Cardiac Angiotensin Converting Enzyme and Endothelin Receptor in Rats With Chronic Myocardial Infarction

Masahiro Kohzuki, M.D., Masayuki Kanazawa, M.D.* Kazunori Yoshida, M.D.
Masahiro Kamimoto, M.D., Xue-Min Wu, M.D., Zhong-Li Jiang M.D.
Minoru Yasujima, M.D.* Keishi Abe, M.D.* Colin I Johnston***
and Tokutaro Sato, M.D.

To ascertain the pathophysiological roles of the renin-angiotensin system and endothelin in heart failure and cardiac hypertrophy, we assessed changes in cardiac angiotensin converting enzyme (ACE) and endothelin-1 (ET-1) receptor using rats in which myocardial infarction was induced by left coronary ligation. The animals were decapitated 1 or 8 months after the operation. Cardiac ACE and ET-1 receptor were quantified by computerized in vitro autoradiography using 125I-MK351A (a lisinopril derivative) and 125I-ET-1. One month after myocardial infarction, cardiac weight and plasma atrial natriuretic peptide had increased in rats with infarction, compared to sham-operated controls, indicating the presence of chronic left ventricular dysfunction, although exchangeable body sodium and plasma renin activity were unchanged. Cardiac ACE increased markedly in the infarcted area and moderately in hypertrophied myocardium without any change in affinity compared to sham-operated rats. On the other hand, there was no change in cardiac ET-1 receptors in infarcted rats. The same results were found even at 8 months after myocardial infarction. The present study indicates that cardiac ACE may participate in tissue repair at the site of myocardial infarction and may also play a role in the pathophysiology of cardiac hypertrophy in rats with chronic heart failure. However, the present results do not reveal whether ET-1 receptor participates in the pathophysiology of cardiac hypertrophy in this model.

(Jpn Circ J 1996; 60: 972–980)

There is increasing evidence that the renin angiotensin system may act as both a circulating hormonal system and as a local autocrine or paracrine system! Most of the components of the renin-angiotensin system have been isolated in cardiac tissue using a variety of biochemical, immunohistochemical and molecular biological techniques. Angiotensin converting enzyme (ACE) has also been demonstrated in the heart and is functionally active in converting angiotensin I to angiotensin II. Angiotensin II is involved in cardiac hypertrophy in vitro as well as in vivo.

Endothelin is a potent vasoconstrictor hormone which may also act as both a circulating hormone and as a local autocrine or

Key words:
Endothelin receptor
Angiotensin converting enzyme
Heart failure
Cardiac hypertrophy

(Received October 20, 1995; accepted May 14, 1996)
Section of Internal Medicine and Disability Prevention, Disability Science, Division of Medicine, Tohoku University Graduate School of Medicine, Sendai, Japan, *Second Department of Internal Medicine, Tohoku University School of Medicine, Sendai, Japan, **Department of Laboratory Medicine, Hiroaki University School of Medicine, Hiroaki, Japan and ***Department of Medicine, Austin Hospital, Melbourne University, Victoria, Australia
Mailing address: Masahiro Kohzuki M.D., Ph.D. Section of Internal Medicine and Disability Prevention, Disability Science, Division of Medicine, Tohoku University Graduate School of Medicine, Seiryo-cho, Aoba-ku, Sendai 980-77, Japan
paracrine hormone? Endothelin (ET) receptors have been demonstrated in rat and human cardiac tissue. Endothelin-1 (ET-1) has also been found to induce hypertrophy of cardiomycocytes in vitro, in association with the induction of muscle-specific genes and a protooncogene. Therefore, it has been suggested that ET-1 may also play an important role in the pathogenesis of cardiac hypertrophy associated with various cardiovascular disease.

However, the pathophysiological roles of renin-angiotensin system and endothelin in heart failure and cardiac hypertrophy in infarcted heart have not been fully elucidated. In this study, we assessed the changes in cardiac ACE and ET-1 receptor using in vitro autoradiography in rats with myocardial infarction that was induced by left coronary ligation.

