Bradykinin Improves Left Ventricular Diastolic Function Under Long-Term Angiotensin-Converting Enzyme Inhibition in Heart Failure

Masanori Fujii, Atsuyuki Wada, Takayoshi Tsutamoto, Masato Ohnishi, Takahiro Isono, Masahiko Kinoshita

Abstract—Both systolic and diastolic cardiac dysfunction coexist in various degrees in the majority of patients with heart failure. Although ACE inhibitors are useful in the treatment of heart failure, the roles of bradykinin in the systolic and diastolic properties of left ventricular function under long-term treatment of ACE inhibitor have not been fully elucidated. We therefore evaluated the changes in left ventricular function, histomorphometry, and the expression of several failing heart related genes, by use of an orally active specific bradykinin type 2 receptor antagonist, FR173657 (0.3 mg/kg per day), with an ACE inhibitor, enalapril (1 mg/kg per day), in dogs with tachycardia-induced heart failure (270 ppm, 22 days) and compared the effects to enalapril alone. Although there were no differences observed in blood pressure, left ventricular dimension, and percentage of fractional shortening, FR173657 significantly increased left ventricular filling pressure (P<0.01), prolonged the time constant of relaxation (P<0.05), and suppressed the expression of endothelial NO synthase and sarcoplasmic reticulum Ca2+-ATPase mRNA (P<0.05). FR173657 also upregulated collagen type I and III mRNA (P<0.05) and increased the total amount of cardiac collagen deposits (P<0.05) in left ventricle compared with that in the enalapril-treated group. In conclusion, endogenous bradykinin contributes to the cardioprotective effect of ACE inhibitor, improving left ventricular diastolic dysfunction rather than systolic dysfunction, via modification of NO release and Ca2+ handling and suppression of collagen accumulation. (Hypertension. 2002;39:952-957.)

Key Words: angiotensin-converting enzyme ■ bradykinin ■ Ca2+-transponding ATPase ■ diastole ■ fibrosis ■ heart failure ■ ventricular function, left

Angiotensin-converting enzyme inhibitors (ACEIs) exert beneficial effects on cardiac contractile function and are useful in improving both the symptoms and survival rate of a broad spectrum of patients with congestive heart failure (CHF).1 In addition to suppressing the formation of angiotensin (Ang) II, the agents also prevent the breakdown of the potent vasodilator bradykinin (BK), which exerts its vasodilative action through BK type 2 (B2) receptors.2 Cardiomyocytes and cardiac fibroblasts have the capacity to generate BK, and they express functional B2 receptors. BK possesses not only vasodilative action but also inotropic and antiproliferative properties.3 Indeed, an ACEI increased the left ventricular (LV) ejection fraction and reduced LV chamber dimension and mass, but these effects were attenuated in B2 receptor knockout mice.4 In the clinical setting, however, the majority of patients with what is currently called “systolic heart failure” also have diastolic dysfunction of various degrees.5 Ang II affects diastolic LV function through the changed composition of the LV wall (ie, increased interstitial fibrosis or fibrous content) and by virtue of the altered activity of the myofibroblasts in CHF. Because BK also possesses antiproliferative properties, ACEIs must participate in cardioprotection through the alteration of localized BK concentrations in cardiac tissue. Captopril caused a selective LV relaxation without altering LV peak positive dP/dt (dP/dt max), and the effects of the ACE inhibitor were attenuated with B2 receptor blockade in the isolated heart.6

In patients with LV hypertrophy, an acute infusion of enalaprilat into the coronary artery improved isovolumic relaxation or LV diastolic chamber distensibility.7,8 Thus, although ACE inhibitors seem to cause the acute enhancement of LV relaxation via the BK pathway, the relative role of BK in LV systolic and diastolic function, under long-term treatment with ACEIs, in the process of CHF has not as yet been well documented. Therefore, we investigated (1) cardiac hemodynamic and geometric changes and (2) the changes in the expression of molecular markers and the histomorphometry in the failing heart with long-term concomitant treatment...
of enalapril and an orally active specific B2 receptor antagonist, FR173657 (FR), in dogs with CHF.

Methods

Animal Preparation

All animal experiments were conducted according to the Guidelines for Animal Experimentation at the Animal Research Committee of Shiga University of Medical Science. Dogs were bred at Kitayama Labes Co, Ltd (Nagano, Japan). CHF was induced by rapid right ventricular pacing in beagles, as previously described.6,10 After the dogs recovered from instrumental surgery for ≥14 days, the pacemaker was programmed at a rate of 270 bpm, and pacing was continued for 22 days.

