Improvement in Baroreflex Function by an Oral Angiotensin Receptor Antagonist with Myocardial Infarction

Masahiko Nishizawa, Hiroo Kumagai, Masashi Ichikawa, Naoki Oshima, Hiromichi Suzuki, Takao Saruta

Abstract Impaired baroreflex function is a factor responsible for poor prognosis in myocardial infarction patients. Using logistic function curves, we calculated the maximal gain of the baroreflex control of renal sympathetic nerve activity (RSNA) and heart rate in conscious Wistar-Kyoto and spontaneously hypertensive rats whose left anterior descending artery had been ligated 4 weeks earlier. We further investigated whether 3-week oral treatment with the angiotensin II type 1 receptor antagonist TCV-116 would improve the baroreflex in rats with myocardial infarction. The maximal gain of the mean arterial pressure-RSNA relation in spontaneously hypertensive rats with myocardial infarction treated with vehicle (1.7 ± 0.1% control per mm Hg) was smaller than the gain in sham-operated hypertensive rats whose left anterior descending artery had been ligated 4 weeks earlier. We also tested the hypothesis that long-term oral treatment with the renin-angiotensin system has a favorable influence on the long-term outcome of patients with MI. Thus, baroreflex dysfunction is considered a critical factor in the poor prognosis of MI patients. Moreover, since a recent study examining the relation between blood pressure and mortality among MI patients showed that the percentage of men with prior MI showed that the percentage of men dying from coronary artery disease was much higher in the hypertensive group, it is important to examine whether high blood pressure exacerbates the influence of MI on baroreflex function.

In the present study, we compared the baroreflex function of conscious WKY and SHR whose left coronary artery had been ligated 4 weeks earlier. We also tested the hypothesis that long-term oral treatment with the renin-angiotensin system has a favorable influence on the long-term outcome of patients with MI. Thus, baroreflex dysfunction is considered a critical factor in the poor prognosis of MI patients. Moreover, since a recent study examining the relation between blood pressure and mortality among MI patients showed that the percentage of men dying from coronary artery disease was much higher in the hypertensive group, it is important to examine whether high blood pressure exacerbates the influence of MI on baroreflex function.

It has been reported that baroreflex function is impaired in hypertension, that MI is associated with an impaired baroreflex, and that the blunted baroreflex in MI is involved in sudden death or fatal arrhythmia. Recently, it has been reported that intravenously injected Ang II receptor antagonist improves the blunted baroreflex in MI in normotensive rats and that improvement in baroreflex by blockade of the renin-angiotensin system has a favorable influence on the long-term outcome of patients with MI. Thus, baroreflex dysfunction is considered a critical factor in the poor prognosis of MI patients. Moreover, since a recent study examining the relation between blood pressure and mortality among MI patients showed that the percentage of men dying from coronary artery disease was much higher in the hypertensive group, it is important to examine whether high blood pressure exacerbates the influence of MI on baroreflex function.

Methods

Male 15-week-old SHR and age-matched WKY were purchased from Charles River Japan Co, Atsugi, Japan. All surgical and experimental procedures were in accordance with institutional animal care guidelines. Rats were housed singly in cages in a room with constant temperature and a 12-hour light/dark cycle.

Myocardial Infarction

We used a previously described technique involving ligation of the left coronary artery to produce chronic MI. With rats under ether inhalation anesthesia, the heart was exteriorized via a left thoracotomy, and the left anterior descending artery was ligated between the pulmonary outflow tract and left atrium. The heart was returned to its normal position, the thorax was closed, and the air was removed. Sham control rats underwent the same procedure but did not have their left coronary artery ligated. Rats were returned to individual cages with free access to normal sodium rat chow (0.38% NaCl, Nippon Clea) and tap water.

