Angiotensin Receptor Blockade Improves Myocardial Beta-Adrenergic Receptor Signaling in Postinfarction Left Ventricular Remodeling

A Possible Link Between Beta-Adrenergic Receptor Kinase-1 and Protein Kinase C Epsilon Isoform

Toshiyuki Takahashi, MD,* Toshihisa Anzai, MD,* Tsutomu Yoshikawa, MD,* Yuichiro Maekawa, MD,* Keitaro Mahara, MD,* Michikado Iwata, MD,* H. Kirk Hammond, MD,† Satoshi Ogawa, MD*

Tokyo, Japan; and San Diego, California

OBJECTIVES
We tested the hypothesis that angiotensin II type 1 receptor blocker (ARB) may improve beta-adrenergic receptor (AR) coupling in heart failure (HF) after myocardial infarction (MI).

BACKGROUND
Beta-AR desensitization is one of the mechanisms underlying the transition from compensated to decompensated HF. Beta-adrenergic receptor kinase-1 (ARK1), which can be induced by protein kinase C (PKC) in vitro, is activated in the failing myocardium, resulting in beta-AR uncoupling.

METHODS
Models of MI in rats were produced by ligation of left coronary artery. Four weeks after surgery, they were randomized to vehicle (MI/control [C]) or candesartan (10 mg/kg/day) treatment (MI/ARB). Sham-operated rats, or shams, served as controls.

RESULTS
After two weeks of treatment, echocardiography and hemodynamics showed that the left ventricular (LV) dimension increased and that the percent of fractional shortening and maximum rate of rise in left ventricular pressure (dP/dt) decreased in MI rats compared with shams. There were no differences in these indexes between MI/C and MI/ARB. An increase in maximum dP/dt under isoproterenol (ISO) stimulation was attenuated in MI/C but improved in MI/ARB. Reductions in the percentage of high-affinity sites of beta-AR and ISO-stimulated cyclic adenosine monophosphate production in noninfarcted myocardium were also improved by ARB treatment. Up-regulation of beta-ARK1 and PKC-epsilon isoform protein levels and activation of PKC in noninfarcted myocardium from MI/C were both inhibited by ARB treatment.

CONCLUSIONS
Treatment with ARB during the chronic phase of MI improved beta-AR coupling in noninfarcted myocardium without affecting basal LV function. Cross-talk between beta-AR and angiotensin signaling through beta-ARK1 and PKC-epsilon may be responsible for the phenomenon.

After a large myocardial infarction (MI), progressive left ventricular (LV) dilation initially occurs as an adaptive phenomenon to maintain cardiac output. In the chronic stage, not only is the LV infarct area impaired but also noninfarcted viable myocardium is affected in response to subsequent mechanical and numerous hormonal stimuli (1). This remodeling process results in global contractile dysfunction and the formation of substrates for lethal arrhythmias. Thus, LV remodeling plays a pivotal role in the pathogenesis of congestive heart failure (HF) after MI. Although the cellular and molecular mechanisms for the development of postinfarction LV remodeling remain to be fully elucidated, structural and functional alternations in noninfarcted myocardium might be responsible for transition from a compensatory state to decompensated HF.

The beta-adrenergic receptor (AR) system is a major regulator of cardiac function. However, long-term stimulation of beta-ARs by overshooting plasma catecholamines leads to blunting of the beta-AR response in HF. This is referred to as beta-AR desensitization, which may be characterized by beta-AR down-regulation and impaired postreceptor signaling, such as uncoupling the beta-AR from Gs, protein, decreased adenylyl cyclase activity, and increased Gi protein (2–4). Among these alternations in the beta-AR system, beta-AR/Gs uncoupling is possibly an early event of HF (5). The process of uncoupling requires phosphorylation of agonist-occupied receptor by beta-
adrenergic receptor kinase-1 (ARK1) (6), which is a member of the G-protein-coupled receptor kinase (GRK) family, translocating from the cytosol to the cell membrane when it is activated (6). Interestingly, cardiac beta-ARK1 activity is shown to increase in human HF and experimental failing myocardium (7,8). Moreover, its activation precedes the alterations of beta-ARs and G proteins in experimental HF models (9). A recent experimental study showed that transgenic mice over-expressing cardiac beta-ARK1 suffered myocardial infarction (7,8). Furthermore, its activation precedes the resultant abnormalities of beta-AR signaling cascade, thereby playing a key role in the development and progression of HF.

