Renin-angiotensin system inhibition on noradrenergic nerve terminal function in pacing-induced heart failure

HIROYA KAWAI, SUZANNE Y. STEVENS, AND CHANG-SENG LIANG
Cardiology Unit, Department of Medicine, and Department of Neurobiology and Anatomy, University of Rochester Medical Center, Rochester, New York 14642

Received 8 March 2000; accepted in final form 14 July 2000

Kawai, Hiroya, Suzanne Y. Stevens, and Chang-Seng Liang. Renin-angiotensin system inhibition on noradrenergic nerve terminal function in pacing-induced heart failure. Am J Physiol Heart Circ Physiol 279: H3012–H3019, 2000.—Chronic angiotensin-converting enzyme (ACE) inhibition has been shown to improve cardiac sympathetic nerve terminal function in heart failure. To determine whether similar effects could be produced by angiotensin II AT1 receptor blockade or ACE inhibition, we administered the ACE inhibitor quinapril or AT1 receptor blocker losartan, or both agents together, to rabbits with pacing-induced heart failure. Chronic rapid pacing produced left ventricular dilation and decline of fractional shortening, increased plasma norepinephrine (NE), and caused reductions of myocardial NE uptake activity, NE histofluorescence profile, and tyrosine hydroxylase immunostained profile. Administration of quinapril or losartan retarded the progression of left ventricular dysfunction and attenuated cardiac sympathetic nerve terminal abnormalities in heart failure. Quinapril and losartan together produced greater effects than either agent alone. The effect of renin-angiotensin system inhibition on improvement of left ventricular function and remodeling, however, was not sustained. Our results suggest that the effects of ACE inhibitors are mediated via the reduced sympathetic nerve terminal abnormalities. In a recent study (19), we found that administration of angiotensin-converting enzyme (ACE) inhibitors, known to reduce mortality and morbidity in chronic heart failure (9, 38, 44), was associated with reductions of the attenuation of cardiac NE uptake, neuronal NE histofluorescence, and tyrosine hydroxylase immunoreactive profiles in dogs with experimental right heart failure. However, it remains to be determined whether the effects of ACE inhibitors are mediated via the reduced conversion of angiotensin I to angiotensin II, or accumulation of bradykinin, both of the mechanisms may lead to reduction of sympathoadrenal release of catecholamines (5, 14, 24, 29).

Angiotensin II AT1 receptor blockers have recently gained popularity because of clinical efficacies like ACE inhibitors and a lack of bradykinin-related side effects (32). These agents could be used to study the direct effect of angiotensin II on AT1 receptors. Furthermore, because the ACE inhibitor and the angiotensin AT1 receptor blocker act at different sites in the renin-angiotensin system activation cascade, they may act synergistically and produce greater beneficial effects than either agent alone (16, 31, 40, 41). In the present study, we sought to study the effects of ACE inhibition and angiotensin II receptor blockade, either alone or in combination, on the cardiac sympathetic nerve terminal function in heart failure.

METHODS

Animal preparations. The study was approved by the University of Rochester Committee on Animal Resources and conformed to the guiding principles approved by the Council of the American Physiological Society and the National Institutes of Health Guide for the Care and Use of Laboratory Animals (DHHS Publication No. NIH 85–23, Revised 1985, Office of Science and Health Reports, Bethesda, MD 20892).

Healthy adult New Zealand White rabbits (2.63–4.0 kg) were chosen for the study. The rabbits were prepared for induction of congestive heart failure (CHF) as previously described (19, 20). The rabbits were anesthetized with intramuscular ketamine (50 mg/kg) and xylazine (3 mg/kg) and artificially ventilated with a respirator (Harvard Apparatus, South Natick, MA). We performed a pericardiotomy via a median sternotomy to incise the heart diaphragm and to gain access to the pericardial cavity. The left atrium was perforated to prevent venous congestion; the pericardium was opened with a scalpel. The coronary arteries were occluded with an inflated balloon catheter. The right atrium was opened and a 26-gauge needle was passed through the inferior vena cava to the dog's right atrium. The right atrial end of the needle was connected to a pressure transducer in the right atrium, and the left atrial end of the needle was connected to a water-filled balloon. The balloon catheter was inflated with 5 ml of saline, and the balloon catheter was then inflated with 10 ml of saline until the mean arterial blood pressure was increased to 200 mm Hg.