MATERIALS AND METHODS

Induction of Myocardial Infarction

Myocardial infarction was induced in female Wistar rats by left coronary artery ligation, using the method of Pfeffer et al. The rats were anesthetized with ether, intubated and ventilated using a rodent respirator. A left thoracotomy was performed from a left intercostal approach, the heart was briefly exteriorized, and the coronary artery was ligated using a 6-0 Prolene suture. The heart was then returned to the thoracic cavity, and the thoracotomy incision was closed with a purse-string suture. The mortality rate within 24 h of this procedure is approximately 50%. Since the left coronary artery cannot be directly visualized, the procedure does not produce myocardial infarction in all of the rats. Those with a silk ligature but without evidence of infarction and those with thoracotomy and heart exteriorization but without a silk ligature served as controls (sham-operated). Surviving rats in this model develop left ventricular free-wall transmural myocardial infarction, which is replaced by a discrete fibrous scar after 1 month. The infarct size was measured histologically by planimetry, using serial sections of the left ventricle taken at 1 mm intervals from base to apex, and expressed as the ratio of infarcted to total left ventricular circumference. In this mod-

el, septal infarction is never seen in surviving rats (either macroscopically or microscopically). Of the survivors, approximately 50% have macroscopic and microscopic evidence of left ventricular free-wall infarction, ranging from 5–60% of the total left ventricular circumference.

Rats with infarction develop hemodynamic abnormalities in proportion to the infarct size! Rats with small infarcts exhibit systolic dysfunction with reduced contractility and peak pressures. Rats with larger infarcts also show diastolic dysfunction with increased filling pressures!

Protocol I: Cardiomegaly and Chronic Myocardial Infarction

Six-week-old female Wistar rats were equilibrated with 22Na for 3 weeks before the induction of myocardial infarction. In the 58 surviving rats, total exchangeable body sodium (NaE) was measured on the day after the operation and at weekly intervals thereafter. Radiolabelled saline was administered on the day after the operation. Four weeks after the operation, the rats were decapitated and their blood was collected to measure plasma atrial natriuretic peptide (ANP).

Measurement of Organ Weights: After decapitation, the hearts and lungs were removed and weighed on an automatic-zero Mettler balance. The atria and right and left ventricles were then individually dissected and weighed. The left ventricles (including the intraventricular septa) were fixed in 10% buffered formalin for histological study. All organ weights were corrected for body weight.

Measurement of Total Exchangeable Body Sodium: Total exchangeable body sodium (NaE) was measured sequentially by isotope dilution using a modification of the method of Dusting et al. This method is simple, accurate, and reproducible, and the results correlate closely with measurements of total body sodium made by ashing or acid digestion. Briefly, the rats were fed a sodium-free solid diet ( < 50 mg/kg dry weight) and allowed to equilibrate for 3 weeks before the operation with a 22Na-labelled saline drinking solution (75 mmol/L). The total exchangeable body sodium of each rat was measured once
a week after the operation throughout the experimental period. The radioisotope equilibrates with endogenous sodium over this period in all sodium-containing body fluids and tissues except bone. The radioactivity of each rat was measured using a Packard Armac large-sample gamma-counter. The result was compared with a counting standard consisting of an accurately weighed 200 ml aliquot of radiolabelled drinking solution. The smallest detectable change in NaE using this method is 0.5 mmol/kg body weight, as calculated from repeated measurements in the same animal over one day. In our laboratory, NaE measured in mature female Wistar rats was 44.3±0.4 mmol/kg body weight (n=13). In 10-week-old male Wistar rats (n=21) there was a close correlation between NaE measured by isotopic dilution and total body sodium measured by subsequent acid digestion (r=0.95, p<0.001). Since most sodium is extracellular, NaE is proportional to extracellular fluid volume and to plasma volume in most pathological conditions.

Measurement of Plasma ANP: To confirm that rats with myocardial infarction had chronic heart failure, plasma ANP was measured at the time of decapitation by radioimmunoassay as described previously. Briefly, ANP was extracted from plasma using Vycor glass, eluted in an acetone:water mixture and evaporated to dryness under an air stream. The extract was reconstituted in buffer and radioimmunoassay was performed using a rabbit antibody and goat anti-rabbit antibody separation. The intra- and inter-assay variances were 5.3% and 16.3%, respectively. The assay sensitivity was 2 pmol/L in rat plasma.