Experimental Protocols

Both the WKY and SHR were divided into four groups of six rats each: a sham-operated, vehicle-treated (sham-vehicle) group, a sham-operated, TCV-116-treated (sham-TCV) group, an MI, vehicle-treated (MI-vehicle) group, and an MI, TCV-116-treated (MI-TCV) group. The MI-vehicle rats and sham-vehicle rats were fed normal sodium rat chow for 4 weeks after the left coronary artery ligation or sham ligation. The MI-TCV and sham-TCV rats were given the specific nonpeptide angiotensin type 1 receptor antagonist TCV-116 (1 mg/kg per day) mixed in ordinary chow for 3 weeks, starting 1 week after the left coronary artery ligation or sham ligation. TCV-116 has been shown to interact with Ang II in bovine adrenal cortical membrane fractions with type 1 receptor in a competitive manner but not to affect the binding of...
Ang II to type 2 receptor in bovine cerebellum. Each group was evaluated for baroreflex function at 19 weeks of age.

**Arterial and Venous Catheterization**

Four weeks after the left coronary artery ligation, with rats under ether inhalation anesthesia, the left and right femoral veins were catheterized with modified polyethylene tubes made of PE-10 tubing (Clay Adams) fused with PE-50 tubing (Clay Adams) for drug infusion, and the left femoral artery was catheterized for measurement of arterial pressure and HR. The catheters were exteriorized at the back of the neck.

**Implantation of Renal Nerve Electrodes**

Rats were anesthetized with pentobarbital sodium (30 mg/kg IV supplemented with 10 mg/kg IV as needed, Abbott Laboratories). Multifiber recordings of postganglionic RSNA were made as described previously. The left kidney was exposed via a retroperitoneal approach through a left flank incision. A renal nerve fascicle was identified under a dissecting microscope and carefully isolated. Polytetrafluoroethylene-coated, multistrand, stainless steel wire electrodes (A-M Systems, Inc) were placed on the nerve. The nerve and electrodes were covered with silicon gel (Sil-Gel 604A and B, Wacker-Chemie) to prevent the nerve from drying. When the gel had hardened, the electrodes were looped in the flank area. The flank incision was closed, and the electrodes were exteriorized at the back of the neck.

**Recording of RSNA**

After surgical preparation, the rats were again housed in individual cages. A minimum of 24 hours later, conscious rats were placed in a nonrestraining holder. The arterial catheter was connected to a transducer (TP-200T, Nihon Kohden Co) for measurement of arterial pressure, MAP, and HR (AT-601G, Nihon Kohden). The nerve recording electrodes were connected to a high-impedance probe (JB1011, Nihon Kohden), which was in turn connected to a differential amplifier (AVB-10, Nihon Kohden) with a band-pass filter (50 to 3000 Hz). The root mean square of RSNA was the whole-nerve activity obtained by recording and integrating the activity using an RMS integrator (EI-601G, Nihon Kohden) with a time constant of 28 milliseconds.

Mean RSNA was defined as the root mean square of RSNA further filtered at 0.08 Hz, and we used this for quantification throughout the studies. Since the RSNA value remaining after maximal inhibition by high-dose phenylephrine was similar to the background noise observed 30 minutes postmortem, the former value was subtracted from RSNA values. Arterial pressure, MAP, HR, original renal neurogram, and mean RSNA were recorded on a thermal array recorder (RTA-1300, Nihon Kohden). MAP, HR, and mean RSNA were sampled at appropriate frequencies (1 to 5 Hz) with an analog-to-digital translation system (MacLab/8s, AD Instruments Pty, Ltd).

**Analysis of Baroreceptor Reflex Function**

After the rats had rested for 60 minutes, arterial pressure, MAP, HR, and RSNA were recorded. Baroreflex curves expressing MAP-RSNA and MAP-HR relations were generated with rats in the conscious state by measurement of RSNA and HR responses to ramp increases and decreases in MAP produced by alternate intravenous infusion of phenylephrine and nitroprusside. Phenylephrine (4.9 mmol/L) was infused at 2.34 to 6.43 µL/mm to raise MAP by 40 mm Hg in 1 to 2 minutes, and nitroprusside (2.2 mmol/L) was infused at 0.1 to 0.39 µL/mm to reduce MAP by 30 to 40 mm Hg in 10 to 20 seconds. At least 30 minutes was allowed between drug infusions. The baseline resting value of mean RSNA immediately before infusion of phenylephrine or nitroprusside was defined as 100%.