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
subxiphoid thoracotomy for placement of a shielded pacing lead (model TPW50, Ethicon, Somerville, NJ) onto the apical region of the left ventricular free wall. A second pacing lead was sutured onto the left pectoral muscle. The leads were routed subcutaneously and exteriorized to the interscapular region, and the wound was closed.

One week later, the pacing leads were connected to a Prevail VHRP programmable pacemaker (model 8086, Medtronic, Minneapolis, MN). Animals were assigned randomly to receive either rapid ventricular pacing at a rate of 360 beats/min (CHF group) or no pacing (sham group). The pacemaker and pacing leads were stored in a pocket of a custom-made rabbit jacket. Electrocardiography was used to confirm proper cardiac pacing weekly.

**Experimental protocol and methods.** CHF and sham rabbits were divided randomly into four groups each, according to the following treatments: 1) control without drug treatment; 2) quinapril (Parke-Davis, Warner-Lambert, Morris Plains, NJ), 10 mg once a day; 3) losartan (Merck, West Point, PA), 50 mg once a day; and 4) quinapril 10 mg and losartan 50 mg each once a day for 8 wk. The doses were chosen on the basis of pilot studies to produce maximal ACE or angiotensin II receptor inhibition. To measure the achieved pharmacological blockade, we measured the pressor responses to three serially increasing doses (0.05, 0.1, and 0.2 μg/kg) of intravenous angiotensin I or II (Sigma Chemical, St. Louis, MO). The peak response of mean arterial pressure was noted after each bolus injection.

During the 8-wk treatment period, echocardiograms were obtained weekly with the rabbits in a left lateral decubitus position, with the use of a scanning system heart sonograph (Toshiba America Medical Systems, Tustin, CA). A 5-MHz transducer was used to measure left ventricular end-diastolic (EDD; mm) and end-systolic dimensions (ESD; mm) at a plane below the mitral valve apparatus. Left ventricular fractional shortening (FS; %) was calculated as ωFS = (EDD − ESD) × 100/EDD.

At the end of the treatment period, cardiac pacing was discontinued, and the rabbits were anesthetized with intramuscular ketamine (28 mg/kg) and midazolam (0.8 mg/kg). A 20-gauge Insite catheter (Deseret Medical, Becton Dickinson, Sandy, UT) was introduced into the left carotid artery and connected to a pressure transducer (model P23 XL, Spectramed, Oxnard, CA) and an eight-channel Brush recorder (model 480, Gould Instruments, Cleveland, OH) for measuring arterial pressure and heart rate. A 2-Fr micromanometer-tipped catheter (Millar Instruments, Houston, TX) was introduced into the left ventricle via the right carotid artery and connected to the Brush recorder for measuring the first derivative of pressure development over time (dP/dt) with the use of an electronic differentiator. Resting hemodynamic measurements were made in triplicate at 5-min intervals at least 1 h after insertion of the Millar catheter. Averages of the triplicates were used for statistical analysis.

Arterial blood samples were taken for plasma NE by a radiosynthetic assay (43).

After the study was completed, the rabbits were given a lethal dose (>100 mg/kg) of intravenous pentobarbital sodium. The heart was removed and weighed. The ventricular free wall was separated from the septum and rinsed in ice-cold oxygenated normal saline. The left ventricular weight included both the septum and left ventricular free wall. Fresh left ventricular muscle blocks were taken for measuring NE uptake activity, as described previously (17, 19). Briefly, fresh tissue slices were incubated in 50 nmol/l L-[7-3H(N)]NE (13.8 Ci/mmol; New England Nuclear, Boston, MA) for 15 min. Specific 3H-uptake activity, defined as the difference in radioactivity between tissue slices incubated in a [3H]NE-containing solution at 37°C and those at 4°C, is considered to approximate NE uptake activity.