Protocol 2: ET Receptor and ACE Measurement

Myocardial infarction was induced in 9-week-old female Wistar rats by left coronary artery ligation. Four weeks (1 month) or 8 months after the operation, the rats (n=6 for each group) were decapitated and blood was collected for measurement of plasma ANP. Their solid diet consisted of standard rat food throughout the experimental period. The hearts from rats with infarcts or sham-operated animals were measured gravimetrically, rapidly removed, and snap-frozen in isopentane at −40 °C. Frozen sections (20 μm) were cut in a cryostat at −20 °C. The sections were thaw-mounted onto gelatin-coated slides, dried in a desiccator for 2 h at 4 °C and then stored at −80 °C.

Quantitative Autoradiography

125I-MK351A binding: The sections were preincubated in 10 mmol/L sodium phosphate buffer, pH 7.4, containing 150 mmol/L NaCl and 0.2% bovine serum albumin (BSA) for 15 min at 20 °C. The sections were then incubated with 11.1 KBq/ml 125I-MK351A in the same buffer for 60 min at 20 °C. Nonspecific binding was determined in the presence of 10−6 mol/L MK351A or lisinopril. Binding isotherms were determined using a set of serial sections incubated with 10−12 to 10−6 mol/L lisinopril for 60 min.

125I-ET-1 binding: The sections were preincubated for 15 min at 20 °C in 20 mmol/L Hepes buffer, pH 7.4, containing 135 mmol/L NaCl, 2 mmol/L CaCl2, 0.2% BSA, and 0.01% bacitracin. The sections were then incubated with 11.1 KBq/ml 125I-ET-1 in the same buffer for 60 min at 20 °C. Nonspecific binding was determined in the presence of 10−6 mol/L ET-1. Binding isotherms were determined using a set of serial sections incubated with 10−12 to 10−6 mol/L unlabelled ET-1 for 60 min.

After incubation, the sections were rapidly dried under a stream of cold air, placed in X-ray cassettes, and exposed to Agfa Scopix CR3 X-ray film (Agfa Gevaert, Australia) for 12–72 h at room temperature. After exposure, the sections were fixed in formaldehyde and stained with hematoxylin and eosin. The optical density of the X-ray films was quantified using an imaging device (Imaging Research Inc, Ontario, Canada) controlled by an IBM AT personal computer. The optical density of the autoradiographs was calibrated in terms of the radioactivity density in dpm/mm2 with reference to standards maintained through the procedure. Binding data were analyzed as described previously. The apparent binding site concentration (Bmax) and binding affinity constant (Kd) in all of the areas (excluding coronary arteries) of the right ventricle, intraventricular septum, the infarcted area in the left ventricle, and
the non-infarcted area in the left ventricle were estimated by an iterative non-linear model-fitting computer program LIGAND. Radioligand: The radioligand used to label ACE was \(^{125}\)I-MK351A. MK351A is a tyrosyl derivative of lisinopril, a potent competitive inhibitor of ACE. MK351A was iodinated by the chloramine T method and separated from free \(^{125}\)I by SP Sephadex C25 (Pharmacia LKB, Uppsala, Sweden) column chromatography. The binding properties of this ligand have been published previously. The specific activity of iodinated MK351A was 59.2 MBq/\(\mu\)g. ET-1 (Protein Research Foundation, Osaka, Japan) was iodinated with \(^{125}\)I-Iodine using Iodogen (Pierce Chemical Co, IL, USA) as described elsewhere. The specific activity of \(^{125}\)I-ET-1 was 23.3 MBq/\(\mu\)g.

**Statistical Methods**

Differences between control (sham) rats and rats with infarction were analysed by a one-way analysis of variance. Pairwise comparisons between groups were made using the Newman-Keuls test, provided a significant F value was obtained. All values are expressed as the mean±standard error of the mean, with \(p<0.05\) considered significant, using Statview 4.0 software (ABACUS Concepts Inc, USA).

