Induction of Cardiac Angiotensinogen mRNA and Angiotensin Converting Enzyme (ACE) Activity in Isoproterenol-Induced Heart Injury

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Effects of isoproterenol (ISO) on the expression of cardiac angiotensinogen mRNA, angiotensin converting enzyme (ACE) activity, and mechanical functions in spontaneously hypertensive rats were investigated. In the acute phase, defined as within 24 h after the subcutaneous injection of ISO 85 mg/kg, cardiac angiotensinogen mRNA was slightly induced, but ACE activity was not. In the subacute phase, defined as within 8 d after ISO treatment on 2 successive d, both angiotensinogen mRNA expression and ACE activity in the heart were markedly induced. ACE activity in serum was not affected by ISO in either phase. In the subacute phase, ISO reduced body weight and blood pressure, increased ventricular weight and calcium content, and impaired cardiac mechanical function. Oral treatment with imidapril (10 mg/kg/d), an ACE inhibitor, 1 h before each ISO treatment and on the following 6 d, improved ventricular hypertrophy, the elevation of the left ventricular end diastolic pressure, the reduction in contractility, and the prolongation of the time constant. Imidapril significantly suppressed both serum and cardiac ACE activity but did not affect cardiac angiotensinogen mRNA expression in the subacute phase. These results indicate that enhancement of cardiac angiotensinogen mRNA and ACE activity is involved in ISO-induced cardiac dysfunction. Imidapril improved ISO-induced cardiac dysfunction, possibly by suppression of the local ACE activity as well as circulating ACE activity. (Hypertens Res 1996; 19: 179-187)

Key Words: imidapril, SHR, isoproterenol, angiotensinogen, ACE

In patients with chronic heart failure, several compensatory mechanisms are activated to maintain circulatory homeostasis. These include the increased release of catecholamines and the activation of the renin-angiotensin system (RAS) (1, 2). It has been recognized that the over-activation of such compensatory mechanisms increases cardiac work load and deteriorates cardiac function. To counteract such deterioration, peripheral vasodilator drugs have been used in the treatment of patients with chronic cardiac failure (3). The efficacy of angiotensin converting enzyme (ACE) inhibitors was first shown by Gavras et al. (4), and subsequently symptomatic and hemodynamic improvement by ACE inhibitors has been demonstrated in patients with congestive heart failure (5-8). Recently, the expression of cardiac ACE has been shown to be upregulated in the failing human heart, indicating that the cardiac RAS is activated in patients with advanced heart failure (9). This finding suggests that the effects of ACE inhibitors include not only vasodilating effects but also inhibition of cardiac RAS.

There are two typical experimental animal models for the study of myocardial ischemia. One is the myocardial infarction model, induced by coronary occlusion, in which the importance of the RAS has been demonstrated (10, 11). The other model is catecholamine-induced cardiac injury, which is often used as a cardiomyopathy model based on activation of the sympathetic nervous system (SNS). Activation of the SNS accelerates inotropy, increases cardiac oxygen consumption, and causes myocardial ischemia. A large dose of isoproterenol (ISO), one of the catecholamines, is well known to exert inotropic effects and cause cardiac injury in experimental animals. Lesions that occur in this model are histochemically comparable to those of human stress-induced cardiomyopathy and myofibrillar degeneration (12-14). Cardiac hemodynamics (15, 16), histochemical changes (13, 17), and calcium overload (18, 19) associated with ISO have also been demonstrated.

Recently, ISO-induced cardiac hypertrophy has been shown to be associated with an increase in cardiac angiotensin II formation. An ACE inhibitor, trandolapril, reduced ventricular weight and the level of angiotensin II (20). These results suggested that the ISO-induced cardiomyopathy is related to augmentation of the cardiac RAS. In order to understand the mechanisms of ISO-induced car-
diomyopathy, it is important to elucidate the relationship between RAS and ISO-induced cardiac disorders.

To this aim, we have used spontaneously hypertensive rats (SHRs), which are vulnerable to ischemia induced by ISO treatment (21-23). Hypertrophied SHR hearts have fewer energy reserves than normotensive rat hearts; therefore, they display greater ST-segment elevation, cardiac insufficiency, and mortality in response to ISO than Wistar Kyoto Rats (21). After volume loading, ISO-treated normotensive rats showed a reduced total peripheral resistance, which may assist in myocardial repair. However, these vascular responses were lacking in SHRs (22). Thus, a large dose of ISO may readily cause deterioration of cardiac function in SHRs.