It is well established that the renin-angiotensin system is associated with cardiac hypertrophy and remodeling. Recent clinical studies demonstrated that an angiotensin II type 1 receptor blocker (ARB) improves symptoms and exercise tolerance in patients with HF (11). The angiotensin II type 1 receptor, which couples Gq protein, activates diacylglycerol. Protein kinase C (PKC), a downstream second messenger of the Gq protein-coupled receptor cascade, is a key mediator of cell signaling in the pathophysiology of cardiac hypertrophy and remodeling. Activation of PKC involves translocation of inactive PKC from the cytosol to the cell membrane, where diacylglycerol activates it. A previous experimental study showed that beta-ARK1 activity and beta-ARK1-dependent receptor homologous desensitization are enhanced in cells after PKC activation in vitro (12), suggesting that PKC activates beta-ARK1 in myocytes in vivo. Therefore, it is postulated that there is intracellular “cross-talk” between angiotensin and beta-AR signaling pathways.

In the present study, we tested the hypothesis that ARB may improve beta-AR responsiveness in HF by affecting beta-AR uncoupling through cross-talk between angiotensin and beta-AR signaling.

METHODS

Animal models and protocol. All procedures were done in accordance with the Keio University animal care guidelines, which conform to the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health. Myocardial infarction was produced in male Wistar rats weighing 250 to 300 g by ligation of the left coronary artery. Briefly, rats were anesthetized with intraperitoneal pentobarbital, 30 mg/kg, and then intubated. The left coronary artery was ligated 2 to 3 mm from the origin with a suture (6-0 silk), and the chest was closed immediately after the distal area paled. The mortality of all animals operated on in this fashion was approximately 40%. Sham-operated rats, or shams (n = 10), were treated similarly, except that the coronary suture was not tied. After these procedures, all rats were freely fed in separate cages. Four weeks after surgery, the MI rats were randomized to receive either vehicle (MI/control [C], n = 10) or candesartan cilextil (Takeda Chemical Industries, Ltd., Osaka, Japan) at 10 mg/kg/day for 2 weeks (MI/ARB, n = 10). None of the animals died during the two weeks of treatment with vehicle or candesartan.

Echocardiographic studies. Echocardiography was performed with the rat under anesthesia, using 30 mg/kg pentobarbital sodium. Two-dimensional and M-mode images were obtained using a 7.5-MHz transducer connected to an ultrasonic echocardiographic system (SONOS 5500, Phillips Medical Systems, Andover, Massachusetts). Images for measurements were obtained from parasternal short-axis views at the mid-papillary muscle level. Measurements were made using criteria from the American Society of Echocardiography (13). The end-diastolic dimension was obtained at the onset of the QRS complex. The end-systolic dimension was taken at the instant on maximal lateral position of the interventricular septum or at the end of the T-wave. Fractional shortening was calculated: (fractional shortening = end-systolic dimension – end-diastolic dimension/end-diastolic dimension) × 100. In addition, the ejection time was measured, and circumferential fiber shortening velocity (Vcf) was calculated: (fractional shortening/ejection time/100 [circ/s]) (14). All parameters were measured on five beats and averaged.