Fresh left ventricular tissue blocks also were rapidly frozen and prepared for glyoxylic acid-induced histofluorescence for catecholamines and immunocytochemistry for tyrosine hydroxylase (17). Histofluorescence specific for catecholamines was performed with the use of a modification (3) of the sucrose-potassium phosphate-glyoxylic acid condensation method of de la Torre (10). A sheep antityrosine hydroxylase primary antibody was used for the immunocytochemical visualization of tyrosine hydroxylase. The sections were photographed at the same magnification (×50) with the use of a 35-mm slide film. The number of stained catecholamine profiles was counted in a 0.221-mm² (0.003536 mm²) field, while the number of tyrosine hydroxylase profiles was counted in a 0.00885-mm² field. The results of six fields were averaged for each ventricle.

**Statistical analysis.** The experimental data were analyzed with RS/1 Research System software (Bolt, Beraney, and Newman, Cambridge, MA) and SYSTAT software (SPSS, Chicago, IL). Data are expressed as means ± SE. The statistical significance of differences among the experimental groups was determined by one- or two-way ANOVA with post hoc Tukey's test comparisons. P < 0.05 was considered significant.

**RESULTS**

**Clinical manifestations of the experimental animals.** The rabbits tolerated the thoracotomy and placement of cardiac pacing wires well. They were monitored closely after the surgery by both the research and vivarium staff. The rabbits received appropriate antibiotics and nutritional supplements under the direction of staff veterinarians. None of the rabbits included in the study exhibited severe sepsis, emaciation, or severe distress. The CHF rabbits showed no significant changes in body weight between the time of thoracotomy (3.16 ± 0.04 kg) and pacer implantation (3.13 ± 0.03 kg). At the end of rapid ventricular pacing 8 wk later, the body weight increased 0.14 ± 0.05 kg (P < 0.05) to 3.27 ± 0.03 kg. The differences in body weight among the various groups of rabbits at the end of the study, however, were small and statistically insignificant (Table 1).

**Efficacies of converting enzyme inhibitor and angiotensin II AT1-receptor blocker.** To determine clinical efficacies of the ACE inhibitor and angiotensin II AT1-receptor blocker, we measured the pressor responses of the animals to angiotensin I and angiotensin II. Aortic blood pressure was increased by bolus injections of angiotensin I and angiotensin II in a dose-dependent manner in control rabbits receiving neither quinapril nor losartan (Fig. 1). Quinapril treatment markedly reduced the pressor response to angiotensin I, whereas losartan attenuated the increase of blood pressure in response to angiotensin II injections. The combination
of quinapril and losartan blocked the pressor effects of both angiotensin I and angiotensin II. However, the combined therapy produced no greater reduction of the pressor responses compared with treatment with a single agent alone.

Effects of rapid cardiac pacing on left ventricular function. Rapid ventricular pacing increased left ventricular EDD and decreased left ventricular FS in control rabbits without drug treatment (Fig. 2). These changes occurred shortly after initiation of pacing and worsened gradually over the 8 wk of pacing. In contrast, no significant changes in echocardiographic parameters occurred in sham rabbits. The early increase in left ventricular EDD and decrease in FS were reduced by quinapril or losartan treatment. Significant dilation of the left ventricle did not occur in rabbits treated with quinapril or losartan until 6 wk after the start of pacing. Administration of both agents together caused further delay of left ventricular dilation, but neither agent either alone nor in combination abolished the late left ventricular systolic dysfunction or dilation. The changes at 8 wk of rapid pacing did not differ statistically among the groups. Quinapril and losartan had no effects in sham rabbits (data not shown).

Resting hemodynamics. Table 1 also shows the systemic and cardiac hemodynamics, heart weights, and plasma NE in sham and CHF animals at the end of study. Rapid ventricular pacing had no statistically significant effects on resting heart rate, mean aortic pressure, or left ventricular weight, but it produced significant increases in right atrial pressure, left ventricular end-diastolic pressure, left ventricular end-diastolic dimension, and fractional shortening. The changes at 8 wk of rapid pacing did not differ statistically among the groups. Quinapril and losartan had no effects in sham rabbits (data not shown).