In this report, we describe the effects of ISO on SHRs in terms of cardiac function, cardiac angiotensinogen mRNA expression, and ACE activity. We also show the effects of imidapril, an ACE inhibitor recently launched in Japan as an anti-hypertensive agent, on this model.

**Materials and Methods**

**Animals and Drug Treatment**

Male SHRs (Charles River Japan, Inc., Atsugi, Japan) 25-32 weeks of age were used. They were given normal rat chow (CRF-1; Oriental Yeast, Tokyo, Japan) and tap water ad libitum throughout the experiment. The rats were allowed approximately 1 wk for acclimatization. At the start of each study, the rats were divided into groups according to their systolic blood pressure (SBP) and heart rate (HR), which were measured by the tail-cuff method (blood pressure monitor MK-1000; Muromachi Kikai Co., Ltd., Tokyo, Japan).

Imidapril at 10 mg/kg/d or vehicle (4 ml/kg/d of distilled water) was orally administered to the animals once a day for 8 d. One hour after the 1st and 2nd doses, the SHRs received a subcutaneous (s.c.) injection of ISO, dissolved in saline, at 85 mg/kg/d. Rats given s.c. injections of 5 ml/kg of saline served as ISO-untreated controls.

**Quantitative Analysis of Angiotensinogen Gene Expression**

The ISO-1-d group consisted of three rats given ISO at 85 mg/kg s.c. and sacrificed by exsanguination under ether anesthesia 24 h after a single ISO treatment. The ISO-8-d group consisted of five rats given ISO for 2 successive d and sacrificed 8 d after the second treatment. Three rats given saline by s.c. injection for 2 d, instead of ISO, served as the control group. The left ventricle of each heart was excised from the animals, blotted on filter paper, weighed, and immediately immersed in liquid nitrogen. Samples were stored at -80°C until use.

Angiotensinogen gene expression was quantified by the technique of RT-PCR (24-26). A digoxigenin-labeled angiotensinogen probe was prepared as follows. The 426 bp DNA fragment encoding the N-terminal region of rat angiotensinogen (27) was PCR-amplified from a rat liver cDNA library (Clontech Laboratories, Inc., Palo Alto, CA, USA) using angiotensinogen-primer 1 (5'-GCT AAG CTT ATG ACT CCC ACG GGG GCA GG-3') containing HindIII site at the 5'-end and angiotensinogen-primer 2 (5'-CTG GGG ATC CAA CGA TCC AAG GTA GAA AGA-3'). The PCR product was subcloned into HindIII BamHI sites of pBluescript II SK (+) (Stratagene Cloning System CA, USA). About 10 ng of the 426 bp DNA fragment was labeled with digoxigenin using a DIG DNA Labeling Kit (Boehringer Mannheim Biochemical, Mannheim, Germany) according to the instructions of the manufacturer. The 2.0 kbp PsI fragment of the β-actin gene was labeled with digoxigenin (28).

The frozen hearts were crushed with a hammer and immediately mixed with ISOGEN solution (Nippon Gene Co., Ltd., Toyama, Japan). The precipitated RNA, obtained according to the ISOGEN protocol, was dissolved in diethyl pyrocarbonate-treated distilled water (DEPC-water) and the amount of RNA was quantitated by measuring the absorbance at 260 nm. The RNA from each sample was diluted with DEPC-water to a concentration of 2 μg/8 μl and used to synthesize the first-strand cDNA, using a First-Strand cDNA Synthesis Kit (Pharmacia Biotech Inc., Uppsala, Sweden) according to the manufacturer’s instructions.

The reaction mixture, containing the two angiotensinogen-primers and the synthesized first-strand cDNA, was then subjected to 45 cycles of amplification in a DNA thermal cycler. After the first 15 cycles, β-actin-primer 1 (5'-TAC ATG GCC GGT TAC ATG ACT CC-3') and β-actin-primer 2 (5'-AAG AGA GGC ATC CTC ACC C-3') were added to the reaction mixture to amplify the 218 bp DNA fragment encoding the rat β-actin gene to serve as an internal control (29).