Hemodynamic measurements. Hemodynamic data were obtained before each rat was killed. Mean aortic, LV systolic and end-diastolic pressures, and maximum and minimum peak rate of rise in left ventricular pressure (dP/dt) were obtained. In the subsets of animals from each group, intravenous isoproterenol (ISO) was administered to assess LV contractile function under beta-agonist stimulation. The response of Vcf under stimulation of ISO was also assessed using echocardiography in the subsets of the animals.

Terminal thoracotomy. The hearts were excised and rinsed, and the coronary arteries were perfused with sterile saline (4°C). The atria, great vessels, and valves were trimmed away. Transthalmic samples were separately taken.
from the infarcted and noninfarcted areas of the LV free wall. Myocardial samples were then frozen (−80°C). Infarct size was measured by LV dissection and determination of the percentage of the LV free wall surface area. If the infarct area was <30%, the samples were excluded from the present study.

**Membrane assessment and preparation.** Frozen transmural samples (−80°C) were powdered in a stainless-steel mortar and pestle (also −80°C), placed in tris(hydroxymethyl)aminomethane (Tris) buffer, and glass-glass homogenized, and the contractile proteins were extracted (0.5 mol/l potassium chloride, 20 min, 4°C). The pellet of a 45,000-g centrifugation was re-suspended in the buffer. The protein concentration was determined by the method of Bradford (15).

**Beta-AR binding studies.** As previously described (16), beta-ARs were identified using the radioligand ([125I]-iodocyanopindolol (ICYP) (Amersham Biosciences, Piscataway, New Jersey) in eight concentrations (5 to 700 pmol/l) in saturation isothermal experiments conducted on crude membrane preparations. Nonspecific binding was defined as binding in the presence of 10−4 mol/l ISO. Data are represented as ICYP specifically bound in femtomoles per milligram membrane protein (fmol/mg). Competition binding experiments were performed in duplicate by incubating 50 pmol/l ICYP with ISO using 16 different concentrations ranging from 10−10 mol/l to 10−3 mol/l. Nonspecific binding was determined using 1 μmol/l propranolol. The ratio of high- to low-affinity binding sites was determined using nonlinear regression analysis with the Prism software program (GraphPad Software Inc., San Diego, California).

**Adenylyl cyclase assays.** Methods for measuring adenylyl cyclase were modified from Salomon et al. (17). The following agents were used to stimulate adenosine 3′,5′-cyclic adenosine monophosphate (cAMP) production in Adenylyl cyclase assays.

**PKC activity assay.** Activity of PKC was measured in supernatant and particulate fractions from 45,000-g centrifugation of crude myocardial homogenate derived from noninfarcted myocardium using a PKC enzyme assay kit (Amersham Biosciences). Tissues were homogenized in a buffer containing 50 mmol/l Tris/hydrogen chloride at pH 7.5, 0.3% (wt./vol.) beta-mercaptoethanol, 5 mmol/l ethylenediamine-tetraacetic acid, 10 mmol/l ethyleneglycol-tetraacetic acid, 50 μg/ml phenylmethylsulfonyl fluoride, and 10 mmol/l benzamidine. The activity of PKC was determined by measuring 32P, transferred from (gamma-32P)-adenosine triphosphate to histone H1, with or without phosphatidylserine and dioleyl-Sn-glycerol.

**Quantification of GRK-2 (beta-ARK-1), GRK-5, and PKC isoform protein content by immunoblotting.** Assessment of GRK2 and GRK5 was conducted using standard sodium dodecyl sulfate-polyacrylamide gel electrophoresis and immunoblotting techniques, as previously described (16). Equal amounts of the denatured protein from each supernatant and particulate fractions from noninfarcted myocardium were electrophoresed on 10% denaturing gel. Proteins were electroblotted onto nitrocellulose membranes (Amersham Biosciences). Transfer efficiency was determined by Ponceau staining. Membranes were incubated in Tris buffered saline containing 3% nonfat dry milk for 1 h to reduce nonspecific binding of antibody and developed by conventional methods using each rabbit polyclonal antibody (Santa Cruz Biotechnology Inc., Santa Cruz, California), followed by exposure to horseradish peroxidase-linked anti-rabbit immunoglobulin (1:5,000 in blocking buffer). The blots were developed by the enhanced chemiluminescence method, and bands were visualized after exposing blots to X-ray film. The densities of each blot were quantified by densitometric scanning.