Table 1. Resting cardiac function, hemodynamics, and plasma NE in sham and CHF animals

<table>
<thead>
<tr>
<th></th>
<th>Sham</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th>CHF</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Quinapril</td>
<td>Losartan</td>
<td>Quinapril + losartan</td>
<td>Control</td>
<td>Quinapril</td>
<td>Losartan</td>
<td>Quinapril + losartan</td>
<td></td>
</tr>
<tr>
<td>Number</td>
<td>10</td>
<td>9</td>
<td>8</td>
<td>6</td>
<td>9</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Body wt, kg</td>
<td>3.3 ± 0.1</td>
<td>3.3 ± 0.1</td>
<td>3.5 ± 0.1</td>
<td>3.4 ± 0.1</td>
<td>3.2 ± 0.04</td>
<td>3.4 ± 0.1</td>
<td>3.3 ± 0.1</td>
<td>3.3 ± 0.1</td>
<td></td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>258 ± 13</td>
<td>257 ± 15</td>
<td>262 ± 19</td>
<td>253 ± 14</td>
<td>254 ± 6</td>
<td>248 ± 8</td>
<td>251 ± 7</td>
<td>242 ± 8</td>
<td></td>
</tr>
<tr>
<td>Aortic pressure, mmHg</td>
<td>96 ± 4</td>
<td>90 ± 2</td>
<td>98 ± 5</td>
<td>88 ± 5</td>
<td>86 ± 3</td>
<td>91 ± 6</td>
<td>96 ± 4</td>
<td>89 ± 5</td>
<td></td>
</tr>
<tr>
<td>Right atrial pressure, mmHg</td>
<td>2 ± 1</td>
<td>2 ± 1</td>
<td>2 ± 1</td>
<td>2 ± 1</td>
<td>6 ± 1*</td>
<td>7 ± 1*</td>
<td>6 ± 1*</td>
<td>4 ± 1*</td>
<td></td>
</tr>
<tr>
<td>LV EDP, mmHg</td>
<td>7 ± 1</td>
<td>7 ± 1</td>
<td>6 ± 1</td>
<td>7 ± 1</td>
<td>23 ± 4*</td>
<td>23 ± 3*</td>
<td>21 ± 2*</td>
<td>19 ± 2*</td>
<td></td>
</tr>
<tr>
<td>LV dP/dt, mmHg/μs × 10³</td>
<td>4.3 ± 0.3</td>
<td>4.3 ± 0.1</td>
<td>4.2 ± 0.2</td>
<td>3.8 ± 0.2</td>
<td>2.8 ± 0.1</td>
<td>2.8 ± 0.3</td>
<td>2.7 ± 0.2</td>
<td>2.6 ± 0.2</td>
<td></td>
</tr>
<tr>
<td>LV weight, g/kg</td>
<td>1.34 ± 0.04</td>
<td>1.27 ± 0.03</td>
<td>1.34 ± 0.03</td>
<td>1.23 ± 0.04</td>
<td>1.52 ± 0.10</td>
<td>1.43 ± 0.05</td>
<td>1.35 ± 0.06</td>
<td>1.46 ± 0.07</td>
<td></td>
</tr>
<tr>
<td>Arterial [NE], pg/ml</td>
<td>99 ± 12</td>
<td>78 ± 24</td>
<td>152 ± 69</td>
<td>134 ± 33</td>
<td>405 ± 119*</td>
<td>139 ± 42†</td>
<td>225 ± 69†</td>
<td>118 ± 23†</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE. CHF, congestive heart failure; LV, left ventricular; EDP, end-diastolic pressure; NE, norepinephrine. *P < 0.05, compared with sham. †P < 0.05, compared with control CHF, by ANOVA and Tukey’s test comparisons.