The PCR products were subjected to 2% agarose gel electrophoresis with TAE buffer (40 mM Tris hydroxymethyl aminomethane, 20 mM acetic acid, 1 mM EDTA) and transferred onto the nylon membrane Hybond™-N+ (Amersham International plc, Buckinghamshire, England). The DNA fragments bound on the membrane were hybridized with a mixture of DIG-labeled angiotensinogen and β-actin probes. DNA was detected by a color reaction using anti-digoxigenin alkaline phosphatase conjugate, provided by DIG Nucleic Acid Detection Kit (Boehringer Mannheim Biochemica, Mannheim, Germany), according to the manufacturer’s instructions. The optical density of the band was measured with a densitograph (AE-6900MFP, ATTO, Osaka, Japan), and angiotensinogen expression was normalized by β-actin expression.

Six SHRs were used for an additional study. Five were treated with imidapril for 8 d and ISO for the first 2 consecutive d and sacrificed 24 h after the last oral treatment. These served as the imidapril+ISO group. The remaining rat, which was treated with vehicle instead of imidapril, served as the ISO-8-d control. The left ventricle was isolated from these rats and stored at -80°C as described in the pre-
vious section. RT-PCR reaction was performed according to the protocol described above. Briefly, RNA was prepared from the six hearts and the three remaining frozen hearts from the ISO-8-d control group used in the previous experiment. First-strand cDNA was synthesized as described. DNA fragments of angiotensinogen and β-actin were amplified individually in a DNA thermal cycler. The PCR products were subjected to 5% polyacrylamide gel electrophoresis in TBE buffer (90 mM Tris hydroxymethyl aminomethane, 90 mM boric acid, 0.01 mM EDTA). The optical density of the bands was measured with a densitograph, and angiotensinogen expression was normalized by β-actin.

Measurement of Weight and Calcium Contents of the Heart, and Urinary Electrolyte Excretion

SHRs were divided into the following four groups: vehicle + saline (n = 8), imidapril + saline (n = 8), vehicle + ISO (n = 16), and imidapril + ISO (n = 10). The body weight was measured every day throughout the experiment. The SBP and HR were measured by the tail-cuff method, 24 h after the 7th oral dose to obtain the post-medication value. Urine was collected using a sanitary cage for 24 h after the final oral dose.

The heart was removed under ether anesthesia after urine collection. The left ventricle (left ventricular free wall and septum) and right ventricle (right ventricular free wall) were removed from the heart, blotted on filter paper, and weighed. The samples were then dried for 24 h at 100°C (column aging unit CE-1; Shimadzu, Kyoto, Japan), and the dry weight was measured. Dried left ventricle was dissolved in 1.5 ml of 60% HNO3 solution at 150-160°C, and this solution was evaporated at the same temperature. Then, 1.5 ml of 60% HClO4 solution was added to the residue, and the mixture was heated to dryness at 200°C. The resulting ash was dissolved in 5 ml of 10 mM LaCl aqueous solution (La2O3, 1.63 g and conc. HCl 6.7 ml/l). The calcium content of the sample was measured by an atomic absorption spectrophotometer (180-80; Hitachi, Tokyo, Japan).

Concentrations of urinary sodium, potassium, and chlorine were measured with a high speed electrolyte analyzer (PVA-αII; Analytical Instrument Co., Ltd., Tokyo, Japan).

Measurement of Cardiac Function

The following three groups were used in hemodynamic studies: vehicle + saline, vehicle + ISO, and imidapril + ISO. Twenty-four hours after the last dose, the animals underwent the following procedure. Under sodium pentobarbital (75 mg/kg i.p.) anesthesia, a micro-tip transducer (SPR-249; Miller Instruments Inc., Houston, USA) was introduced into the left ventricle through the right carotid artery to measure left ventricular pressure (LVP). The rising rate of LVP (LVdP/dt) was also measured using an amplifier and a differentiator (AP-601G and ED-601G; Nihon Kohden, Tokyo, Japan). A polyethylene catheter (PE-50; Becton Dickinson, New Jersey, USA), connected to a pressure transducer (TF-101T; Nihon Kohden, Tokyo, Japan), was placed in the femoral artery to monitor arterial pressure and HR (pressure amplifier AP-601G, blood pressure meter AP-611G, and heart rate meter AT-601G; Nihon Kohden, Tokyo, Japan). These hemodynamic variables were simultaneously recorded on a polygraph (RM-6000; Nihon Kohden, Tokyo, Japan) and on a serial printer (RP-80FTI; EPSON, Tokyo, Japan) at a sampling rate of 30 s.