This investigation conformed to the principles outlined in the Declaration of Helsinki.

**RESULTS**

**Protocol 1: Cardiomegaly and Chronic Myocardial Infarction**

Healed myocardial infarcts were present in 12 of 23 surviving rats. Mean infarct size was 32.3±3.3% in rats with infarction (Table I). Plasma ANP was significantly increased in rats with infarction (\(p<0.05\)). Exchangeable body sodium was unchanged in rats with infarction compared to the controls. Total cardiac weight and left and right ventricular weight were significantly increased in rats with infarction compared to sham-operated controls (\(p<0.01\), \(p<0.05\), \(p<0.01\) respectively) (Table I). Lung weight was also increased in rats with infarction (\(p<0.05\)). These results confirm that the rats with myocardial infarction in the present study had chronic heart failure.

**TABLE I**

<table>
<thead>
<tr>
<th></th>
<th>SHAM</th>
<th>INFARCT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>12</td>
<td>11</td>
</tr>
<tr>
<td>Infarct size (%)</td>
<td>–</td>
<td>32.3±3.3</td>
</tr>
<tr>
<td>NaE (mmol/kg)</td>
<td>44.8±0.7</td>
<td>45.5±0.8</td>
</tr>
<tr>
<td>ANP (pmol/L)</td>
<td>79±10</td>
<td>171±40†</td>
</tr>
<tr>
<td>Organ Weight</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cardiac Weight</td>
<td>3.48±0.08</td>
<td>4.11±0.17 lem</td>
</tr>
<tr>
<td>L. Ventricle</td>
<td>2.55±0.35</td>
<td>2.76±0.07 lem</td>
</tr>
<tr>
<td>L. Atrium</td>
<td>0.69±0.02</td>
<td>0.95±0.07 lem</td>
</tr>
<tr>
<td>R. Atrium</td>
<td>0.11±0.01</td>
<td>0.17±0.03</td>
</tr>
<tr>
<td>Lung (mg/g)</td>
<td>5.84±0.30</td>
<td>7.51±0.63 lem</td>
</tr>
</tbody>
</table>

Infarct size, exchangeable body sodium (NaE), plasma atrial natriuretic peptide (ANP) and organ weights (corrected for body weight) in rats with infarction and sham-operated rats.

\(^{*}=p<0.05\) compared with sham-operated controls, \(^{†}=p<0.01\) compared with sham-operated controls.

**TABLE II**

<table>
<thead>
<tr>
<th>Time after the operation</th>
<th>SHAM</th>
<th>INFARCT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 M</td>
<td>8 M</td>
</tr>
<tr>
<td>Number</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Infarct size (%)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Left ventricle (infarcted area)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Left ventricle (non-infarcted area)</td>
<td>1.7±0.3</td>
<td>1.5±0.6</td>
</tr>
<tr>
<td>Intraventricular septum</td>
<td>1.9±0.4</td>
<td>1.7±0.3</td>
</tr>
<tr>
<td>Right ventricle</td>
<td>2.2±0.9</td>
<td>1.3±0.7</td>
</tr>
<tr>
<td>Plasma ANP (pmol/L)</td>
<td>71±8</td>
<td>189±27†</td>
</tr>
</tbody>
</table>

The \(^{125}\)I-MK351A binding affinity constant (\(K_A; \times 10^9\)M) in the left ventricle, intraventricular septum and right ventricle, and plasma atrial natriuretic peptide (ANP) the sham-operated rats and in rats with chronic heart failure 1 month or 8 months after the induction of myocardial infarction.

\(^{*}=p<0.05\) compared with sham-operated controls (same duration after the operation).

*Japanese Circulation Journal Vol.60, December 1996*
Fig 1. Computer-generated pseudo-color images of autoradiographs of $^{125}$I-labeled MK351A-binding to rat heart. Red, highest receptor density; blue, low or undetectable receptor density. Top panel: sections from rats with myocardial infarction (INFARCT: 1 month or 8 months). Bottom panel: sections from rats without myocardial infarction (SHAM: 1 month or 8 months).

