coronary vasodilatory effect of ACE inhibition in the present heart failure model seemed to be partly mediated through endothelium-derived nitric oxide (NO) and prostacyclin, caused by accumulated bradykinin (32–34). In previous studies using a similar pacing-induced heart failure model in dogs, plasma levels of bradykinin were elevated fourfold (34), and plasma levels of bradykinin were 4- to 10-fold increased by enalaprilat (35). The different responses to ACE inhibition of control animals and those with CHF in this study seem to be related to bradykinin levels attained after ACE inhibition, but not to endogenous bradykinin before the administration of the ACE inhibitor, as suggested from the study using HOE-140. In contrast, it has been reported that coronary flow decreased after treatment with HOE-140 in a similar CHF model (34). The reason for the different results is unclear, but methodologic differences must be considered.

In this study, in the late stage of CHF after five weeks of rapid pacing, the difference in coronary flow with and without the ACE inhibitor appeared to be attenuated, as compared with that in the early stage of CHF after three weeks of rapid pacing. According to our previous study using the same model, an increase in coronary blood flow through endothelium-derived NO was preserved until three weeks, but was attenuated after five weeks of rapid ventricular pacing (36). The NO-releasing capacity of the vascular endothelium, presumably through bradykinin release, might also participate in the alteration of the vasodilatory effect of ACE inhibition (37).

**Effect of AT$_2$R on coronary flow dynamics.** Asano et al. (15) demonstrated that AT$_1$R, but not AT$_2$R, densities are decreased significantly in ventricles with idiopathic dilated cardiomyopathy. However, the localization and function of AT$_2$R in the coronary circulation of the failing heart have not been elucidated. The results of this study suggest that regul-
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In contrast to the action of angiotensin II in the peripheral circulation, the vasoconstrictive effect of the angiotensin II receptor in the coronary circulation would be greatly attenuated in CHF, which might protect against myocardial injury through coronary artery constriction, as expected from significantly increased angiotensin II. Enhanced bradykinin accumulation by ACE inhibition, as well as desensitization to increased angiotensin II, would increase coronary blood flow at least in the short term in CHF. It is necessary to clarify whether the beneficial effect of ACE inhibition on the coronary circulation may also be expected in clinical settings, irrespective of the severity of CHF, and whether long-term treatment with ACE inhibition promotes this vasodilatory action in the coronary circulation.

References