**Statistics.** Data are expressed as the mean value ± SEM. Intergroup comparisons were performed using one-way analysis of variance and repeated measures analysis of variance, as appropriate. When a statistical difference was detected by analysis of variance, the Bonferroni multiple comparisons procedure was used to define differences between the results. Statistical significance was defined as p < 0.05. All statistical analyses were performed using Statview version 5.0 software (SAS Institute Inc., Cary, North Carolina).

**RESULTS**

**Animal characteristics.** As shown in Table 1, the ratio of LV to body weight was greater in MI/C rats than in shams, but it was not significantly different between shams and MI/ARB rats. The infarct weight was similar in MI/C and MI/ARB rats. Hemodynamic measurement showed that mean blood pressure was lower and LV end-diastolic pressure was higher in MI rats than in shams. Echocardiographic findings indicated that the LV dimension increased and percent fractional shortening decreased in MI rats more

### Table 1. Body Weight, LV Weight, Hemodynamics, LV Size, and LV Function

<table>
<thead>
<tr>
<th></th>
<th>Sham Operation</th>
<th>MI/C Group</th>
<th>MI/ARB Group</th>
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</thead>
<tbody>
<tr>
<td><strong>Group</strong> (n = 8)</td>
<td></td>
<td>(n = 8)</td>
<td>(n = 8)</td>
</tr>
<tr>
<td>BW (g)</td>
<td>418 ± 7</td>
<td>388 ± 19</td>
<td>413 ± 17</td>
</tr>
<tr>
<td>LVW/BW (mg/g)</td>
<td>2.1 ± 0.1</td>
<td>2.7 ± 0.1*</td>
<td>2.3 ± 0.1†</td>
</tr>
<tr>
<td>Infarct weight (g)</td>
<td>—</td>
<td>0.30 ± 0.03</td>
<td>0.30 ± 0.02</td>
</tr>
<tr>
<td>HR (beats/min)</td>
<td>405 ± 15</td>
<td>363 ± 14</td>
<td>389 ± 10</td>
</tr>
<tr>
<td>mBP (mm Hg)</td>
<td>105 ± 3</td>
<td>88 ± 3*</td>
<td>88 ± 2*</td>
</tr>
<tr>
<td>LVEDP (mm Hg)</td>
<td>4 ± 1</td>
<td>17 ± 1*</td>
<td>14 ± 1*</td>
</tr>
<tr>
<td>LVEDD (mm)</td>
<td>5.8 ± 0.4</td>
<td>9.7 ± 0.5*</td>
<td>8.9 ± 0.4*</td>
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<tr>
<td>FS (%)</td>
<td>47 ± 3</td>
<td>15 ± 1*</td>
<td>18 ± 1*</td>
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*p < 0.05 vs sham; †p < 0.05 vs MI/C. Data are presented as the mean value ± SE. BW = body weight; FS = fractional shortening; HR = heart rate; LV = left ventricular; LVEDD = left ventricular end-diastolic dimension; LVEDP = left ventricular end-diastolic pressure; mBP = mean blood pressure; MI/ARB = rats with myocardial infarction (MI) treated with the angiotensin II type 1 receptor blocker candesartan; MI/C = MI rats randomized to vehicle (control).
than in shams, although there was no difference in these indexes between the MI/C and MI/ARB groups.