Experimental Protocol

Study 1: Inhibitory Pressor Effect of FR in Response to Exogenous BK Injection

The selective nature of FR (Fujisawa Pharmaceutical Co) as a B2 receptor antagonist has been demonstrated to be orally active and to have long duration of action in vivo, high affinity, and optimal selectivity and specificity.11 To confirm the in vivo effect of FR, we observed its inhibitory effect on mean arterial pressure response to exogenously administered human BK. Six dogs were randomly selected and studied after completely recovering from the effects of surgery. After hemodynamics were stabilized, BK (30, 100, and 300 ng) was injected as a bolus. After 3 days of the first study, FR (0.3 mg/kg) was orally administered to each dog 6 hours before 3 doses of BK were injected.

Study 2: Cardiohormonial Effects of ACEI With or Without B2 Receptor Antagonist in Dogs With CHF

To compare the long-term effects of an ACEI with or without a B2 antagonist on the changes in LV geometry, hormones, and LV gene expression during the progression of CHF, the dogs were divided into 4 groups in a randomized fashion: (1) the ACEI group (n=6) received enalapril (1 mg/kg per day orally once a day); (2) the combination group (n=6) received enalapril and a B2 receptor antagonist, FR (1 and 0.3 mg/kg per day orally once a day, respectively); (3) the vehicle group (n=6) received only placebo and constituted time controls; and (4) the normal group (n=6) received the same operation without pacing. Drug treatment commenced on day 8 after the initiation of pacing and continued for 2 weeks. On the 22nd day after the initiation of pacing, the pacemaker was deactivated, and hemodynamic and echocardiographic measurements were subsequently performed with the dogs in the conscious state as described in our previous reports.12,13 Blood was collected for hormonal assays of plasma atrial natriuretic peptide and aldosterone concentrations and plasma renin activity, as previously described.6,13 Thereafter, a high-fidelity micromanometer catheter (SPC-350, Millar Instruments Inc) was placed into the LV under anesthesia. We subsequently measured dP/dtmax (maximal rate of pressure development/maximum first derivative of LV pressure) as an isovolumic phase index of LV maximum contractility, LV end-diastolic pressure (LVEDP) as an index of reduced LV diastolic distensibility, and the time constant of LV pressure decay, τ, which is an indicator of slow isovolumic LV relaxation.14

Quantification of mRNA

After the in vivo measurements were completed, the dogs were deeply anesthetized and euthanized by bleeding. The heart was rapidly removed, and portions of the LV free walls were immediately frozen in liquid nitrogen. For the analysis of myocardial canine sarco(endo)plasmic reticulum Ca2+-ATPase (SERCA2), endothelial NO synthase (eNOS), and collagen type I and III gene expression, total RNA was extracted from the frozen LV myocardium as previously reported.6,15 The levels of mRNA were evaluated by the quantitative reverse transcription–polymerase chain reaction (RT-PCR) procedure. Briefly, first-strand cDNA was synthesized from 5 μg of total RNA, and 1-μg aliquots of cDNA were amplified as previously described.16 Canine SERCA2- and eNOS-specific primer pairs were synthesized according to the published literature.17,18 Putative canine PCR primers collagen type I and III and GAPDH genes were designed based on homology with the highly conserved regions of the coding sequence of the human genes.19,20 The PCR products were then resolved by electrophoresis and were quantified. The intensity of each band was normalized relative to the GAPDH band density.

Histomorphometric Analysis: Interstitial Fibrosis

The specimens of LV were stained with picrosirus red stain to evaluate the degree of fibrosis, according to our previous report.21 Twenty microscope fields were randomly selected and digitized at a magnification of ×100. In the LV, any fields containing vessels, minor scars, or artifacts were excluded from the study.

Statistical Analysis

Data are expressed as mean±SEM. Differences among the 4 groups were assessed by use of ANOVA with the Scheffe’s F test when appropriate. A value of P<0.05 was considered significant.

An expanded Methods section can be found in an online data supplement available at http://www.hypertensionaha.org.

Results

Blocking Effect of FR on BK-Induced Hypotension

The inhibitory effects of FR on BK-induced maximal changes in mean arterial pressure are shown in Figure 1. Although stepwise-administered BK elicited depressor responses within 1 minute in a dose-dependent fashion and the blood pressure rapidly returned to baseline levels within 2 minutes, oral pretreatment with FR at a dose of 0.3 mg/kg significantly blocked these BK-induced depressor responses.