Rate pressure product (RPP; HR X left ventricular systolic pressure) and coronary perfusion pressure index (CPPi; diastolic pressure of the femoral artery minus left ventricular end diastolic pressure) were calculated. + LVdP/dt/P50 (LVdP/dt at a developing pressure of 50 mmHg on a rising phase) and left ventricular end diastolic pressure (LVEDP) were determined from the chart of the polygraph. The time constant, τ, was calculated according to the method described by Weiss et al. (30). Briefly, from the time maximum negative LVdP/dt was attained, pressure was plotted and fitted to the function $P = e^{At+B}$ (ln $P = At + B$). The negative number A represents the slope of ln $P$ vs. time in s$^{-1}$. An index of relaxation, τ, characterizes this phase of pressure fall and is equal to $1/\tau$. B is ln $P$ at maximum negative LVdP/dt.

Measurement of Serum and Cardiac ACE Activity

The following groups were prepared: vehicle + saline, vehicle + ISO, and imidapril + ISO. Each ISO-treated group was subdivided into two groups; one was sacrificed 24 h after the 1st oral treatment and the other was sacrificed 24 h after the 8th oral treatment. SHRs in the vehicle + saline group were sacrificed 24 h after the 8th oral treatment.

A blood sample was collected from the abdominal aorta under ether anesthesia and centrifuged to obtain serum, which served as a crude enzyme preparation. The heart was removed from each animal. The atria and the right ventricular free wall were removed, and the remaining left ventricle was blotted on filter paper and weighed. The serum and left ventricle were stored at −20°C until use.

ACE activity was determined from histidine productivity by a bioassay system described by Itoh et al. (31). Briefly, after thawing, the ventricle was cut in pieces with scissors and homogenized with 9 volumes of 0.01 M Tris-HCl 0.2 M sucrose (pH 7.4) on ice. The ventricle was homogenized with a polytron homogenizer at setting 9 for 20 s on ice. The homogenate was centrifuged (700 × g, 20 min, 4°C), and the supernatant was used as a crude enzyme preparation. Ten μl of 0.2 M Tris-HCl/0.2 M NaCl (pH 7.4) and 10 μl of distilled water were added to 20 μl of the crude enzyme fraction and mixed. The reaction was started by adding 10 μl of the substrate solution to the mixture at 37°C. The reaction time was set at 5 h for the heart and 30 min for the serum. The reaction was terminated by adding 50 μl of 1 N HCl to the preparation on ice, and the solution was neutralized by adding 50 μl of 1 N NaOH. Blank solution was pre-
pared by adding distilled water instead of the substrate solution to the mixture. One thousand μl of distilled water and 1,250 μl of culture medium free from histidine were added to the reaction mixture, and the mixture was autoclaved at 115°C for 5 min. After the addition of 50 μl of Leuconostoc mesenteroides P60 suspension, the mixture was incubated at 37°C for 16-18 h. The optical density of the mixture at 660 nm was measured. Histidine production was determined by the optical density obtained from standard dilutions of 0-200 μM of histidine in culture medium. The activity of ACE was expressed as nmol of histidine production by 1 mg of the wet heart tissue for 1 h or nmol of histidine production by 1 ml of serum for 1 min.

**Drugs Used**

Imidapril hydrochloride was synthesized at the Research Laboratory of Applied Biochemistry, Tanabe Seiyaku Co., Ltd., Osaka, Japan. Heparin sodium salt and hippuryl-L-His-L-Leu were purchased from Mochida (Tokyo, Japan) and Peptide Institute, Inc. (Osaka, Japan), respectively. All other chemicals were obtained from Nakarai Tesque, Inc. (Kyoto, Japan) or Katayama Chemical (Osaka, Japan).

**Statistical Analysis**

Data are expressed as the mean and standard errors of the mean. Differences between ISO— and ISO+ control groups or ISO+ control and ISO+ imidapril groups were evaluated by F-test followed by Student's or Aspin-Welch’s t-test. Differences were considered statistically significant when p<0.05.

