Altered Effects of Angiotensin II Type 1 and Type 2 Receptor Blockers on Cardiac Norepinephrine Release and Inotropic Responses During Cardiac Sympathetic Nerve Stimulation in Aorto-Caval Shunt Rats

Hitoshi Suzuki, MD; Kazuhiro Maehara, MD; Hiroyuki Yaoita, MD; Yukio Maruyama, MD

Background  Inhibition of the sympathetic nervous and renin–angiotensin systems has become an important strategy in the treatment of chronic heart failure. However, direct evidence of how inhibition of the renin–angiotensin system alters sympathetic activity in a diseased heart is lacking.

Methods and Results  Four weeks after abdominal aorto-caval (AV) shunting or sham operation in rats, the hearts were retrogradely perfused in vivo and the left ventricles contracted isovolumetrically at 300 beats/min. Sympathetic nerve stimulation (SNS) was performed in the baseline state and repeated with an infusion of the angiotensin II (A-II) type 1 receptor (AT1-R) blocker, losartan, the A-II type 2 receptor (AT2-R) blocker, PD123319, or A-II. Norepinephrine (NE) overflow and left ventricular (LV) inotropic responses during baseline SNS were lower in the AV shunt rats. Losartan did not change the NE overflow or the LV inotropic responses to SNS in the sham rats, but did increase them in the AV shunt rats. PD123319 changed neither parameter in the sham rats, but decreased both in the AV shunt rats. A-II enhanced the NE overflow but attenuated the LV inotropic responses to SNS in the sham rats, but attenuated both in the AV shunt rats.

Conclusions  The effects of A-II via the AT1-R and AT2-R on the adrenergic drive in the heart were altered significantly in volume overload hypertrophy induced by AV shunting. (Circ J 2004; 68: 683–690)

Key Words: Angiotensin; Cardiac hypertrophy; Sympathetic nervous system

Neurohumoral activation is one of the hallmarks of chronic heart failure (CHF) and contributes to its development and progression. Inhibition of the activated neurohumoral systems, in particular the sympathetic nervous system and the renin–angiotensin system are important targets for treating CHF. The results of multiple large clinical trials have shown that therapy with angiotensin-converting enzyme (ACE) inhibitors and/or angiotensin II (A-II) type 1 receptor (AT1-R) blockade improve the survival of patients with CHF. These drugs inhibit the protein synthesis stimulated by endogenously produced A-II, which results in myocyte hypertrophy and interstitial fibrosis, leading to myocardial failure. In addition, it has been speculated that these drugs reduce further activation of sympathetic activity because exogenous A-II potentiates norepinephrine (NE) release from sympathetic nerve terminals via AT1-R in the normal heart during sympathetic nerve stimulation. However, facilitating the effect of endogenous A-II on NE release during sympathetic nerve stimulation has not been examined directly in the failing heart and needs to be verified in the heart displaying cardiac dysfunction under sympathetic activation, although significant alterations of the effects of A-II via AT1-R on myocyte contractility have been reported as well as the basal coronary vascular tone in the failing heart without sympathetic activation.

On the other hand, exogenous A-II has an inhibitory effect on the positive inotropic action of sympathetic receptor stimulation in the normal heart by activation of protein kinase C via AT1-R. This inhibitory effect, if present in the failing heart, would be important pathophysiologically, because endogenous A-II might further reduce myocardial systolic function through this mechanism. However, this effect also has not been verified in the failing heart. These 2 issues were investigated in the present study.

We created an abdominal aorto-caval (AV) shunt in rats to induce left ventricular (LV) volume overload hypertrophy with cardiac dysfunction. Four weeks after the operation, the rat was pithed and the heart was perfused retrogradely from the aorta (in vivo Langendorff preparation) to study the interaction between endogenous A-II and activation of the sympathetic nerve system at the peripheral level in the heart. We studied the effect of the AT1-R blocker, losartan, on NE overflow in the coronary effluent and LV contractility under isovolumic contraction, and on the sequel of electrical stimulation of the thoracic sympathetic outflow. In this model, the influences of changes in heart rate, preload and afterload are excluded.