**Beta-AR responsiveness to ISO stimulation.** In the basal condition, maximum dP/dt was lower in MI rats than in shams, although it was no different between MI/C and MI/ARB. An increase in maximum dP/dt under ISO stimulation was attenuated in MI/C but improved in MI/ARB (Fig. 1). Furthermore, basal and ISO-stimulated (100 pg/g intravenously) Vcf was measured (n = 4 in each group). Basal Vcf was lower in the MI groups than in sham group (sham: 6.0 ± 0.1 circ/s; MI/C: 1.7 ± 0.3 circ/s; MI/ARB: 2.1 ± 0.1 circ/s); however, under ISO stimulation, Vcf was significantly increased in shams and MI/ARB rats but not in MI/C rats (sham: 8.7 ± 0.3 circ/s; MI/C: 1.8 ± 0.2 circ/s; MI/ARB: 2.6 ± 0.1 circ/s). The percentage of increase in Vcf by ISO stimulation was greater in MI/ARB than in MI/C rats (24 ± 1% vs. 5 ± 1%, p = 0.01).

**Radioligand binding studies.** The beta-AR number was not affected by HF after MI (Fig. 2A). The characteristic reduction in high-affinity binding, denoting uncoupling of beta-ARs, was noted in myocardial samples from noninfarcted regions of untreated myocardial infarction (MI) animals (MI/control [C]) compared with shams. Treatment of MI animals with candesartan (MI/angiotensin II type 1 receptor blocker [ARB]) increased the proportion of receptors exhibiting high-affinity agonist binding. *p < 0.01 MI/ARB vs. MI/C; †p < 0.01 MI/ARB vs. MI/C; n = 6 in each group.

**Adenyl cyclase activity.** Basal cAMP production of noninfarcted myocardium was not affected by HF after MI. Isoproterenol-stimulated cAMP production was reduced in MI/C rats but restored by ARB treatment (50% increase vs. MI/C, p < 0.05). On the other hand, cAMP production under stimulation by 5′-guanylylimidodiphosphate and forskolin was not significantly different among the three groups (Fig. 3).

**Activity of PKC.** Myocardial PKC activity in the particulate fraction from noninfarcted viable myocardium of MI/C animals showed a 46% increase compared with shams (p < 0.05). Treatment with ARB of rats with MI (MI/ARB) was associated with a 29% reduction in PKC activity in the particulate fraction, compared with MI/C rats (p < 0.05) (Fig. 4). Activity of PKC in the supernatant fraction did not significantly differ among the three groups (sham: 32.2 ± 5.1 nmol/mg/min; MI/C: 32.5 ± 5.8 nmol/mg/min; MI/ARB: 30.8 ± 2.2 nmol/mg/min; n = 5 in each group).

**Beta-ARK1 and GRK5 protein content.** Immunoblotting showed that the beta-ARK1 content in the particulate fraction was increased by 1.5-fold in noninfarcted viable myocardium of MI/C animals compared with shams (p < 0.001) (Fig. 5A), even though there was no significant difference in beta-ARK1 content in the supernatant fraction between the two groups (Fig. 5B). Animals in the MI/ARB...
group exhibited a significant reduction in beta-ARK1 protein level of noninfarcted viable myocardium, compared with animals in the MI/C group (23% decrease vs. MI/C, \( p < 0.01 \)) (Fig. 5A), indicating that ARB treatment four weeks after surgery reversed the beta-ARK1 protein level of noninfarcted viable myocardium. In contrast, there was no significant difference in the GRK5 protein content of the particulate fraction among the three groups (sham: 99 ± 3 arbitrary densitometric units; MI/C: 98 ± 5 arbitrary densitometric units; MI/ARB: 100 ± 4 arbitrary densitometric units; \( n = 6 \) in each group) (Fig. 5C).