**Results**

**Effects of ISO on Angiotensinogen Gene Expression**

ISO-induced cardiac hypertrophy is associated with an increase in angiotensin II formation, indicating the activation of the cardiac RAS. To study kinetic aspects of RAS-activation, we measured the amount of angiotensinogen mRNA in two phases of ISO-treatment. One was 24 h after a single ISO treatment, at which acute changes can be observed. The other was 8 d after two ISO treatments, at which changes occurring after the establishment of myopathy can be observed. Two of 5 SHRs in the ISO-8-d group died on the 3rd day of the experiment, while all SHRs in control and the ISO-1-d groups survived. The amount of angiotensinogen mRNA was determined by the quantitative RT-PCR method from the hearts of the rats that survived the entire experimental period (Fig. 1). The amount of angiotensinogen mRNA increased in 1 rat 1 d after ISO-treatment. The expression of angiotensinogen mRNA was augmented in all rats in the ISO-8-d group, whose optical density normalized with β-actin was significantly higher than that in control (p < 0.01). These results indicate that ISO-treatment induces prolonged augmentation of the cardiac RAS.

**The Weight and Calcium Content of the Heart, and Urinary Electrolyte Excretion**

Four of the 16 rats in the vehicle + ISO group died on the 3rd day of the experiment, and one of the 10 rats in the imidapril + ISO group died on the 5th day, while all SHRs in the two ISO-untreated groups survived. To evaluate the hypothesis that ISO-induced RAS is involved in the establishment of cardiac insufficiency, we examined the effects of imidapril, an ACE inhibitor, in the ISO-8-d group. Body weight, SBP, and HR before the start of the experiment and 24 h after the 7th dose are shown in Table 1. In all ISO-untreated SHRs, body weight increased slightly during the experimental period. In all ISO-treated SHRs, body weight decreased to about 370 g in the first 4 days, and thereafter gradually increased but remained below that of ISO-untreated SHRs. ISO treatment significantly decreased SBP but did not affect HR. The rats in the imidapril + ISO group tended to show lower SBP and HR. Imidapril decreased blood pressure in ISO-untreated SHRs.
Because ISO treatment significantly decreased body weight, we examined the effects of imidapril on absolute heart weight (Table 2). In the ISO-uninjected control group, imidapril did not affect the ventricular water content or Ca content. Wet weight, water content, and Ca content of the ventricle were significantly increased by ISO. Imidapril did not affect the Ca content but suppressed ISO-induced hypertrophy and edema. In the ISO-uninjected control group, wet ventricular weight was slightly reduced and dry weight was significantly decreased by imidapril. Urinary sodium, potassium, and chloride excretion and urine volume were almost equal in all groups (data not shown).

### Cardiac Function

ISO impaired cardiac function is shown in Table 3. ISO slightly decreased mean arterial pressure (MAP), left ventricular systolic pressure (LVSP), RPP, maximum +LVdP/dt, and CPPI. ISO significantly decreased +LVdP/dt/P50 and increased LVEDP and τ, but did not affect HR. Imidapril further decreased MAP, LVSP, RPP, and +LVdP/dt with no significant differences. Imidapril improved ISO-induced elevation of LVEDP, decrease in +LVdP/dt/P50, and prolongation of τ.

### Serum and Cardiac ACE Activity

Twenty-four hours after a single treatment with ISO, serum and cardiac ACE activities were not significantly different from those of ISO-untreated control (Fig. 2). Eight days after the two ISO treatments, serum ACE activity was virtually equal to that of the ISO-untreated control level, although cardiac ACE activity was significantly increased. Both serum and cardiac ACE activities in the imidapril group were significantly suppressed, as compared with those in the ISO-treated and -untreated controls on both the 1st and 8th days.

### Effects of Imidapril on Cardiac Angiotensinogen Gene Expression

In the subacute phase, the impairment of cardiac function and the augmentation of cardiac angiotensinogen gene expression were observed in the ISO-treated control group. Effects of imidapril on cardiac angiotensinogen gene expression in the subacute phase were additionally studied because imidapril improved ISO-induced cardiac dysfunction. The amount of angiotensinogen mRNA in the imidapril + ISO group was almost equal to that of the ISO-8-d control (Fig. 3).