Because the involvement of the A-II type 2 receptor (AT2-R) in A-II induced facilitation of sympathetic neurotransmission has been demonstrated recently in the normal heart, we also examined the effect of the AT2-R blocker, PD123319, on NE overflow and LV contractility.

(Received August 18, 2003; revised manuscript received April 15, 2004; accepted April 20, 2004)
First Department of Internal Medicine, Fukushima Medical University, Fukushima, Japan
Mailing address: Yukio Maruyama, MD, Professor and Chairman, First Department of Internal Medicine, Fukushima Medical University, 1 Hikarigaoka, Fukushima 960-1295, Japan. E-mail: maruyama @fmu.ac.jp
Methods

A total of 62 male Wister rats were used in this study, which was carried out under the supervision of the Animal Research Committee in accordance with the Guideline on Animal Experiments of Fukushima Medical University and the Japanese Government Animal Protection and Management Law (No. 115).

Animal Model

An AV shunt was created to induce volume overload cardiac hypertrophy (AV shunt rats, n=31). Briefly, after anesthesia with sodium pentobarbital (50 mg/kg ip), the abdominal aorta and inferior vena cava were carefully exposed. The abdominal aorta was clamped just caudal to the left renal artery and then punctured with an 18 gauge disposable needle at a site between the renal artery and the aortic bifurcation. The needle was advanced into the inferior vena cava. After the needle was withdrawn, the aortic puncture was sealed with cyanoacrylate glue. Identical operations were performed in control rats without making the fistulas (sham rats, n=31). After the operation, the rats were caged individually for 4 weeks during which they had free access to normal rat chow and water.

Echocardiographic Assessment

After light anesthesia with pentobarbital (20 mg/kg, ip), M-mode echocardiograms at the papillary muscle level using a 10-MHz sector scan probe (Model SONOS 100CF, Hewlett-Packard, USA) were recorded 4 weeks after the shunt operation. LV end-diastolic diameter (LVEDD), LV end-systolic diameter (LVESD), interventricular septal thickness (IVST), and LV posterior wall thickness (LVPWT) were measured and the values in 15 beats were averaged.

Surgical Preparation for the Main Experiment

Following echocardiographic examination, a midline laparotomy was performed and 3 ml of blood was sampled from the inferior vena cava to measure plasma A-II and NE concentrations. Then, atropine sulfate (1 mg/kg) and heparin sodium (200 units) were injected to inhibit parasympathetic nervous activation by electrical field stimulation and blood clotting, respectively. The chest was opened by a midline incision and a polyethylene cannula (PE 205, Clay Adams, Parsippany, NJ, USA) was inserted retrogradely into the ascending aorta for retrograde perfusion of the heart, as previously described.15,16 The heart was then perfused by a peristaltic pump (Micro Tube Pump MP-3B, Tokyo Rikakikai Co Ltd, Tokyo, Japan) with oxygenated modified Krebs-Henseleit solution containing (in mmol/L): NaCl 118, KCl 4.7, CaCl2 2.5, KH2PO4 1.2, MgSO4 1.2, NaHCO3 2.5, glucose 5, and sodium pyruvate 5. The perfusate was continuously bubbled with 95% O2–5% CO2 and it was continuously bubbled with 95% O2–5% CO2 and 10 mmHg. LV systolic pressure (LVSP) and the first derivative of LV pressure (LV dp/dt) were monitored continuously by a pressure and differential amplifier (Polygraph 360 SYSTEM, NEC, Japan). Finally, a polyethylene cannula (PE 205) was inserted from the inferior vena cava into the right atrium to collect coronary effluent.15,16

Sympathetic Nerve Stimulation

After the perfusion system was set up, the spine was cut at C1, and the spinal cord from C1 to the sacral end was carefully pithed using a steel wire. A platinum bipolar electrode was inserted and placed at the T1–T5 level to stimulate the cardiac sympathetic nerve system.15,16 Cardiac sympathetic nerve stimulation (SNS) was performed by electrical field stimulation from the thoracic spinal canal for 45 s with a series of monophasic square pulses of 1 ms duration, 3 Hz, and supramaximal voltage. The coronary perfusate from the right atrium was collected for 60 s from the start to 15 s after SNS for analysis of NE overflow. The reproducibility of the LV inotropic response and NE overflow from repeated SNS with an interval of 15 min was confirmed in both sham and AV shunt rats (n=6, each) by comparing them with the first SNS. In the second SNS, LVSP was 99±1% in sham rats and 101±1% in AV shunt rats, and NE overflow was 97±7% in sham rats and 104±8% in AV shunt rats (NS, each).