### TABLE III

<table>
<thead>
<tr>
<th>Time after the operation</th>
<th>SHAM</th>
<th>INFARCT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 M</td>
<td>8 M</td>
</tr>
<tr>
<td>Number</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Infarct size (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Left ventricle (infarcted area)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Left ventricle (non-infarcted area)</td>
<td>26±5</td>
<td>34±11</td>
</tr>
<tr>
<td>Intraventricular septum</td>
<td>23±5</td>
<td>30±11</td>
</tr>
<tr>
<td>Right ventricle</td>
<td>34±4</td>
<td>43±8</td>
</tr>
</tbody>
</table>

The $^{125}$I-MK351A binding site concentration ($B_{max}$; f mol/mm$^2$) in the left ventricle, intraventricular septum and right ventricle in sham-operated rats and in rats with chronic heart failure 1 month or 8 months after induction of myocardial infarction.

The $^{125}$I-MK351A binding site concentration in the left ventricle (infarcted area) was compared to that in the non-infarcted area in sham-operated rats.

$a = p < 0.05$ compared with sham-operated controls.

$b = p < 0.01$ compared with sham-operated controls.

$^c = p < 0.001$ compared with sham-operated controls.

---

**Protocol 2: ET Receptor and ACE Measurement**

Healed myocardial infarcts were present in 6 of 12 surviving rats decapitated 1 month after the operation and in 6 of 12 surviving rats decapitated 8 months after the operation. Mean infarct size was 31.5±6.2% in the former and 38.9±5.4% in the latter (Table II). Plasma ANP was significantly increased, indicating the presence of chronic heart failure (1 M: 71±8 vs 189±27 pg/ml in SHAM and CHF (p<0.05), 8 M: 77±10 vs 258±27 pg/ml in SHAM and CHF (p<0.001).
ET-1 RECEPTOR IN RAT HEART

Fig 2. Computer-generated pseudo-color images of autoradiographs of $^{125}$I-labeled ET-1 binding to rat heart. Red, highest receptor density; blue, low or undetectable receptor density. Top panel: sections from rats with myocardial infarction (INFARCT: 1 month or 8 months). Bottom panel: sections from rats without myocardial infarction (SHAM: 1 month or 8 months).

<table>
<thead>
<tr>
<th>Time after the operation</th>
<th>SHAM</th>
<th>INFARCT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 M</td>
<td>8 M</td>
</tr>
<tr>
<td>Number</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Infarct size (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Left ventricle (infarcted area)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Left ventricle (non-infarcted area)</td>
<td>4.2±1.6</td>
<td>3.2±0.9</td>
</tr>
<tr>
<td>Intraventricular septum</td>
<td>4.9±2.0</td>
<td>3.5±1.1</td>
</tr>
<tr>
<td>Right ventricle</td>
<td>6.2±2.2</td>
<td>5.8±2.1</td>
</tr>
</tbody>
</table>

The $^{125}$I-endothelin-1 binding affinity constant ($K_a\times10^{9}$M) in the left ventricle, intraventricular septum and right ventricle in sham-operated rats and in rats with chronic heart failure 1 month or 8 months after the induction of myocardial infarction. The $^{125}$I-endothelin-1 binding affinity constant in the left ventricle (infarcted area) was compared to that in the non-infarcted area in sham-operated rats.

pg/ml in SHAM and CHF (p<0.01).

Ventricular myocardium showed a low, but detectable, density of ACE in the sham-operated rats (Fig 1). The binding parameters of $^{125}$I-MK351A in different cardiac chambers in CHF and SHAM are shown in Tables II and III. ACE concentration, determined by the number of binding sites for $^{125}$I-MK351A, was significantly increased in all of the chambers of the heart in rats with infarction. This concentration was particularly high in the scar tissue of the infarcted area 1 month after myocardial infarction, without any changes in the affinity compared to the sham-operated rats (Fig 1).

