Role of Cardiac Angiotensin II in Isoproterenol-Induced Left Ventricular Hypertrophy

Masahiro Nagano, Jitsuo Higaki, Fumiaki Nakamura, Koichi Higashimori, Noriko Nagano, Hiroshi Mikami, and Toshio Ogihara

Angiotensin II (Ang II) has been shown to induce proliferation of cardiac myocytes. To examine the role of Ang II in left ventricular (LV) hypertrophy, isoproterenol was infused subcutaneously into 9-week-old male Wistar rats at 4.2 mg/kg/day for 7 days. Infusion of isoproterenol increased LV weight and Ang II concentrations in plasma and in LV tissue. In anephric rats, LV weight and tissue Ang II were increased similarly, but plasma Ang II was not changed by isoproterenol. Concomitant oral administration of trandolapril and isoproterenol prevented increases in both LV Ang II and LV weight. Treatment with hydralazine decreased blood pressure in a similar way as trandolapril but did not affect either LV weight or LV Ang II. Plasma Ang II was not decreased by either trandolapril or hydralazine when administered in combination with isoproterenol. These results suggest that cardiac tissue Ang II regulates myocyte growth in isoproterenol-induced LV hypertrophy, and the reduction of Ang II partly explains the prevention of cardiac hypertrophy by the converting enzyme inhibitor. (Hypertension 1992;19:708-712)

KEY WORDS • angiotensin II • hypertrophy • angiotensin converting enzyme inhibitors • isoproterenol

The extrarenal renin-angiotensin system has been demonstrated in the heart, and its possible role in cardiac function is suggested. Angiotensin converting enzyme (ACE) inhibitors have been reported to exert their effects when administered by intracoronary infusion and in isolated cardiac tissue, suggesting the direct effect of ACE inhibitors on the heart. However, it is not clear whether ACE inhibitors produce their cardiac effects via suppression of the tissue renin-angiotensin system, because ACE inhibitors also block kininase II.

We previously assessed the role of the cardiac renin-angiotensin system by direct measurement of its active peptide, angiotensin II (Ang II), in cardiac tissue and reported its importance in left ventricular (LV) hypertrophy. In this study, to further evaluate the role of the cardiac renin-angiotensin system, Ang II was measured during the progression and prevention of LV hypertrophy induced by isoproterenol.

Methods

Materials and Drug Administration

Nine-week-old male Wistar rats, obtained from Charles River, Japan (Atsugi, Kanagawa), were used in this study. An osmotic minipump (Alzet, model 2001, Alza Corp., Palo Alto, Calif.) was implanted into the subcutaneous tissue of the back just below the lower left costal margin, under pentobarbital anesthesia (40 mg/kg i.p.). Vehicle (0.1% ascorbic acid in 0.9% sodium chloride, n=6) or isoproterenol (4.2 mg/kg/day, n=6) was then infused subcutaneously for 7 days.

To examine the role of renal renin on plasma and cardiac Ang II, bilateral nephrectomy was performed by dorsal approach under pentobarbital anesthesia (n=12). Another group of 12 rats underwent sham operation as a control. Those rats were then administered isoproterenol or vehicle infusion as described above. Forty hours after the operation, the rats were used for the experiment.

Another series of experiments was performed to examine the effect of antihypertensive drugs on isoproterenol-induced LV hypertrophy. The converting enzyme inhibitor trandolapril or the vasodilator hydralazine was administered in the drinking water. The concentrations were adjusted to achieve a daily intake of 3 mg/kg for trandolapril and 8 mg/kg for hydralazine. Three days after drug treatment was started, isoproterenol or vehicle was infused concomitantly for 7 days as described above. Systolic blood pressure and pulse rate were measured by a tail-cuff method.

Measurements of Plasma and Cardiac Angiotensin II

Seven days after isoproterenol infusion or 40 hours after nephrectomy, blood was collected by decapitation and placed in chilled tubes containing EDTA disodium (final concentration, 5 mM) and ortho-phenanthroline (1 mM).

Cardiac Ang II was measured by our previously reported method. Briefly, blood was washed from cardiac tissue by perfusion with heparinized saline (20 units/ml). The heart was quickly removed and divided
into the atria and right and left ventricles including the interventricular septum. The tissue was frozen on dry ice and stored at −70°C until use. On the day of extraction, tissue was thawed and its wet weight was measured and expressed as milligrams wet weight per gram body weight. Tissue was then homogenized by polytron in 0.1N hydrochloric acid and centrifuged at 20,000 g for 30 minutes at 4°C. The tissue supernatant and plasma were applied to an octyl minicolumn (Amprep C8, 500 mg, Amersham, Buckinghamshire, UK). After the column was washed with 0.1% trifluoroacetic acid (TFA), Ang II was eluted with 2 ml methanol/water/TFA (80:19.9:0.1 [vol/vol/vol]).