### Discussion

It has been established that the circulating RAS is

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**Table 1. Effects of Imidapril on Body Weight, Systolic Blood Pressure, and Heart Rate in Isoproterenol Treated SHRs**

<table>
<thead>
<tr>
<th>Drug</th>
<th>ISO treatment</th>
<th>Pre-medication</th>
<th>8th day</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td></td>
<td></td>
</tr>
<tr>
<td>−</td>
<td>8</td>
<td>401 ± 5</td>
<td>413 ± 4</td>
</tr>
<tr>
<td>Imidapril</td>
<td>−</td>
<td>407 ± 6</td>
<td>416 ± 5</td>
</tr>
<tr>
<td>−</td>
<td>+</td>
<td>395 ± 4</td>
<td>386 ± 4***</td>
</tr>
<tr>
<td>Imidapril</td>
<td>+</td>
<td>404 ± 5</td>
<td>391 ± 5</td>
</tr>
</tbody>
</table>

Values are given as the mean ± SEM. ISO, isoproterenol; SBP, systolic blood pressure; HR, heart rate; BW, body weight; bpm, beats per min. ***p < 0.001 vs. ISO-uninjected control by unpaired Student’s t-test, **p < 0.01 vs. vehicle-administered control groups between ISO-untreated groups by unpaired Student’s t-test.

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**Table 2. Effects of Imidapril on Heart Weight, Water Content, and Calcium Content in Isoproterenol Treated SHRs**

<table>
<thead>
<tr>
<th>Drug</th>
<th>ISO treatment</th>
<th>Wet weight</th>
<th>Dry weight</th>
<th>Water content (%)</th>
<th>Ca content (μg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>1325 ± 22</td>
<td>317.7 ± 4.1</td>
<td>76.0 ± 0.2</td>
<td>38.9 ± 6.5</td>
</tr>
<tr>
<td>Imidapril</td>
<td>−</td>
<td>1267 ± 32</td>
<td>299.8 ± 6.3*</td>
<td>76.3 ± 0.1</td>
<td>50.1 ± 2.4</td>
</tr>
<tr>
<td>−</td>
<td>+</td>
<td>1427 ± 41*</td>
<td>311.5 ± 10.1</td>
<td>78.2 ± 0.1***</td>
<td>82.8 ± 6.0***</td>
</tr>
<tr>
<td>Imidapril</td>
<td>+</td>
<td>1269 ± 22***</td>
<td>287.0 ± 5.1</td>
<td>77.4 ± 0.1***</td>
<td>83.3 ± 6.0</td>
</tr>
</tbody>
</table>

Water content of the heart was calculated by the following formula: [(wet weight of the heart) − (dry weight of the heart)]/(wet weight of the heart). Calcium (Ca) content was expressed by micrograms of Ca per 1 g of dry heart. Ca content of tissue sample was measured by an atomic absorption spectrometry. Values are expressed as the mean ± SEM. ISO, isoproterenol; *p < 0.05, ***p < 0.001 vs. ISO-uninjected control by unpaired Student’s or Aspin-Welch’s t-test; **p < 0.01, ###p < 0.001 vs. the vehicle-administered control groups between ISO-injected groups by unpaired Student’s or Aspin-Welch’s t-test. n = 8–12.
involved in controlling blood pressure. Recently, renin and ACE have been shown to be expressed in hypertension-related organs such as the heart and kidney (32). Enhanced intracardiac angiotensin II has also been reported to be involved in coronary contraction, impairment of diastolic relaxation, myocyte enlargement, and interstitial fibrosis (33, 34). It was suggested that the augmented cardiac RAS not only increases cardiac work but also causes an additional energy deficiency and mechanical insufficiency, even after the coronary circulation has been restored (16).

We have shown in this report that angiotensinogen mRNA expression is activated in 1 of 3 rats 1 d after ISO-treatment. Such an augmentation of angiotensinogen mRNA was much stronger in SHRs 9 d after ISO-treatment. Furthermore, the effect on cardiac ACE was increased in the sub-

Stretch stress and pressure overload have been shown to induce angiotensinogen and ACE mRNA expression and angiotensin II formation in both cultured cardiac myocytes and whole heart (33, 35). The rise in HR and contractility is known to be induced by the inotropic action of ISO as an early event (36). These results indicate that the induction of angiotensinogen mRNA in the acute phase (1 d after ISO-treatment) in our experiment was caused by stretch stress in the early phase of ISO-treatment in 1 rat. In contrast to this early event, ISO increased neither the HR nor contractility in the subacute phase (9 d after ISO-treatment). This indicates that marked increases in both angiotensinogen mRNA and ACE activity, observed in the subacute phase, are related to later events of cardiomyopathy rather than to those of the initial stretch stress.