The data of each rat for the MAP-RSNA and MAP-HR relations after infusions of phenylephrine and nitroprusside were individually fitted to a logistic function curve using a nonlinear regression program (JMP version 3.0) on a Macintosh computer. The best fit of the curve was obtained with the above computer program. Four parameters were derived from the following equation: RSNA or HR = P1 / (1 + exp [P2(MAP - P3)]) + P4, where P1 is the range of RSNA or HR, P2 is the slope coefficient (independent of the range), P3 is the MAP corresponding to half the RSNA or HR range, and P4 is the lower plateau of RSNA or HR. Baroreceptor reflex sensitivity was defined as the Gmax values of the logistic function curve. Gmax was calculated as -P1 x P2 / P4. In the present study, the goodness of fit, which was determined by the percentage of the total sums of squares that were accounted for by the model, was greater than 95%.

**Measurement of Left Ventricular End-Diastolic Pressure, Heart Weight, and Infarct Size**

After the experiment to test baroreflex function, the rat was anesthetized with pentobarbital. Another catheter, connected to a transducer (TP-200T, Nihon Kohden) for measurement of left ventricular end-diastolic pressure (LVEDP), was inserted into the left ventricle via the right carotid artery. The rat was then killed with an overdose of pentobarbital. The heart was removed, and the left ventricle and septum were separated from the free wall.

<p>| TABLE 1. Baseline Characteristics in Myocardial Infarction and Sham-Operated Rats |
|---------------------------------|---------|---------|-----------------|--------|---------|-----------------|--------|</p>
<table>
<thead>
<tr>
<th></th>
<th>MAP, mm Hg</th>
<th>HR, bpm</th>
<th>Mi Size, %</th>
<th>LVEDP, mm Hg</th>
<th>BW, g</th>
<th>LV/BW, x10^-3</th>
<th>RV/BW, x10^-4</th>
</tr>
</thead>
<tbody>
<tr>
<td>WKY</td>
<td>110±7</td>
<td>320±14</td>
<td>3±1</td>
<td>432±5</td>
<td>1.6±0.08</td>
<td>4±0.5</td>
<td></td>
</tr>
<tr>
<td>Sham-veh</td>
<td>104±7</td>
<td>340±11</td>
<td>3±1</td>
<td>417±21</td>
<td>1.6±0.08</td>
<td>4±0.4</td>
<td></td>
</tr>
<tr>
<td>Sham-TCV</td>
<td>102±5</td>
<td>324±11</td>
<td>34±3</td>
<td>428±5</td>
<td>2.1±0.01</td>
<td>7±0.2</td>
<td></td>
</tr>
<tr>
<td>Ml-veh</td>
<td>104±3</td>
<td>330±12</td>
<td>33±4</td>
<td>428±10</td>
<td>1.8±0.07</td>
<td>5±0.3</td>
<td></td>
</tr>
<tr>
<td>Ml-TCV</td>
<td>113±15</td>
<td>331±12</td>
<td>30±5</td>
<td>330±7</td>
<td>2.3±0.06</td>
<td>9±0.3</td>
<td></td>
</tr>
<tr>
<td>SHR</td>
<td>143±3</td>
<td>323±8</td>
<td>5±1</td>
<td>333±5</td>
<td>2.3±0.04</td>
<td>6±0.1</td>
<td></td>
</tr>
<tr>
<td>Sham-TOV</td>
<td>113±2</td>
<td>346±10</td>
<td>4±1</td>
<td>300±22</td>
<td>2.0±0.03</td>
<td>6±0.1</td>
<td></td>
</tr>
<tr>
<td>Ml-TOV</td>
<td>98±7</td>
<td>324±7</td>
<td>28±3</td>
<td>330±5</td>
<td>2.7±0.06</td>
<td>8±0.03</td>
<td></td>
</tr>
<tr>
<td>Ml-TCV</td>
<td>113±16</td>
<td>331±12</td>
<td>30±5</td>
<td>330±7</td>
<td>2.3±0.06</td>
<td>9±0.3</td>
<td></td>
</tr>
</tbody>
</table>