**Protein content of PKC-epsilon and other isoforms.** Immunoblotting showed that the PKC-epsilon content in the particulate fraction was increased in noninfarcted viable myocardium of MI/C animals by 1.8-fold, compared with shams (\( p < 0.001 \)) (Fig. 6A), even though there was no significant difference in PKC-epsilon content in the supernatant fraction among the groups (Fig. 6B). In contrast, the protein content of other PKC isoforms in either particulate (alpha: 139 ± 5 vs. 138 ± 3 arbitrary densitometric units; beta-2: 109 ± 3 vs. 108 ± 3 arbitrary densitometric units; delta: 150 ± 4 vs. 152 ± 5 arbitrary densitometric units; zeta: 176 ± 3 vs. 167 ± 3 arbitrary densitometric units) or supernatant fraction (alpha: 129 ± 3...
DISCUSSION

The current study demonstrated that beta-AR desensitization in postinfarction LV remodeling was associated with reductions in the percentage of high-affinity sites of beta-AR and in ISO-stimulated cAMP production and with increased protein levels of beta-ARK1 in noninfarcted viable myocardium. The epsilon isoform of PKC, but not other PKC isoforms, was up-regulated at the protein level in noninfarcted myocardium of untreated post-MI animals with increased total PKC activity. Treatment with an ARB for two weeks did not reverse LV size and fractional shortening in the basal condition, but beta-AR–mediated inotropic responsiveness was improved by ARB treatment, accompanied by an increase in the proportion of myocardial beta-ARs that are coupled to Go. In addition, we observed that up-regulation of beta-ARK1 and PKC-epsilon levels and activation of PKC in noninfarcted myocardium were inhibited by ARB treatment. These data suggest that treatment with an ARB improves beta-AR desensitization associated with HF after MI, specifically by affecting beta-AR/Go coupling in noninfarcted myocardium during the development of LV remodeling.

Beta-AR desensitization in HF after MI. Adrenergic activation in HF is associated with altered beta-AR signaling, including beta-AR down-regulation, uncoupling of beta-AR from the Go protein, and decreased adenylyl cyclase activity (3,4,18). The desensitization of beta-AR caused by these factors facilitates a further activation of the sympathetic nervous system, resulting in myocyte damage due to some toxic effects of catecholamines, such as calcium overloading. Furthermore, activation of the sympathetic nervous system is related to the susceptibility to fatal arrhythmia, sudden death, and induction of ischemia (19). Thus, long-term adrenergic activation and desensitization of the beta-AR system may play a role as a trigger for the vicious cycle seen in decompensated HF. We confirmed the occurrence of beta-AR desensitization in the present study, using an animal model of post-MI HF that mimics many of the features of clinical HF.

The precise molecular mechanisms for depressed beta-AR responsiveness in HF have not been fully clarified. Since the original description of beta-AR desensitization in the human myocardium (18), various abnormalities of its signal transduction pathway have been shown in different models of HF. Maurice et al. (20) reported functional uncoupling of myocardial beta-ARs in a rabbit post-MI model with increased beta-ARK1 levels and activity, compared with sham-operated animals. In the present study, there was a significant reduction in the proportion of beta-ARs coupled to the Go protein, and immunoblotting showed that the membrane level of beta-ARK1, but not GRK5, was up-regulated in noninfarcted myocardium of untreated post-MI rats compared with shams, which is consistent with their findings. Although the precise mechanisms for beta-AR desensitization associated with HF are
still unclear, our results suggest that beta-AR/G_s uncoupling induced by up-regulation of the beta-ARK1 level is responsible for beta-AR desensitization in noninfarcted myocardium, rather than down-regulation of the global myocardial beta-ARs.