Experimental Protocol

Both groups of rats (sham rats and AV shunt rats, n=25 each) were divided into 4 sub-groups, and used for the following experimental protocols.

1. SNS with infusion of the AT1-R blocker, losartan (n=7, in each group). After making the experimental preparation, 15 min was allowed for stabilization and the first SNS was performed in the baseline state (SNS1). Ten min after SNS1, a continuous infusion of 10–6 mol/L losartan (Merck Co, USA) into the coronary perfusate, which causes virtually 100% occupancy of the AT1-R,20 was started using a microinjector (CFV-2100, Nihon kohden). After 5 min of losartan infusion, the second SNS (SNS2), identical to SNS1, was performed in each group.

2. SNS with infusion of the AT2-R blocker, PD123319 (n=6, in each group). Ten min after SNS1, a continuous infusion of 10–6 mol/L PD123319 (Sigma Chemical Co, St Louis, MO, USA), which also causes almost 100% occupancy of the AT2-R,20 was started using a microinjector. Five min after the start of PD123319 infusion, SNS2 was performed.

3. SNS with A-II infusion (n=7, in each group). Ten min after SNS1, a continuous infusion of 10–6 mol/L A-II (Sigma Chemical Co) was started using a microinjector. The concentration of A-II infused in this protocol was determined as the maximum concentration not causing a significant decrease in CorF in a preliminary study. Five min after the start of A-II infusion, SNS2 was performed.

4. NE infusion study (n=5, in each group). To clarify whether the action of A-II on the myocyte inotropic response to NE was altered, the LV inotropic response to NE was measured in both sham and AV shunt rats with or without concomitant

Circulation Journal Vol.68, July 2004
Altered Adrenergic Function by A-II in CHF

A-II infusion. After the infusion of $5 \times 10^{-7}$ mol/L NE for 5 min, $10^{-6}$ mol/L NE was infused into the coronary inflow line, at $100\text{nl/min}$ in both cases. The concentration of NE infused in this protocol was determined as the concentration causing maximum LV positive inotropic steady-state response in a preliminary study. Ten min after NE infusion, the coronary perfusate was switched to Krebs-Henseleit solution containing $10^{-9}$ mol/L A-II. Five min after the start of A-II infusion, NE infusion was repeated.

These 4 experimental groups were used because we wanted to investigate changes in LV systolic function and NE release via the interaction between A-II and sympathetic nerve activation, and to compare those responses without and with an AT1-R or AT2-R blocker in order to analyze how the AT1-R- or AT2-R-mediated action is involved in each result. We also wanted to clarify these responses after administering high doses of A-II exogenously, because the endogenous level of A-II may be high in the AV shunt heart, leading to different responses in LV contractility or NE release caused by SNS. Also, NE release following SNS with or without A-II stimulation may not be the same in normal and diseased hearts, leading to different responses. For these reasons, the interaction between NE and A-II administered exogenously was investigated not only in protocol (3), but also in protocol (4) under which the same amount of NE was infused to exclude the possibility that different NE release caused by SNS with or without A-II may lead to misunderstanding of results.

LV Mechanical Data Analyses

LV volume was calculated by the Pombo method (ie, the cube of the LV short-axis internal diameter obtained from an echocardiogram). Cardiac output and LV ejection fraction were calculated as follows: ($LVEDD^3 - LVESD^3$)/$LVEDD^3$, respectively.

LVSP and maximal positive LV dp/dt (LV +dp/dt) were measured and averaged for 5 s at the baseline steady state and at the end of SNS or NE infusion. NE overflow was calculated as the product of NE concentration in the coronary perfusate and total CorF in 60 s, and was normalized according to the heart weight, which was measured after the experiment.