**Measurement of Angiotensin II**

The eluate was dried, the resultant residue was resuspended in 100 μl of 0.1% TFA, and chromatography was performed using a Vydac C18 reversed-phase column (0.46x25 cm, Separations Group, Hesperia, Calif.). The elution was carried out with an exponential gradient of methanol from 30% to 50% in 10 mM sodium acetate buffer, pH 5.6, over a period of 15 minutes at a flow rate of 1 ml/min. The resolution of angiotensins by high-performance liquid chromatography with authentic standards is illustrated in Figure 1. Immunoreactive Ang II was measured by radioimmunoassay. The sensitivity of this assay was 0.1 pg per tube. The cross-reactivities were 100% for angiotensin III and less than 0.1% for angiotensin I and rat atrial natriuretic factor-(99–126).

**Statistical Analysis**

Data are expressed as mean±SEM. The difference between the vehicle and isoproterenol treatment groups was analyzed by two-tailed Student's t test. The effects of isoproterenol and the three interventions (nephrectomy, trandolapril, or hydralazine) were assessed by two-factor analysis of variance. When the interaction was significant, Bonferroni’s t test was applied to examine the difference between groups. Values of p<0.05 were considered significant.

**Results**

Subcutaneous infusion of isoproterenol increased heart rate (488±15 versus 378±10 beats per minute by vehicle, p<0.01), whereas systolic blood pressure remained unchanged (120±7 versus 128±2 mm Hg). Increases in plasma Ang II (8.06±1.61 versus 2.84±0.45 pg/ml), LV weight (253±9 versus 218±4 mg/100 g body wt), and tissue Ang II concentration (16.2±2.0 versus 7.3±0.8 pg/g tissue) were observed in rats treated with isoproterenol compared with vehicle-infused rats (p<0.01).

Bilateral nephrectomy caused a marked decrease in plasma renin activity (0.05±0.02 versus 2.66±0.79 ng/
ml · hr for sham operation, p<0.01) and Ang II (2.2±0.2 versus 7.9±1.7 pg/ml, p<0.01). Infusion of isoproterenol in an anephric rat did not alter plasma renin (0.08±0.02) or Ang II (3.0±0.5). In contrast, LV Ang II and LV weight were not altered by nephrectomy but were increased by infusion of isoproterenol, similar to sham-operated rats (Figure 2).

The changes in body weight, blood pressure, heart rate, and plasma Ang II by concomitant administration of trandolapril or hydralazine are summarized in Table 1. Trandolapril and hydralazine caused similar changes in body weight, blood pressure, and plasma Ang II of vehicle- and isoproterenol-infused rats; however, trandolapril and hydralazine had different effects on heart rate. Although administration of trandolapril suppressed the increases in LV Ang II and LV weight by isoproterenol (Figure 3), administration of hydralazine did not affect these changes (Figure 4).

**Discussion**

The existence and participation of the extrarenal renin-angiotensin system for some pathophysiological conditions has been documented. In the heart, gene expression of angiotensinogen, renin, and ACE has been demonstrated. Recent studies have shown that Ang II has a direct proliferative effect on cultured cardiac myocytes.

The present study demonstrated that isoproterenol produced LV hypertrophy, which was associated with increases in plasma and tissue Ang II. Infusion of isoproterenol caused increases in blood pressure and heart rate, while trandolapril and hydralazine did not.

**Table 1. Effect of Isoproterenol and Concomitant Administration of Trandolapril or Hydralazine on Body Weight, Blood Pressure, Heart Rate, and Plasma Angiotensin II**

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Body weight (g)</th>
<th>Blood pressure (mm Hg)</th>
<th>Heart rate (bpm)</th>
<th>Plasma Ang II (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle (n=12)</td>
<td>338±5</td>
<td>130±2</td>
<td>389±12</td>
<td>3.9±0.6</td>
</tr>
<tr>
<td>Vehicle+ISO (n=12)</td>
<td>320±8</td>
<td>121±5</td>
<td>471±12</td>
<td>13.3±3.2</td>
</tr>
<tr>
<td>Trandolapril (n=12)</td>
<td>339±8</td>
<td>109±3</td>
<td>398±7</td>
<td>6.6±0.6</td>
</tr>
<tr>
<td>Trandolapril+ISO (n=12)</td>
<td>320±6</td>
<td>109±4</td>
<td>483±11</td>
<td>18.5±2.6</td>
</tr>
</tbody>
</table>

*p*  
Interaction NS NS NS NS  
ISO <0.05 NS <0.01 <0.01  
Trandolapril NS <0.01 NS NS  
Vehicle (n=6) 339±7 127±2 373±17 5.6±2.5  
Vehicle+ISO (n=6) 315±1 115±5 469±15† 17.6±5.5  
Hydralazine (n=6) 336±20 107±4 450±20† 11.4±2.2  
Hydralazine+ISO (n=6) 303±10 114±7 459±13† 15.2±3.2  

*p*  
Interaction NS NS <0.05 NS  
ISO <0.05 NS ... <0.05  
Hydralazine NS <0.05 ... NS  

Values are mean±SEM. ISO, isoproterenol; bpm, beats per minute; Ang II, angiotensin II.  
*Values obtained from two-factor analysis of variance; †p<0.05 vs. vehicle by Bonferroni's modified *t* test.
terenol in anephric rats similarly increased LV weight and tissue Ang II but did not affect plasma Ang II. From these results, it is suggested that Ang II in the heart rather than in plasma may be partly responsible for LV hypertrophy induced by isoproterenol.