Fig. 1. Increases in mean aortic pressure produced by either angiotensin I (top) and angiotensin II (bottom) in the control, quinapril, losartan, and quinapril + losartan groups. The numbers of experiments were 14, 14, 16, and 14 for the control, quinapril, losartan, and quinapril + losartan groups, respectively. *P < 0.05, compared with the control group.

Fig. 2. Effects of rapid cardiac pacing and quinapril, losartan, and quinapril + losartan on left ventricular diastolic dimension (top) and fractional shortening (FS) in congestive heart failure (CHF) rabbits. See Table 1 for no. of rabbits in each group. *P < 0.05, compared with baseline (week 0). †P < 0.05, compared with CHF control.
tricular end-diastolic pressure, and plasma NE. Concomitantly, left ventricular dP/dt decreased. Administration of quinapril and losartan either alone or in combination reduced plasma NE in CHF rabbits but did not affect any other indexes in sham or CHF rabbits.

Myocardial NE uptake activity and sympathetic nerve terminal profiles. Myocardial NE uptake activity was reduced in CHF rabbits compared with sham rabbits (Fig. 3). This change was associated with reductions of NE histofluorescence (Fig. 4) and tyrosine hydroxylase profiles (Fig. 5). Administration of quinapril and losartan attenuated the reductions of NE uptake activity and neuronal terminal transmitter profiles, also shown in the figures. The restoration appeared to be more complete when both quinapril and losartan were administered together. Figure 6 shows the representative photographs from each rabbit group. In contrast, neither quinapril nor losartan affected myocardial NE uptake activity or adrenergic terminal profiles in the sham rabbits (Figs. 3–5).

**DISCUSSION**

The present study confirms our recent report (19) with experimental right-sided heart failure that sympathetic nerve terminal abnormalities in the failing myocardium were attenuated by converting enzyme inhibition in cardiomyopathy. Furthermore, our present study showed that like ACE inhibitors, the angiotensin II AT1 receptor blocker losartan attenuated the reductions of myocardial NE uptake activity, catecholaminergic histofluorescence, and tyrosine hydroxylase immunoreactive profiles in CHF. These results support the concept that ACE inhibitors exert an effect on cardiac sympathetic nerve terminal function predominantly via an action of angiotensin II common to both agents. Additionally, combined treatment with ACE inhibitor and angiotensin II AT1 receptor blocker produced a greater protective effect on cardiac sympathetic nerve terminal function than either agent alone.

The blocking effect of quinapril on ACE activity was determined by the pressor response of the rabbits to intravenous angiotensin I in our present study. The conversion of angiotensin I to angiotensin II in the intravascular space is determined entirely by circulating and vascular ACE (1). In our present study, quinapril at the dose employed probably produced maximal ACE inhibition in the vasculature, because addition of losartan produced no further reduction of pressor response to angiotensin I. However, tissue ACE level may be much greater from that in the intravascular space and could not be totally inhibited by the dose of ACE inhibitor employed.

Chronic ACE inhibition in humans with CHF has been shown to improve cardiac function and hemodynamics (21) and reduce plasma NE (4) and skeletal muscle sympathetic nerve activity (15). Plasma NE concentration was also reduced in our present study in CHF rabbits treated with quinapril and losartan, as well as in an earlier study (22) that used benazeprilat, an ACE inhibitor, or valsartan, an angiotensin II receptor blocker. The decrease of NE probably is related to reduced production or effect of angiotensin II, which has a presynaptic effect to facilitate release of catecholamines from the sympathetic nerve endings and adrenal medulla (13, 24). This effect of angiotensin II is mediated via the AT1 receptor, because the angiotensin II-mediated catecholamine release is inhibited by an-

---

**Fig. 3.** Effects of quinapril, losartan, and quinapril + losartan on myocardial norepinephrine (NE) uptake activity in sham and CHF rabbits. See Table 1 for no. of rabbits in each group. *P < 0.05, compared with sham control. †P < 0.05, compared with CHF control.

**Fig. 4.** Effects of quinapril, losartan, and quinapril + losartan on catecholaminergic histofluorescence profiles in sham and CHF rabbits; n = 6–10 rabbits in each group. *P < 0.05, compared with sham control. †P < 0.05, compared with CHF control.