Biochemical Analyses

Plasma A-II concentrations were determined by radioimmunoassay. NE was measured by high-performance liquid chromatography (HPLC-725, Tosoh, Tokyo, Japan) with a limit of detection of 0.1 pmol/ml.15

<table>
<thead>
<tr>
<th>Variables</th>
<th>Baseline (n=31)</th>
<th>SNS (n=31)</th>
<th>Baseline (n=31)</th>
<th>SNS (n=31)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak LVSP (mmHg)</td>
<td>95±2</td>
<td>159±4*</td>
<td>96±3</td>
<td>139±4*</td>
</tr>
<tr>
<td>(% change)</td>
<td>(100)</td>
<td>(107±5)</td>
<td>(100)</td>
<td>(145±7)</td>
</tr>
<tr>
<td>LV +dp/dt (mmHg/s)</td>
<td>2.23±1.06</td>
<td>4.97±246*</td>
<td>2.25±141</td>
<td>3.92±159*</td>
</tr>
<tr>
<td>(% change)</td>
<td>(100)</td>
<td>(223±12)</td>
<td>(100)</td>
<td>(175±14)</td>
</tr>
<tr>
<td>CorF (ml/min)</td>
<td>12.3±0.6</td>
<td>12.6±0.6</td>
<td>15.9±1.2*</td>
<td>15.9±1.2*</td>
</tr>
<tr>
<td>NE (pmol/g)</td>
<td>&lt;0.1</td>
<td>58±13*</td>
<td>&lt;0.1</td>
<td>21±3*</td>
</tr>
</tbody>
</table>

Values are mean±SEM. SNS, sympathetic nerve stimulation; LVSP, left ventricular systolic pressure; LV +dp/dt, left ventricular +dp/dt; CorF, coronary flow; NE, norepinephrine overflow.

*p<0.01 vs baseline, †p<0.01, and ‡p<0.05 vs sham rats. NE of coronary effluent was not detected in the baseline state before SNS.

Results

Table 1 shows the body weights, heart weights, plasma concentrations of A-II and NE, and the echocardiographic findings in the AV shunt and sham groups. Heart weight was increased by approximately 90% in the AV shunt group (p<0.01). Plasma A-II and NE concentrations were significantly higher in the AV shunt group than in the sham group (p<0.01). LVVEDD and LVEDV were significantly increased in the AV shunt group (p<0.01), indicating the development of LV volume overload hypertrophy. LV ejection fraction was slightly but significantly smaller in the AV shunt group (p<0.01); however, the AV shunt rats showed no signs of congestive heart failure such as peripheral edema.

LV Contractile Responses to and NE Overflow From SNS

In the basal state, peak LVSP and LV +dp/dt were simi-

Table 2 Left Ventricular Contractile Response, Coronary Flow Change and Norepinephrine Overflow in Response to SNS at a Fixed Heart Rate (300 Beats/Min) in Sham and AV Shunt Rats

<table>
<thead>
<tr>
<th>Variables</th>
<th>Sham (n=31)</th>
<th>AV shunt (n=31)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>SNS</td>
<td>Baseline</td>
</tr>
<tr>
<td>Peak LVSP (mmHg)</td>
<td>95±2</td>
<td>159±4*</td>
</tr>
<tr>
<td>(% change)</td>
<td>(100)</td>
<td>(107±5)</td>
</tr>
<tr>
<td>LV +dp/dt (mmHg/s)</td>
<td>2.23±1.06</td>
<td>4.97±246*</td>
</tr>
<tr>
<td>(% change)</td>
<td>(100)</td>
<td>(223±12)</td>
</tr>
<tr>
<td>CorF (ml/min)</td>
<td>12.3±0.6</td>
<td>12.6±0.6</td>
</tr>
<tr>
<td>NE (pmol/g)</td>
<td>&lt;0.1</td>
<td>58±13*</td>
</tr>
</tbody>
</table>

Values are mean±SEM. SNS, sympathetic nerve stimulation; LVSP, left ventricular systolic pressure; LV +dp/dt, left ventricular +dp/dt; CorF, coronary flow; NE, norepinephrine overflow.

*p<0.01 vs baseline, †p<0.01, and ‡p<0.05 vs sham rats. NE of coronary effluent was not detected in the baseline state before SNS.