Effects on Hemodynamics and LV Function and Dimensions After Long-Term ACEI Treatment With or Without B2 Receptor Antagonist

As shown in the Table, after 3 weeks of pacing, mean arterial pressure and cardiac output decreased and heart rate increased in the vehicle group compared with normal values. Although mean arterial pressure and heart rate did not differ among the 3 pacing groups, enalapril and the concomitant with enalapril and FR group significantly improved cardiac output associated with the decreased in systemic vascular resistance compared with that of the vehicle group and tended to increase dP/dtmax. LV dimension and contractile measure-
Effects of the ACEI and the Coadministration of B₂ Receptor Antagonist on Hemodynamics, LV Geometry, LV Function, and Hormonal Factors in Normal and CHF Dogs

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Normal</th>
<th>CHF</th>
<th>ACEI</th>
<th>ACEI + FR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean arterial pressure, mm Hg</td>
<td>122±8</td>
<td>90±4†</td>
<td>96±7‡</td>
<td>95±3†</td>
</tr>
<tr>
<td>LVEDP, mm Hg</td>
<td>4.3±0.6</td>
<td>21.7±2.23‡</td>
<td>11.9±1.4§</td>
<td>20.0±0.7</td>
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<tr>
<td>Heart rate, bpm</td>
<td>108±4</td>
<td>160±7†</td>
<td>148±4‡</td>
<td>154±9‡</td>
</tr>
<tr>
<td>Cardiac output, L/min</td>
<td>2.99±0.11</td>
<td>1.46±0.09†</td>
<td>2.13±0.27‖</td>
<td>1.90±0.10‖</td>
</tr>
<tr>
<td>Systemic vascular resistance, dynes · sec · cm⁻²</td>
<td>2755±162</td>
<td>4908±456‡</td>
<td>3210±363‡</td>
<td>3320±177‡</td>
</tr>
<tr>
<td>LV end-diastolic diameter, mm</td>
<td>30.9±0.5</td>
<td>39.8±1.8*</td>
<td>35.8±0.5*‡</td>
<td>35.7±1.3*‡</td>
</tr>
<tr>
<td>% FS</td>
<td>27.6±1.1</td>
<td>8.6±1.2*</td>
<td>17.6±1.0*‡</td>
<td>15.1±1.2*‡</td>
</tr>
<tr>
<td>dP/dt max, mm Hg/sec</td>
<td>5688±471</td>
<td>1671±131*</td>
<td>2262±138*</td>
<td>2022±206*</td>
</tr>
<tr>
<td>α, macc</td>
<td>26.5±2.4</td>
<td>41.2±4.5*</td>
<td>27.9±1.6‡</td>
<td>40.4±3.5∗‡</td>
</tr>
<tr>
<td>Plasma renin activity, ng · mL⁻¹ · hr⁻¹</td>
<td>2.0±0.3</td>
<td>7.3±0.7*</td>
<td>15.8±2.6*‡</td>
<td>15.6±1.1*‡</td>
</tr>
<tr>
<td>Aldosterone, pg/mL</td>
<td>6±2</td>
<td>506±82†</td>
<td>45±7§</td>
<td>25±8§</td>
</tr>
<tr>
<td>Atrial natriuretic peptide, pg/mL</td>
<td>40±6</td>
<td>514±29†</td>
<td>298±30‡</td>
<td>456±33†‡</td>
</tr>
</tbody>
</table>

Values are mean±SEM.

*P<0.05; †P<0.01 vs normal; ‡P<0.05; §P<0.01 vs CHF; ||P<0.05; ¶P<0.01 vs ACEI.

Effects of ACEI Treatment With or Without B₂ Receptor Antagonist on Cardiohormonal Factors

Regarding the renin-angiotensin system, both types of treatment increased plasma renin activity and decreased plasma aldosterone levels significantly, as shown in the Table. These results indicate that the renin-angiotensin-aldosterone system may be equally suppressed in both groups. Plasma atrial natriuretic peptide levels, which are secreted in proportion to atrial stretch or pressure, increased in the vehicle group compared with the normal group, and enalapril significantly decreased the concentrations compared with those of the vehicle group. However, the plasma atrial natriuretic peptide levels of the combination group were significantly higher than those of the ACEI group.