**Fig. 5.** Effects of quinapril, losartan, and quinapril + losartan on tyrosine hydroxylase immunoreactive profiles in sham and CHF rabbits; n = 6–10 rabbits in each group. *P < 0.05, compared with sham control. †P < 0.05, compared with CHF control.
giotensin II AT\textsubscript{1} receptor blockers (13). Additionally, because angiotensin II reduces baroreflex sensitivity via an action on the area postrema (26), inhibition of the renin-angiotensin system in CHF has been shown to improve baroreflex control of heart rate (26, 34) and renal sympathetic outflow (33); this effect is expected to reduce the release of NE to the heart. Furthermore, bradykinin, which is expected to increase in the tissue
not only after ACE inhibition but also after angiotensin II AT1 receptor blockade by an action of the unopposed AT2 receptor (27), may alter sympathetic NE release. Bradykinin has been shown to increase electrically induced NE release from sympathetic nerves via a prejunctional bradykinin B2 receptor (6, 7, 12, 23). However, bradykinin also has been shown to reduce the NE outflow elicited by sympathetic nerve stimulation (29) or coronary artery occlusion and reperfusion (45). The latter effect of bradykinin is mediated via the activation of bradykinin B1 receptors (5). Whether the increase in bradykinin in CHF produces net production or inhibition of NE release is unknown; it may vary depending on the various experimental conditions or relative contributions of bradykinin B1 and B2 receptors in the tissue.

Rapid cardiac pacing produced progressive left ventricular systolic dysfunction and chamber dilation in rabbits. Administration of an ACE inhibitor, angiotensin II AT1 receptor blocker, or combination of the two, attenuated the early progression of left ventricular remodeling. The effect of quinapril appeared to be greater than that of losartan. By the end of the experiment (week 8 of pacing), the magnitude of reduction of left ventricular FS and increase in left ventricular dimension was quantitatively smaller in the rabbits treated with renin-angiotensin system inhibitors than the control CHF rabbits without the blocking agents, but the differences among the groups did not reach statistical significance. Renin-angiotensin system inhibition also did not produce statistically significant changes on resting hemodynamics in chronic CHF rabbits at week 8. These changes are similar to what we reported previously (19) in right heart failure dogs that despite the recovery of noradrenergic nerve terminal function, ACE inhibitors produced no significant changes in resting hemodynamics. Like our present study, losartan alone had no effect on left ventricular remodeling after rapid ventricular pacing in pigs (8). On the other hand, Spinale et al. (40, 41) reported that the renin-angiotensin system inhibition improved cardiac function and myocyte contractile function in pacing-induced cardiomyopathy. The reasons for the discrepancies cannot be stated with certainty, but because the duration of pacing was only 3 wk in the latter studies, we speculate that their results are applicable only to early stages of pacing-induced cardiomyopathy.

Several studies have compared the relative benefits of ACE inhibitors and angiotensin II receptor blockers on cardiac output and hemodynamics in CHF. In pigs with pacing-induced cardiomyopathy, chronic administration of either benazeprilat or valsartan for 3 wk has been shown to increase cardiac output (22). Cardiac output also increases after acute administration of an ACE inhibitor or angiotensin II receptor blocker in a pig model of heart failure induced by serial myocardial infarctions followed by repeated rapid ventricular pacing (37). Under both conditions, total peripheral vascular resistance fell. A greater effect on cardiac output and total peripheral vascular resistance is observed when both agents are combined. Similar beneficial effects also have been shown to occur after a chronic (2 mo) ACE inhibitor or angiotensin II AT1 receptor blocker therapy in rats with CHF induced by myocardial infarction (27). However, there are conflicting results. Murakami et al. (35) reported that acute administration of captopril, but not losartan, increased cardiac output in pacing-induced heart failure in conscious dogs. Also, unlike the ACE inhibitor fosinopril, the angiotensin II AT1 receptor blocker irbesartan had no protective effect on left ventricular geometry or myocyte contractile function in pacing-induced CHF rabbits (40, 41). Nor did administration of the angiotensin II AT1 receptor blocker valsartan attenuate the left ventricular dilation or decline of the systolic shortening of the left ventricle in pigs with pacing-induced cardiomyopathy (39). Valsartan did not affect the relative content of left ventricular fibrillar collagen in the pacing CHF rabbits. Valsartan also had only marginal effects on the left ventricular remodeling parameters in dogs with moderate heart failure induced by coronary embolization (42). Furthermore, although both quinapril and losartan reduced left ventricular end-diastolic pressure in rats with large myocardial infarctions, only quinapril increased cardiac output and reduced left ventricular weights in rats with large myocardial infarctions (18). In the latter study (18), the beneficial effects of quinapril on cardiac performance and weight are attributed to a bradykinin-dependent mechanism, because the effects were blocked by concomitant administration of a bradykinin B2-receptor blocker.