Table 1 Body Weight, Heart Weight, Plasma Concentrations of A-II and NE and Echocardiographic Findings in Sham and AV Shunt Rats

<table>
<thead>
<tr>
<th>Variables</th>
<th>Sham (n=31)</th>
<th>AV shunt (n=31)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BW (g)</td>
<td>292±17</td>
<td>299±28</td>
</tr>
<tr>
<td>HW (g)</td>
<td>0.82±0.08</td>
<td>1.55±0.26*</td>
</tr>
<tr>
<td>A-II (pmol/L)</td>
<td>78±15</td>
<td>223±38*</td>
</tr>
<tr>
<td>NE (pmol/L)</td>
<td>779±212</td>
<td>1.820±74*</td>
</tr>
<tr>
<td>LVEDD (mm)</td>
<td>5.2±0.21</td>
<td>7.45±0.97*</td>
</tr>
<tr>
<td>LVEDV (mm)</td>
<td>5.09±0.36</td>
<td>4.40±0.81*</td>
</tr>
<tr>
<td>LVST (mm)</td>
<td>1.23±0.08</td>
<td>1.26±0.14</td>
</tr>
<tr>
<td>LVPTW (mt)</td>
<td>1.22±0.11</td>
<td>1.28±0.25</td>
</tr>
<tr>
<td>LVEF (%)</td>
<td>86.4±6.1</td>
<td>80.6±5.6*</td>
</tr>
<tr>
<td>CO (ml/min)</td>
<td>68±24</td>
<td>189±14*</td>
</tr>
</tbody>
</table>

Values are mean±SEM. BW, body weight; HW, heart weight; A-II, angiotensin II; NE, norepinephrine; LVEDD, left ventricular end-diastolic diameter; LVEDV, left ventricular end-systolic diameter; LVST, interventricular septal thickness; LVPTW, left ventricular posterior wall thickness; LVEF, left ventricular ejection fraction; CO, cardiac output. *p<0.01 vs sham rats.

Statistical Analyses

The data are presented as means±SEM. The unpaired Student's t-test was used for comparisons between the AV shunt group and the sham group. Sequential changes in LV mechanical indices, CorF and NE overflow were compared by one-way ANOVA, followed by Fisher's least significant difference method as a post hoc test. Comparisons of the rate of increase in LVSP and LV +dp/dt before and after each pharmacological intervention were performed by paired Student's t-test. Differences were considered significant at a level of p<0.05.
Fig 1. Comparisons of the increases in peak left ventricular systolic pressure (peak LVSP, Left panel), maximal left ventricular +dp/dt (LV +dp/dt, Middle panel) and norepinephrine (NE) overflow (Right panel) during sympathetic nerve stimulation (SNS1 and SNS2) without and with losartan treatment in the sham rats (Upper panel, n=7) and the AV shunt rats (Lower panel, n=7). SNS2 with losartan significantly potentiated the increases in peak LVSP, LV +dp/dt and NE overflow in the AV shunt rats, but did not alter them in the sham rats. *p<0.01, †p<0.05 vs corresponding SNS1 without losartan in the same group.

Fig 2. Comparisons of the increases in peak left ventricular systolic pressure (peak LVSP, Left panel), maximal left ventricular +dp/dt (LV +dp/dt, Middle panel) and norepinephrine (NE) overflow (Right panel) during sympathetic nerve stimulation (SNS1 and SNS2) without and with PD123319 treatment in the sham rats (Upper panel, n=6) and the AV shunt rats (Lower panel, n=6). SNS2 with PD123319 significantly attenuated the increases in peak LVSP, LV +dp/dt and NE overflow in the AV shunt rats, but did not alter them in the sham rats. *p<0.01, †p<0.05 vs corresponding SNS1 without PD123319 in the same group.
lar in the sham and AV shunt groups (Table 2). However, CorF was higher in the AV shunt than in the sham group (p<0.05), reflecting the increase in LV mass. During SNS, peak LVSP and LV +dp/dt significantly increased in both groups (p<0.01, each), but the increases were significantly smaller in the AV shunt group than in the sham group (p<0.01, each). NE was not detected in the coronary effluent of either group in the baseline state. The increase in NE overflow caused by SNS was significantly greater in the sham group than in the AV shunt group (p<0.01).