Effects of ACEI Treatment With or Without B₂ Receptor Antagonist on the Expression of Cardiac Genes in CHF

As shown in Figures 2 and 3, the mRNA levels of SERCA2 in LV significantly decreased in the vehicle group compared with the normal group. Although enalapril significantly increased SERCA2 mRNA, the coadministration of FR attenuated the improvement of the expression. Furthermore, eNOS mRNA in LV was significantly decreased in the vehicle group compared with the normal group. Although ACEI treatment normalized the eNOS mRNA level, the concomitant B₂ receptor antagonist significantly decreased the eNOS mRNA level compared with that of ACEI treatment alone.

Effects of ACEI Treatment With or Without B₂ Receptor Antagonist on Collagen Type I and III Gene Expressions and Histomorphometry

As also shown in Figures 2 and 3, the expression of collagen type I and III mRNA increased significantly in the vehicle group compared with the normal group, and ACEI treatment suppressed both levels of mRNA. Combined treatment, however, significantly upregulated the gene expression.

Representative photomicrographs of histological sections of the LV from each group stained with picrosirius red are shown in Figure 4. Long-term rapid pacing caused the accumulation of a diffuse collagen network and increased the amount of interstitial fibrosis that is expressed as the increase in the collagen volume fraction. Enalapril significantly decreased the collagen volume fraction. In contrast, collagen
density in the combination group remained significantly higher than that of the ACEI group.

**Discussion**

To elucidate the role of BK, we administered an orally active B2 receptor antagonist, FR, to dogs with CHF under long-term concomitant treatment with enalapril. To commence, we selected a dosage of FR that could apparently attenuate the hypotensive effect of exogenous BK, a response we believe can serve as a marker of considerable inhibition of endogenous BK activity in intact animals. The coadministration of FR does not alter the systemic blood pressure, LV chamber dimension, or LV fractional shortening; however, concomitant treatment with FR elevated both LVEDP and the plasma atrial natriuretic peptide level, which are activated in proportion to atrial stretching, and prolonged the \( \tau \). FR also decreased the gene expression of eNOS and SERCA2 in the failing myocardium. In addition, combined FR treatment significantly increased collagen type I and III gene expression and cardiac collagen deposition compared with that of enalapril alone. The important role of endogenous BK on LV diastolic properties can be distinct not only in relation to hemodynamics but also in regard to the cellular and molecular levels in CHF. ACEIs can exert favorable cardioprotective action through the suppression of the renin-angiotensin-aldosterone system and, in part, via the augmentation of cardiac endogenous BK action in CHF.

In the vasculature, BK stimulates eNOS via endothelial B2 receptors, thus increasing NO and causing vasodilation. In the present study, the combination of FR and enalapril did not alter the systemic blood pressure and systemic vascular resistance compared with that of enalapril alone. In patients with CHF who received long-term ACEI therapy, a B2 receptor antagonist had no discernible vasoconstrictive effects on forearm blood flow; conversely, a combined B1 and B2 receptor antagonist produced vasoconstriction. In the present study, we did not verify the role of B1 receptors in the vasculature; however, in our tachycardia-induced CHF model, which is quite similar to the clinical findings of the dilated cardiomyopathy seen in humans, endogenous BK did not appear to participate in arterial blood pressure regulation, at least via a B2 receptor.

The heart has the capacity to generate Ang II and BK, and their specific receptors have been identified in this organ. Therefore, locally generated hormones could alter the tissue structure in an autocrine and/or paracrine manner. Because we could not detect any conspicuous differences in LV geometry and systolic function between the 2 treatment groups, the effect of the ACEI on both the LV contractile state and geometry seems to be more closely related to the inhibition of Ang II rather than the potentiation of BK action. There are 3 major diastolic functional abnormalities,
mainly slowed relaxation, reduced LV filling, and altered passive elastic properties, especially collagen architecture.\textsuperscript{27} We found a significant elevation of LV filling pressure and a prolonged time constant of relaxation in the present study. High LV filling pressure in the setting of unaltered LV chamber size and the slowed relaxation after the concomitant treatment with FR indicate that BK may be involved in the LV diastolic properties in long-term treatment with the ACEI in dogs with CHF.