In the present study, we also examined the role of the renin-angiotensin system in the prevention of LV hypertrophy induced by isoproterenol. Our data showed that the concomitant administration of trandolapril prevented the increases in both LV weight and tissue Ang II. A decrease in blood pressure by trandolapril may prevent LV hypertrophy induced by isoproterenol. However, it is unlikely, because hydralazine did not prevent the increase in LV weight although it produced similar blood pressure reduction. Previous studies have also demonstrated dissociation of blood pressure reduction and regression of LV hypertrophy with antihypertensive treatment. Thus, these data and ours suggest that mechanisms other than blood pressure reduction account for the prevention of cardiac hypertrophy by ACE inhibitors.

In contrast to blood pressure, parallel changes in LV Ang II and LV weight were observed with concomitant administration of antihypertensive drugs. Our previous study in spontaneously hypertensive rats demonstrated that the reduction in LV weight after treatment with ACE inhibitors was related to a decrease in cardiac Ang II. From these results, we conclude that the reduction of LV Ang II by trandolapril may be a mechanism of prevention of LV hypertrophy.

In this study, plasma Ang II was increased by trandolapril. Mento and Wilkes have also demonstrated that chronic oral treatment with enalapril increased plasma Ang II from 9.9±1.8 to 28.9±8.7 pg/ml, whereas plasma ACE activity was undetectable. They speculated that an induction of the alternative pathways for Ang II formation or mass action via the classic cascade may account for the increase in plasma Ang II by ACE inhibitors.

FIGURE 3. Bar graphs show effects of vehicle (VEH) or isoproterenol (ISO) infusion in left ventricular angiotensin II and weight. Concomitant administration of trandolapril suppressed the increases in left ventricular angiotensin II and weight by isoproterenol. *p<0.05 vs. vehicle by Bonferroni's modified t test; NS, not significant; n=12 in each group. BW, body weight.

FIGURE 4. Bar graphs show left ventricular angiotensin II and weight in hydralazine-treated rats. Treatment with hydralazine did not affect the increases in either left ventricular angiotensin II or weight induced by isoproterenol (ISO). *p<0.05 vs. vehicle (VEH) by two-factor analysis of variance; n=6 in each group. BW, body weight.
inhibitor. The mechanisms responsible for high plasma Ang II will require further study.

Mechanisms other than cardiac Ang II may also be involved in the prevention of LV hypertrophy. Kohlmann et al. demonstrated that treatment with hydralazine increased plasma norepinephrine and norepinephrine turnover in the heart, whereas enalapril did not change the plasma level of norepinephrine and decreased its cardiac turnover. Sen et al showed that cardiac catecholamine levels were increased by hydralazine and were unchanged by captopril. Therefore, the difference in the effect of ACE inhibitor and hydralazine on cardiac sympathetic nervous system may also explain the different effect on LV weight.

It appears that trandolapril prevents LV hypertrophy via hemodynamic changes such as cardiac contractility, although it did not affect either blood pressure or heart rate in isoproterenol-infused rats. We did not assess cardiac function in this study; however, data reported by Zierhut and Zimmer did not support this hypothesis. In norepinephrine-induced cardiac hypertrophy, concomitant administration of verapamil prevented norepinephrine-induced hemodynamic changes including LV dP/dt max, but it did not affect LV weight, suggesting that hemodynamic changes have a minor role in the prevention of LV hypertrophy by catecholamine.

It was reported that exogenously administered or endogenously increased Ang II in plasma caused necrosis of cardiac myocytes. Because Ang II receptors have not been demonstrated on the surface of the adult rat myocyte, Ang II derived from adjacent myocytes or plasma cannot act on the myocyte by the paracrine mechanism or as a hormone. In contrast, receptors for Ang II have been shown to be present in the nuclei. Therefore, it is possible that Ang II in the myocyte binds its nuclei by the autocrine mechanism and stimulates cell proliferation.

In conclusion, progression and prevention of LV hypertrophy induced by isoproterenol were associated with changes in LV tissue Ang II. Because Ang II has a proliferative effect on cardiac myocytes, we conclude that cardiac tissue Ang II may be one of the factors that regulate the growth of cardiac myocytes.

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