Ventricular myocardium showed a moderate density of ET receptors in sham-operated rats (Fig 1). In contrast to cardiac ACE, there was no change in cardiac ET receptors...
in infarcted rats (Fig 2, Tables IV and V). The binding parameters of $^{125}$I-ET-1 in different cardiac chambers in CHF- and SHAM-rats are shown in Tables IV and V.

The same results were found at 8 months after myocardial infarction (Figs 1 & 2, Tables II–V).

**DISCUSSION**

Coronary artery ligation in the rat is a reliable model of ventricular dysfunction and closely mimics left ventricular failure due to myocardial infarction in man. Rats with infarction show reduced cardiac output with a rise in peripheral resistance. Plasma renin activity and the plasma angiotensin II concentration, as well as the renal renin mRNA level, renal angiotensinogen mRNA level and renal angiotensin concentration have been reported to be increased in rats with myocardial infarction. Angiotensin mRNA levels have been reported to be significantly elevated in the non-infarcted portion of left ventricles in this model. In this study, we assessed the changes in cardiac ACE and ET-1 receptor in this model using in vitro autoradiography.

One month after myocardial infarction, ACE was markedly increased in the infarct area and in hypertrophied myocardium without any change in affinity compared to sham-operated rats. On the other hand, there was no change in cardiac ET receptors in infarcted rats. The plasma ANP level was significantly high, indicating the presence of chronic heart failure. The same results were found 8 months after myocardial infarction. Thus, the present study indicates that cardiac ACE may participate in tissue repair at the site of myocardial infarction and might contribute at least in part to the pathophysiology of cardiac hypertrophy in rats with chronic heart failure.

Whether or not there is an increase in ET in plasma in animals with myocardial infarction and patients with myocardial infarction is controversial. If there is such an increase, the extent of the increase is too small to draw definite conclusions. Therefore, additional experimental studies are needed to provide clearer evidence. In our present study, there was no change in cardiac ET receptors in infarcted rats. Whether or not ET receptors participate in the pathophysiology of cardiac hypertrophy in this model requires further investigation.

The progressive development of cardiac hypertrophy in heart failure may be a consequence of increased afterload and neurohumoral activation. We previously reported an attenuated response to ANP in rats with heart failure and that successful treatment of heart failure with a reduction in atrial distention tends to normalize the ANP level. In the present study plasma ANP was significantly elevated in rats 8 months after myocardial infarction, indicating the presence of chronic heart failure as much as 8 months after myocardial infarction. In previous studies, we confirmed that the ACE inhibitor enalapril caused a marked reduction in cardiac hypertrophy and lung congestion, while preload reduction using salt
restriction or chlorothiazide had no effect on cardiac or lung weight\textsuperscript{31,32}. These findings suggest that preload reduction has less beneficial hemodynamic effects than afterload reduction in this model of heart failure.

In the present study, a large amount of ACE was found in the infarct area in the thin scar tissue of the left ventricle. In addition to myocardium, ACE is found in macrophages, endothelial cells and fibroblast-like cells (type I collagen-producing cells)\textsuperscript{33}. It has not yet been determined whether one of its functions is to degrade inflammatory mediators, since ACE is identical to kininase II, which degrades kinin, or to form fibrous tissue.

Cardiac hypertrophy is a major risk factor for heart failure and the degree of cardiac hypertrophy correlates well with mortality in patients with heart failure\textsuperscript{34,35}. The present study indicates that cardiac ACE may participate in tissue repair at the site of myocardial infarction and might contribute at least in part to the pathophysiology of cardiac hypertrophy in chronic heart failure. This study raises the possibility that the beneficial cardiac effects of ACE inhibitors, including the reduction of left ventricular hypertrophy, may be associated not only with a reduction in circulating angiotensin II but also with the inhibition of local cardiac ACE.

Acknowledgements

This work was supported by grants from the Scientific Research Fund of the Ministry of Education, Japan, and by a grant from Jyunkanki-gaku Kenkyu Shinkou Zaidan, Japan. Part of this work was presented at the 3rd International Conference on Endothelin, Houston, Texas, February, 1993.

REFERENCES