Two large s.c. doses of ISO have been established to induce an impairment of the heart in rats (15, 17, 21, 37, 38). The mechanism of ISO-induced cardiac injury is well known. In the acute phase of treatment, ISO exerts an inotropic effect initially, and the heart falls into ischemia due to elevated oxygen consumption. Soon after ischemia, the myocardium is overloaded with calcium, which is followed by histological changes (15, 18, 19, 39). During the subacute phase, the work-load and cell growth increase in the viable myocardium to compensate for the malfunctioning necrotic areas (40, 41). In agreement with this scheme, increases in cardiac weight and calcium content and cardiac dysfunction were observed in our experiment. The elevation of heart weight by ISO was attributed to cardiac edema, since ISO increased ventricular wet weight but not dry weight. Hirsch et al. reported that deterioration of urinary parameters was observed 45 d after myocardial infarction (11). No such effect was observed in our experiment because of a shorter study period.

The cardiac RAS has been suggested to be augmented to compensate for the reduced cardiac pump function in the failing heart, which occurs as an adaptive reaction (42). It has been suggested that such compensatory reactions have harmful effects on the heart. In our experiment, a potent ACE inhibitor, imidapril, improved both cardiac pump function and hypertrophy. These results further support the notion that the cardiac RAS is involved in ISO-induced mechanical dysfunction of the heart. We observed that pretreatment with imidapril did not suppress ISO-induced cardiac calcium overload in the acute phase. The induction of angiotensinogen mRNA in the acute phase was much less than that in the subacute phase. Furthermore, ACE activity did not increase in the acute phase of the ISO-treatment. These results suggest that the involvement of the cardiac RAS in cardiac dysfunction is confined to the subacute phase.

Previous studies have reported that an increase in cardiac angiotensin II is accompanied by cardiac dysfunction in cardiomyopathic hamsters and that chronic treatment with an angiotensin II receptor antagonist improves the cardiac function (43). We confirmed that imidapril improved cardiac contractility and prolonged survival in cardiomyopathic hamsters (44). Besides ISO-induced ischemia models, it has been suggested that the development of diastolic impairment due to cardiomyopathy is also related to the augmentation of RAS.

Imidapril treatment suppressed the progression of ISO-induced cardiac functional insufficiency. However, it did not suppress the induction of cardiac angiotensinogen mRNA on the 9th day. The induction of angiotensinogen mRNA due to either ACE inhibition, as a feedback reaction (45), or ISO treatment may counteract the suppression of angiotensinogen mRNA induction.

Reduction of afterload of the heart via the systemic vasodilating effects of ACE inhibitors has been suggested to be an important factor in the improvement of heart failure (46). The serum ACE activity
was significantly suppressed by imidapril as early as 24 h after the final dose. Suppression of serum ACE activity would have caused a significant reduction in peripheral resistance, although the hypertensive effect of imidapril was only slight due to a reduction in blood pressure by ISO. Mitigation of the workload of the failing heart via a marked reduction in afterload by imidapril partly alleviated the cardiac damage induced by adaptive mechanisms. It has been reported that hydralazine did not affect left ventricular hypertrophy induced by ISO but decreased blood pressure to a similar extent as with trandolapril (20). This result suggests that a hypotensive effect is not always necessary to reduce ISO-induced heart damage.

Studies using a bradykinin B2 receptor antagonist have confirmed that ACE inhibitors improve cardiac function by inhibition of bradykinin degradation in stroke-prone SHR and in dogs with coronary ligation (47, 48). Imidapril has been reported to augment bradykinin-induced hypotension in conscious rats and bradykinin-induced vasodilation in various arteries and veins in dogs (49-51). In this study, the effect of bradykinin may have been involved in the improvement of cardiac function and the reduction of cardiac workload.

Prostaglandin synthesis inhibition by aspirin has been reported to counteract the systemic arterial vasodilation induced by enalapril in severe heart failure (52). The cardioprotective effect of ramiprilat in vivo was abolished by inhibiting nitric oxide synthesis in a myocardial ischemia model (53). These data suggest that increased prostaglandin and nitric oxide synthesis by ACE inhibition may also play a part in the cardioprotective effects of imidapril.

In conclusion, our results strongly suggest that enhancement of cardiac angiotensinogen and ACE activity is involved in ISO-induced cardiac mechanical dysfunction and hypertrophy. Imidapril improved the cardiac insufficiency by inhibition of both cardiac and circulating ACE activity.

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