Definitions are as in Abbreviations and Acronyms box and LVEDP, left ventricular end-diastolic pressure, BW, body weight, LV/BW, ratio of left ventricular to body weight, RV/BW, ratio of right ventricular to body weight, sham-veh, sham-operated and vehicle-treated, sham-TOV, sham-operated and TOV-116-treated, Mi-veh, Mi and vehicle-treated, and Mi-TCV, Mi and TOV-116-treated

*p<0.05 vs sham-veh, **p<0.01 vs sham-veh, #p<0.05 vs Mi-veh
of the right ventricle. The left and right ventricles were weighed separately. The left ventricle was cut into four slices perpendicular to the cardiac long axis. The slices were stained for 4 to 7 minutes at 37°C in a 1% solution of triphenyltetrazolium chloride (TTC, Sigma Chemical Co) in phosphate buffer. TTC stained the normal tissue red, but necrotic tissue remained unstained. All slices were photographed, and the area of myocardium unstained by TTC was measured with a planimeter (MAP 100, Casio Co). The TTC-unstained area was expressed as a fraction of the total cross-sectional area of the left ventricle.

**Statistical Analysis**

Data were analyzed with StatView 4.02 for Macintosh computers. Statistical analysis was performed with Student's t test for unpaired data. One-way ANOVA and Scheffe's F test were used for group comparisons. Values are expressed as mean±SE, a value of \( P < 0.05 \) was considered statistically significant.

**Results**

Baseline characteristics of the WKY and SHR are given in Table 1. All rats that underwent coronary artery ligation developed moderate MI, with infarct size ranging from 25% to 40% of left ventricular circumference. There were no significant differences in infarct size in the various MI groups. The MAPs of SHR with MI-vehicle and with MI-TCV were lower than the MAPs of SHR with sham-vehicle, whereas the MAPs of WKY in all groups were similar. Both right ventricle weight-to-body weight ratios (RV/BW) and left ventricle weight-to-body weight ratios (LV/BW) were significantly larger in MI-vehicle SHR and MI-vehicle WKY than that in sham-vehicle rats or MI-TCV rats. The LVEDPs of MI-vehicle WKY and SHR were significantly higher than in sham-vehicle rats or MI-TCV rats (Table 1).

Representative logistic function curves that fit to actual data are shown in Figure 1. The MAP-RSNA and MAP-HR relation curves, which reflect baroreflex function, are shown in Figures 2 and 3, and the \( G_{max} \) values of MAP-RSNA and MAP-HR relations are shown in Figures 4 and 5, respectively. Four parameters of logistic function curves are provided in Tables 2 and 3. The \( G_{max} \) of the MAP-RSNA relation in MI-vehicle WKY (3.4±0.3% control per mm Hg) was smaller \( (P < 0.05) \) than the \( G_{max} \) in sham-vehicle WKY (4.7±0.4) (Fig 4). By 3 weeks of oral treatment with TCV-116, the \( G_{max} \) in MAP-RSNA in MI rats was 4.6±0.1 and was significantly greater than the \( G_{max} \) in MI-vehicle rats. Baroreflex control of HR in WKY with MI (MI-WKY) was more severely impaired \( (P < 0.01) \) versus sham-operated rats, Figure 5) than that of RSNA \( (P < 0.05) \) versus sham-operated rats, Fig 4). As shown in Figure 5, the \( G_{max} \) of the MAP-HR relation in MI-TCV rats (3.3±0.1 beats per minute/mm Hg) was larger than the \( G_{max} \) in MI-vehicle rats (1.8±0.3).

The \( G_{max} \) of the MAP-RSNA relation in MI-vehicle SHR (1.7±0.1) was smaller \( (P < 0.05) \) than the \( G_{max} \) in sham-vehicle SHR (2.3±0.1) (Fig 4). After TCV-116 treatment, the \( G_{max} \) of MAP-RSNA in SHR with MI (MI-SHR) was 2.3±0.1 and significantly greater than the \( G_{max} \) in MI-vehicle SHR. This value did not differ from the \( G_{max} \) in sham TCV-treated SHR. In MI-SHR, baroreflex regulation of HR \( (P < 0.005) \) versus sham-operated, Fig 5) was more seriously impaired than baroreflex regulation of...
Nishizawa et al
Baroreflex and Angiotensin Receptor Antagonist

WKY

- sham-veh
- sham-TCV
- MI-veh
- MI-TCV

SHR

- sham-veh
- sham-TCV
- MI-veh
- MI-TCV

250 ----_
200 1 1
50 100 150 200
Mean Arterial Pressure (mmHg)

FIG 3. MAP-HR relation curves in WKY and SHR. Definitions as in Fig 2 legend. Circles and squares demonstrate resting points.