Effects of Losartan on LV Contractile Responses to and NE Overflow From SNS
Losartan did not alter baseline peak LVSP (93±2 to 93±2 mmHg in the sham group, 100±4 to 100±4 mmHg in the AV shunt group, NS each), and NE overflow remained undetectable after losartan in both groups. The responses of peak LVSP, LV +dp/dt and NE overflow to SNS before and after losartan infusion at a fixed heart rate (300 beats/min) are shown in Fig 1. In the sham group, losartan did not affect the responses of peak LVSP, LV +dp/dt and NE overflow from SNS2. On the contrary, losartan infusion significantly attenuated the responses of peak LVSP (p<0.01) and LV +dp/dt (p<0.01) compared with SNS2 in the AV shunt group, and decreased NE overflow in SNS2 (p<0.05).

Effects of PD123319 on LV Contractile Responses to and NE Overflow From SNS
PD123319 did not alter baseline peak LVSP (97±2 to 97±2 mmHg in the sham group, 92±3 to 92±3 mmHg in the AV shunt group, NS each), and NE overflow remained undetectable after PD123319 in both groups. The responses of peak LVSP, LV +dp/dt and NE overflow to SNS before and after PD123319 infusion at a fixed heart rate (300 beats/min) are shown in Fig 2. In the sham group, PD123319 did not affect the responses of peak LVSP, LV +dp/dt and NE overflow from SNS2. In contrast, PD123319 infusion significantly attenuated the responses of peak LVSP (p<0.05) and LV +dp/dt (p<0.01) to SNS2 in the AV shunt group, and decreased NE overflow in SNS2 (p<0.05).

Effects of A-II on LV Inotropic Responses to and NE Overflow From SNS
Infusion of A-II in the basal state had no significant effect on peak LVSP (97±2 to 97±2 mmHg in the sham group, 92±3 to 92±3 mmHg in the AV shunt group, NS each), and NE overflow remained undetectable after A-II in both groups. The responses of peak LVSP, LV +dp/dt and NE overflow to SNS before and after A-II infusion at a fixed heart rate (300 beats/min) are shown in Fig 3. NE overflow significantly increased (p<0.05), but the rate of increase in peak LVSP and LV +dp/dt was attenuated in SNS2 during the administration of A-II (p<0.01, each) compared with SNS1 in the sham group. In contrast, A-II infusion significantly attenuated NE overflow in SNS2 in the AV shunt group (p<0.01), and attenuated the responses of peak LVSP and LV +dp/dt to SNS2 (p<0.01, each). The rate of increase in peak LVSP during SNS2 was reduced by 14±2% compared with that during SNS1 in the sham group, and by 21±5% in the AV shunt group (NS vs sham group).
LV Contractile Responses to NE Infusion Without and With A-II

The responses of peak LVSP and LV +dp/dt to NE infusion at a fixed heart rate (300 beats/min) are shown in Table 3 and Fig 4. NE infusion increased peak LVSP and LV +dp/dt in a dose-dependent manner in both the sham and AV shunt groups. Peak LVSP and LV +dp/dt at each dose (5×10⁻⁷ mol/L and 10⁻⁶ mol/L) were not different in the 2 groups, although both tended to be greater in the sham group. In the sham group A-II significantly decreased the increase in peak LVSP and LV +dp/dt at both doses (5×10⁻⁷ mol/L and 10⁻⁶ mol/L), and the contractile response to NE increase was unchanged. In contrast, A-II did not alter these responses in the AV shunt group.

Discussion

In this study, LV volume overload hypertrophy with mild systolic dysfunction was induced 4 weeks after AV shunting (AV shunt rats). Both the inotropic response and NE release following SNS were markedly reduced (Table 2), and neurohumoral activation was prominent with significantly increased plasma concentrations of A-II and NE in AV shunt rats compared with sham rats (Table 1). Although it is difficult to define, the hearts of the AV shunt rats may have been in the transitional phase from compensated hypertrophy to the early phase of myocardial failure. The present results clearly showed the interaction between sympathetic nerve activation and A-II irrespective of car-
Altered Adrenergic Function by A-II in CHF