Administration of a bradykinin B2-receptor blocker icatibant also has been shown to inhibit the reversal by ACE inhibitors of cardiac remodeling after myocardial infarction in rats (27). Similar changes occur in rats after knockout of the kininogen gene (28), further demonstrating that the bradykinin system plays an important role in mediating the reversal of cardiac remodeling by ACE inhibitors. Likewise, Liu et al. (27) showed that the beneficial effect of angiotensin II AT1 receptor blockers on reversal of cardiac remodeling in rats after myocardial infarction could be inhibited by both an AT2 antagonist and icatibant. The results suggest that angiotensin II can activate the AT2 receptor and cause an increase of tissue kinins and autocoids. This bradykinin-mediated mechanism probably is responsible for an AT2-receptor-mediated depressor response to angiotensin II (36).

Like the effect of losartan on the cardiac sympathetic nerve terminal function in our present study, addition of angiotensin receptor blockade has been shown to produce an additive effect on the exercise capacity in CHF patients treated with maximally recommended doses of ACE inhibitors (16). This additive effect of angiotensin II receptor blockers may be related to the fact that maximally recommended doses of ACE inhibitors do not result in complete ACE inhibition when the renin-angiotensin system is activated in CHF. Furthermore, alternative pathways of conversion of angiotensin I to angiotensin II, such as chymase, exist and may elevate circulating or tissue level of angiotensin II.
in CHF despite ACE inhibition (46). However, the relative contributions of ACE and chymase to angiotensin II formation in the heart differ among animal species (2). Studies have shown that while chymase predominates over ACE activity in human heart, it has only a limited role for forming angiotensin II in rabbit heart (2). Thus the greater effects of the combination therapy in rabbits in this study probably was not related to the “escape” formation of angiotensin II by chymase.

The hemodynamic measurements in our present study were obtained in rabbits under anesthesia with ketamine and midazolam. Although these agents cause minimal cardiorespiratory depression, they may increase heart rate (30) and thus obscure the expected increase in heart rate in CHF rabbits.

In conclusion, chronic ACE inhibition and angiotensin II AT1 receptor blockade are both effective in attenuating cardiac sympathetic nerve terminal abnormalities in rabbits with pacing-induced heart failure, and the combination treatment with the two classes of agents may produce greater protection than that by each agent alone. These findings indicate that angiotensin II plays a pivotal role in modulating cardiac sympathetic nerve terminal function during development of heart failure. The beneficial effects of the combination therapy on cardiac sympathetic nerve terminal function may contribute to the beneficial clinical outcome in patients with heart failure. Studies are in progress to determine the effects of the combined therapy on mortality and cardiovascular morbidity in CHF patients.

The authors thank Amy Mohan, Jacqueline Armstrong, and Jason Hagen for technical assistance.

This study was supported in part by an American Heart Association grant. This study was presented in part before the Annual Scientific Sessions of the American Heart Association in Dallas, TX, on November 11, 1998.

REFERENCES

23. Kurz T, Tolg R, and Richardt G. Bradykinin B1-receptor mediated stimulation of exocytotic noradrenaline release from