RSNA (P<.05 versus sham-operated, Fig 4). Dysfunction of baroreflex control of HR in MI-SHR (P<.005) was more severe than dysfunction of HR control in MI-WKY (P<.01, Fig 5). The $G_{max}$ of the MAP-HR relation in MI-TCV SHR (1.5±0.1) was also larger than the $G_{max}$ in MI-vehicle SHR (0.3±0.1), but it was still smaller ($P<.01$) than the $G_{max}$ in sham-TCV SHR (2.7±0.2, Fig 5).

Discussion

We investigated the effects of 3 weeks of oral treatment with the Ang II receptor antagonist TCV-116 on the arterial baroreflex control of RSNA and HR in conscious MI-SHR and MI-WKY by using logistic function curves. The principal findings obtained were as follows: (1) in WKY and SHR, the $G_{max}$ values of baroreflex control of RSNA and HR were smaller in vehicle-treated MI than that in sham-operated, vehicle-treated rats; (2) baroreflex regulation of HR was more severely impaired by MI than baroreflex control of RSNA in both WKY and SHR; (3) oral treatment with an Ang II receptor antagonist restored the baroreflex control of RSNA and HR in MI-WKY to a level comparable to that in sham-operated, vehicle-treated WKY; (4) baroreflex regulation of RSNA was favorably restored by the Ang II receptor antagonist in MI-SHR; and (5) improvement in baroreflex control of HR by the Ang II receptor antagonist in MI-SHR did not reach the level in sham-operated SHR.

In previous studies,1-3, we showed that baroreflex function is impaired in hypertensive animals. Many articles have reported that the baroreflex is also impaired in animals and humans after MI.4-7,12-18,19 Schwartz et al3 demonstrated that the degree of baroreflex dysfunction in MI was closely correlated with the occurrence of fatal arrhythmia. There are some reports claiming that administration of an ACE inhibitor or Ang II receptor antagonist attenuates cardiac remodeling and improves cardiac function in MI,20-22 and other studies have shown that angiotensin-converting enzyme inhibition improves the long-term prognosis of MI patients.8,9,20 Deck et al23 demonstrated that treatment with the angiotensin-converting enzyme inhibitor captopril improved baroreflex control of HR in normotensive Sprague-Dawley rats with MI, and the results obtained in captopril-treated heart failure rats showed that the improvement in baroreflex was associated with a decrease in Ang II. Thus, baroreflex dysfunction seems to be an important factor in worsening the long-term outcome of MI, and endogenous Ang II may play an important role in baroreflex impairment.

In the present study, we found that baroreflex control of HR in MI-SHR was more severely impaired than in MI-
WKY (Fig 5). This suggests that baroreflex dysfunction due to MI can be exacerbated by high blood pressure. This may be one reason why hypertensive MI patients have a higher mortality rate than normotensive MI patients.10,23

The sympathetic nervous system has been shown to be stimulated in MI and cardiac ischemia, and both the suppressed parasympathetic and the enhanced sympathetic nervous systems may be involved in the etiology of ventricular fibrillation or sudden death.6,18,25,26 Moreover, some studies examining baroreflex control of HR in MI in humans or animals have revealed a clear correlation between the degree of baroreflex dysfunction and the occurrence of arrhythmia or high mortality.4,5,26 The results of the present study are compatible with those of previous reports, since our data show that baroreflex regulation of HR is more severely impaired than that of RSNA in MI rats, suggesting that the parasympathetic nervous system as well as the sympathetic system is impaired by the effects of MI.