The reason why A-II, losartan and PD123319 changed neither LV contractility nor NE release in the basal condition without SNS in both sham and AV shunt rats, would be related to the very low basal sympathetic tone in the pithed rats in which the NE concentrations in the coronary effluent were negligibly small (Table 2). The major findings of this study are as follows. First, it was confirmed that A-II has both a facilitative effect on NE release and an inhibitory effect on the positive inotropic action of sympathetic stimulation in the normal heart, because A-II infusion further increased NE overflow, but reduced the positive inotropic response to SNS in the sham rats (Fig 3). We speculate that the reason the AT1-R and AT2-R blockers had no effect on either NE overflow or LV contractility after SNS in sham rats (Figs 1,2) is that there was insufficient endogenous tissue A-II to be blocked in the normal heart. Second, the facilitative effect of A-II via AT1-R on NE release would be reversed completely and the inhibitory effect on the positive inotropic action of sympathetic stimulation would disappear in AV shunt rats. As a result, A-II infusion decreased NE overflow and the LV positive inotropic response to SNS (Fig 3), and did not affect the positive inotropic action of NE infusion in the AV shunt rats (Fig 4). In contrast, losartan further increased NE overflow and the LV positive inotropic response to SNS (Fig 1). The significant effect of losartan indicates there was a sufficient amount of endogenous A-II in the hypertrophied heart of the AV shunt rat. Third, A-II facilitated NE release via AT2-R in AV shunt rats, as previously reported in the normal heart but this effect would be overwhelmed by the inhibition of NE release via AT1-R in the AV shunt rats (Figs 1,2). This is the first report to clarify the altered effect of A-II via AT1-R on the sympathetic nervous system in the heart with volume overload hypertrophy and mild systolic dysfunction. The overall effect is that endogenous A-II in the LV contractility during augmented sympathetic nerve activity. However, the contractility response to NE infusion, attenuates the positive inotropic response in the AV shunt rat, although it was not significant in the normal heart. Accordingly, it is likely that endogenous tissue A-II has a positive inotropic effect via AT2-R; however, from the results of our study it is not known whether A-II has any effect via AT2-R on the receptor mediated positive inotropic response, because LV contractility and NE release during SNS were decreased concomitantly by an AT2-R blocker (Fig 2).

Study Limitations

First, it remains unknown whether the altered effects of A-II via AT1-R or AT2-R also occur in other types of heart disease or in the severely decompensated contractile state. Second, we did not investigate the intracellular signaling transduction downstream of the A-II receptor. It has been reported that inhibitory G protein increases in the hypertrophied heart so reconstitution of the intracellular signal of the AT1-R might also be involved in the alterations reported here. The intracellular mechanisms should be examined in a future study. Third, we assumed there was a sufficient amount of endogenous tissue A-II in the heart of AV shunt rats as previously reported but we did not directly measure tissue A-II. Fourth, it has been reported that AT2-R increases from approximately 40% in normal rat ventricle to approximately 60% in the AV shunt rat heart. Therefore, it is possible that the enhancement of the effect of A-II via AT2-R was caused by the increase in AT2-R in the AV shunt rat heart; however, the densities of AT1- and AT2-R were not investigated in this study. Fifth, the present study was an acute administration study. It has been reported that losartan treatment for CHF gradually reduces plasma NE concentration. Therefore, it should be taken into consideration that the effects of A-II obtained in this study were acute effects on untreated hypertrophy with mild systolic dysfunction.

Clinical Implication

The reduction of further activation of sympathetic activity has been suggested as a mechanism for the beneficial effects of ACE inhibitor and AT1-R blocker therapies for CHF. However, the results of this study indicate that losartan facilitates NE release with a concomitant increase in LV contractility during augmented sympathetic nerve activation, at least as an acute effect. It is known that the reserve of NE for release by cardiac sympathetic stimulation is reduced in CHF, which correlates with decreased exercise capacity as well as resting LV function and prognosis. Losartan might improve the reduction in NE release, and concomitantly LV dysfunction and/or exercise.
capacity, at least acutely. However, it is not known whether this action of an AT₁-R blocker is essential for a beneficial effect on morbidity or mortality in CHF. A chronic administration study of AT₁-R and AT₂-R blockers is needed to validate the results of this study.

References