We further investigated the molecular markers of myocardial diastolic properties using the failing canine myocardium. In human heart failure, reduced expression of SERCA2, the key enzyme sequestering $\text{Ca}^{2+}$ into the sarcoplasmic reticulum (SR) during diastole, is thought to be ultimately responsible, at least in part, for dysfunctional SR $\text{Ca}^{2+}$-handling. Even though we did not measure the activity or protein level of SERCA2 in the present study, steady-state mRNA levels could be assumed to be predictive of $\text{Ca}^{2+}$ uptake by SR in comparisons of nonfailing and failing myocardium. Captopril attenuated the reduction in the SR $\text{Ca}^{2+}$ pump and phospholamban protein contents, and the mRNA levels for SERCA2 and phospholamban in the failing rat heart, because of myocardial infarction.\textsuperscript{28} In the present study, enalapril significantly improved the changes in the expression of SERCA2; however, FR attenuated the effect of enalapril on the upregulation of the SERCA2 gene. Endogenous BK may exert a beneficial effect on the status of SR $\text{Ca}^{2+}$ transport in the failing heart and improve LV diastolic properties under long-term treatment of an ACEI.

Although NO produced by either eNOS or inducible NO may affect myocardial contractility and chronotropism, NO may also mediate the enhancement of diastolic relaxation. In isolated ejecting guinea pig hearts, captopril acutely reduced the time constant of early LV relaxation without affecting early systolic parameters, and a B\textsubscript{2} receptor antagonist and an NO scavenger, hemoglobin, inhibited the effects of captopril on LV relaxation.\textsuperscript{6} In the present study, enalapril upregulated decreases in LV eNOS mRNA in CHF; however, the concomitant BK antagonist attenuated the effects of this agent. Although ACEIs seem to improve the NO system in the myocardium via their inhibitory effects on the renin-angiotensin-aldosterone system,\textsuperscript{29} the endogenous BK-NO pathway also exerts an important role regulating the failing myocardial relaxation. Because NO inhibitors have been shown to cause LV remodeling, more long-term downregulation of eNOS mRNA in the LV should contribute to the further development of myocardial structural changes.

Because both Ang II and aldosterone enhance collagen synthesis in cardiac fibroblasts, the antifibrotic actions of ACE inhibitors are thought to be the result of blocking the production of these factors, whereas BK can decrease mRNA and protein levels for fibronectin and collagen type I and III.\textsuperscript{30} In B\textsubscript{2} receptor knockout mice, perivascular and reparative fibrosis was observed in the LV during development.\textsuperscript{31} In the present study, we confirmed that enalapril reduced collagen type I and III gene expression and decreased the deposition of collagen, whereas concomitant treatment with a B\textsubscript{2} antagonist significantly attenuated the myocardial antifibrotic effects of the ACEI. A change in collagen architecture, with abnormal alignment and/or endomyocardial fibrosis, appears to be responsible for the increase in myocardial stiffness, and the beneficial effects of BK on diastolic properties could partially result from altered collagen turnover and deposition in the failing heart.

There are several limitations in relation to the present study. First, we used a single dosing regimen because FR is both an expensive and scarce compound to obtain. Therefore, we could not address the possible contributions of BK augmented with enalapril to LV geometry and contractile function in large animals, even though we administered FR at doses that significantly affected the pharmacological dose of BK on blood pressure. Second, endogenous BK has been shown to acutely produce coronary vasodilation and enhance LV relaxation and contractile function in CHF without an ACEI.\textsuperscript{32} Unfortunately, we did not measure the changes in coronary blood flow in the present study; therefore, we could not estimate to what extent BK chronically exerted a beneficial effect on diastolic function through the regulation of coronary flow. Finally, in the present study, we could not evaluate constant myocardial stiffness, even though we examined the deposition of collagen in the LV, because we did not analyze the LV pressure–volume relation.

In conclusion, inhibition of BK metabolism under long-term ACEI treatment seems to be critical for cardiac diastolic properties through local cellular and molecular function rather than by virtue of systolic function in CHF.

The majority of patients with CHF are not the mere consequence of systolic heart failure, but in fact, diastolic failure also coexists in the progression of CHF. The optimal treatment for diastolic dysfunction, however, has not been sufficiently determined. Our results indicate that cardiac BK can exert cardioprotective action via B\textsubscript{2} receptors concerning LV diastolic properties under long-term ACE inhibition, and ACEIs may be one of the most efficacious strategies for diastolic HF. Although current clinical efforts have been directed to evaluate whether combined ACE inhibition and Ang II receptor antagonism can further attenuate LV remodeling, when an Ang II type1 receptor is blocked, the Ang II may act on Ang II type 2 receptors, which could release BK and NO. Therefore, combination therapy may further augment the endogenous BK activity compared with that of ACEI alone. In the near future, it will be necessary to clarify the clinical significance of BK under the long-term combined treatment with ACEI and Ang II receptor blockade from the dual aspects of molecular and cellular mechanisms.

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