The present study has demonstrated that baroreflex control of RSNA and HR in normotensive WKY with MI was restored to a level comparable to that of sham-operated WKY by 3 weeks of oral treatment with an Ang II receptor antagonist. DiBona et al.7 reported improved function of baroreflex function in response to intravenous losartan, another Ang II receptor antagonist, in normotensive Sprague-Dawley rats with MI. However, in that study, the G_{max} of baroreflex regulation of RSNA in MI treated by acute administration of the Ang II receptor antagonist did not reach the G_{max} in sham-operated rats. The difference between the study of DiBona et al. and ours may be attributable to differences in the duration of treatment with the Ang II receptor antagonist.

In hypertensive rats with MI, the baroreflex control of HR was more profoundly impaired than the baroreflex control of RSNA. By 3 weeks of oral treatment with the Ang II receptor antagonist DIBona et al.7 reported improvement of baroreflex function in normotensive WKY by 3 weeks of oral treatment with an Ang II receptor antagonist, baroreflex regulation of RSNA in MI treated by acute administration of the Ang II receptor antagonist did not reach the G_{max} in sham-operated rats. The difference between the study of DiBona et al. and ours may be attributable to differences in the duration of treatment with the Ang II receptor antagonist.

In summary, baroreflex control of HR was more severely impaired than that of RSNA in MI-WKY as well, and the control of RSNA was favorably restored by the Ang II receptor antagonist in MI-SHR, we speculate that the sympathetic nerves and vagal efferent nerves to the heart as well as the afferent limb of the baroreflex arch were impaired. Alternatively, an unknown factor other than endogenous Ang II may have played an important role in affecting the cardiac vagal efferent nerves.

<table>
<thead>
<tr>
<th>TABLE 2. Four Parameters of Logistic Function Curve in Myocardial Infarction and Sham Operated Wistar-Kyoto Rats</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>RSNA</strong></td>
</tr>
<tr>
<td>Sham-veh</td>
</tr>
<tr>
<td>Sham-TCV</td>
</tr>
<tr>
<td>MI-veh</td>
</tr>
<tr>
<td>MI-TCV</td>
</tr>
<tr>
<td><strong>HR</strong></td>
</tr>
<tr>
<td>Sham-veh</td>
</tr>
<tr>
<td>Sham-TCV</td>
</tr>
<tr>
<td>MI-veh</td>
</tr>
<tr>
<td>MI-TCV</td>
</tr>
</tbody>
</table>

**RSNA** indicates range of RSNA or HR, **P2**, circle coefficient (independent of range), **P3**, MAP corresponding to half of RSNA or HR range, **P4**, lower plateau of RSNA or HR, and definitions as in Table 1.

**Acknowledgment**

This research was supported by research grant No. 06770871 from the Ministry of Education, Science, and Culture in Japan.

**References**


Shibouta Y, Isada Y, Ojima M, Wada T, Noda M, Sanada T, Kubo K, Kohara Y, Naka T, Nishikawa K. Pharmacological profile of highly potent and long-acting angiotensin II receptor antagonist, 2-ethoxy-1-[[2'-(1H-tetrazol-5-yl)biphenyl-4-yl)methyl]-1H-benzimidazole-7-carboxylic acid (CV-11974), and its prodrug, (t)-1-(cyclohexyloxycarbonyloxy)-ethyl 2-ethoxy-1-[[2'-(1H-tetrazol-5-yl)biphenyl-4-yl)methyl]-1H-benzimidazole-7-carboxylate (TCV-116). *J Pharmacol Exp Ther* 1993;266:114-120


Kjellgren O, Gomes JA. Heart rate variability and sensitivity in myocardial infarction. *Am Heart J* 1993;125:204-215


Improvement in Baroreflex Function by an Oral Angiotensin Receptor Antagonist in Rats With Myocardial Infarction

Masahiko Nishizawa, Hiroo Kumagai, Masashi Ichikawa, Naoki Oshima, Hiromichi Suzuki and Takao Saruta

Hypertension. 1997;29:458-463
doi: 10.1161/01.HYP.29.1.458

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1997 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/29/1/458

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Hypertension can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Hypertension is online at:
http://hyper.ahajournals.org//subscriptions/