Role of PKC in cardiac hypertrophy and failure. Activation of PKC is important for multiple cardiovascular functions and pathophysiological conditions, including myocardial protection against ischemic injury, cardiac hypertrophy, interstitial fibrosis, and HF (21). The distribution of the various PKC isoforms is tissue- and species-dependent. In adult rat cardiomyocytes and myocardium, PKC isoforms epsilon and delta seem to be maintained with age, whereas other PKC isoforms may decline (22,23). In human myocardium, PKC isoforms alpha, beta-1/2, delta, and epsilon have also been reported (24). In the present study, PKC-epsilon, but not other PKC isoforms, was up-regulated in noninfarcted myocardium of the untreated post-MI rats. To date, PKC-epsilon, which is a major hormonally responsive PKC isoform in mammalian cardiac myocytes, is believed to be involved in cardiomyocyte growth and ischemic preconditioning (25,26). Acute mechanical stretch of the LV induces translocation of PKC-epsilon (27) and chronic pressure overload by aortic banding activated PKC-alpha and -epsilon in the guinea pig heart (28). Activation of PKC-epsilon has also been found to contribute to G_{m,1} overexpression-induced cardiac hypertrophy and failure (29). Based on these findings, it is possible that PKC-epsilon plays an important role in cardiac hypertrophy and failure in noninfarcted myocardium during postinfarction LV remodeling.

Effect of ARB on beta-AR signaling in HF. Recently, Makino et al. (30) reported that angiotensin-converting enzyme inhibitor or ARB had an effect of preserving myocardial beta-AR signaling in an MI model using rabbits. In their study, ARB treatment was started immediately after coronary ligation and continued for three weeks, resulting in limited cardiac remodeling and improved systolic dysfunction. Therefore, the effect of ARB on beta-AR signaling might be secondary to hemodynamic improvement and limited LV remodeling. In contrast, we started administration of ARB to post-MI rats four weeks after surgery, when LV remodeling had definitely developed. Treatment with ARB for two weeks did not influence basal LV function and hemodynamics but improved myocardial beta-AR responsiveness. These findings may suggest a primary effect of ARB on myocardial beta-AR signaling. Ramos-Ruiz et al. (31) have demonstrated that the activity of the beta-ARK1 promoter is stimulated by activation of the G_{m,1}/PKC signaling pathway in aortic smooth muscle cells. Although the mechanism for the increased beta-ARK1 level in HF is not certain, existing evidence suggests that PKC can directly regulate beta-ARK activity to enhance beta-AR phosphorylation and initiate desensitization (12). In the present study, the inhibitory effect of treatment with candesartan on cardiac beta-ARK1 expression could be potentially explained by altering cardiac PKC activity and expression. We also found that PKC-epsilon was selectively increased in noninfarcted viable myocardium from post-MI rats; treatment of HF animals with candesartan was associated with a reduced cardiac PKC-epsilon content. These data suggest a possible mechanism by which an ARB influences the cardiac beta-ARK1 content: treatment with candesartan, by reducing angiotensin II-mediated PKC activation, may reduce beta-ARK1 translocation.

Study limitations and implications. The long-term effect of ARB on the development of LV remodeling after MI was not determined in the present study. However, we have found that short-term ARB treatment during the chronic phase of MI is associated with a reduced myocardial beta-ARK1 level, resulting in enhanced beta-AR responsiveness. These data suggest a plausible explanation for the observation that an ARB has a favorable effect on exercise tolerance and symptoms in patients with HF (32). Although beta-ARK1 and PKC-epsilon may play important roles in the mechanisms of this phenomenon, multiple pathways could have been independently influenced by ARB treatment. Further experiments using PKC-epsilon knockout mice or selective inhibitor of PKC-epsilon would be needed to confirm the intracellular cross-talk between beta-AR and angiotensin signaling through beta-ARK1 and PKC-epsilon.

Conclusions. Treatment with ARB during the chronic phase of MI improved beta-AR coupling in noninfarcted myocardium, even though there was no change in basal LV function. Cross-talk between beta-AR and angiotensin signaling through beta-ARK1 and PKC-epsilon may be responsible for the phenomenon.

Reprint requests and correspondence: Dr. Toshihisa Anzai, Cardiopulmonary Division, Department of Medicine, Keio University School of Medicine, 35 Shinanomachi, Shinjuku-ku, Tokyo 160-8582, Japan. E-mail: anzai@cpnet.med.keio.ac.jp.

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