Perindopril Effect on Uncoupling Protein and Energy Metabolism in Failing Rat Hearts

Kazushi Murakami, Katsufumi Mizushige, Takahisa Noma, Teppei Tsuji, Shoji Kimura, Masakazu Kohno

Abstract—Uncoupling proteins, inner mitochondrial membrane proton transporters, are important for regulating myocardial energy efficiency. We investigated the effects of the ACE inhibitor perindopril on cardiac performance, myocardial energy efficiency, and uncoupling protein expression in an aortic regurgitation rat model. Twenty male Sprague-Dawley rats, in which aortic regurgitation was produced, were divided into untreated and perindopril-treated (5 mg · kg⁻¹ · d⁻¹) rats. The treatments were initiated 3 days after operation. Ten control rats were sham-operated. Measurements of blood pressure and echocardiography were repeated before and 100 days after operation (endpoint). Left ventricular uncoupling protein-2 expression, creatine phosphate, and adenosine triphosphate were measured at endpoint. In perindopril-treated rats, systolic and diastolic blood pressure decreased after treatment (92±4/65±2 mm Hg). At endpoint, left ventricular end-diastolic dimension in untreated (10.7±0.2 mm) and treated rats (9.2±0.2 mm) was increased, and fractional shortening was reduced in untreated rats (28±1%) but did not change in treated rats (36±2%). Uncoupling protein-2 mRNA expression increased in untreated rats (3.7-fold) and was suppressed by perindopril (1.5-fold). The creatine phosphate was reduced in untreated rats (10.6±0.7 μmol/g) but not in treated rats (15.9±2.0 μmol/g). In the chronic stage of aortic regurgitation, perindopril improved cardiac performance and myocardial energy efficiency, in which the suppression of uncoupling protein-2 may play an important role. *(Hypertension. 2002;40:251-255.)*

**Key Words:** uncoupling proteins ■ heart failure ■ metabolism ■ angiotensin-converting enzyme ■ rats ■ cardiac function

Uncoupling proteins (UCPs) are inner mitochondrial membrane proton transporters and decrease the proton electrochemical gradient across the inner mitochondrial membrane. Decrease in electrochemical gradient reduces energy force for adenosine triphosphate (ATP) biosynthesis during respiration spending oxygen and stimulates heat production (UCP-1).1,2 In our previous study of aortic regurgitation (AR) model rats, the myocardial expression of UCP-2 was significantly increased and the high-energy phosphate creatine phosphate (CrP) was decreased at the chronic stage of heart failure. These results suggested that the alteration of energy efficiency through the expression of UCP-2 in the heart has an important role in regulating cardiac function during the process of developing heart failure.3 It has been reported that ACE inhibitors improve cardiac function and prevent myocardial remodeling in patients4,5 and animal models6-8 with heart failure. Although this mechanism of beneficial effects has been investigated,9-11 the effects of ACE inhibitors on myocardial energy efficiency in the failing heart have not been elucidated. The aim of the present study was to investigate the effect of the ACE inhibitor perindopril on myocardial energy metabolism, expression of UCP, and its relation with cardiac performance in AR model rats.

**Methods**

**Animals**

Thirty male Sprague-Dawley rats (body weight, 366 to 572 g) were maintained at the Kagawa Medical University animal experiment center. They were kept in a pathogen-free facility under controlled temperature (23±2°C) and humidity (55±5%) with a 12-hour/12-hour artificial light/dark cycle, and were given free access to standard laboratory rat chow (MF, Oriental Yeast Corp) and tap water. All procedures were in accordance with institutional guidelines for animal research.

**Experimental Aortic Regurgitation Model**

Rats were anesthetized with ketamine (50 mg/kg intraperitoneal) and xylazine (10 mg/kg intraperitoneal). In 20 rats, the distal site of right common carotid artery was ligated, the tip of a catheter (P50) was introduced to the ascending aorta, and the cusp of the aortic valve was punctured.12 Rats that had a massive AR jet confirmed using color Doppler echocardiography were enrolled as AR model rats. Ten sham-operated rats in which AR was not induced were prepared.

**Experimental Protocols**

Three days after the development of AR, the rats were divided into 3 groups: (a) untreated AR rats (n=10), (b) perindopril-treated (2...
mg · kg⁻¹ · d⁻¹) AR rats (n=10), and (c) sham-operated rats (n=10). Perindopril was administered in the drinking water, and the concentration of perindopril was adjusted to the individual drinking habits to ensure an appropriate dose based on the measurement of body weight and daily fluid intake.

At the baseline (before operation) and at 30 and 100 days after operation, blood pressure, heart rate, and body weight were measured. Systolic and diastolic blood pressure and heart rate were measured in conscious rats by the tail-cuff method (automatic blood pressure measurement system, Softron Corp; mini thermal printer, Saneienduki Corp). The mean values of ≥3 measurements were used.

At 100 days after operation, rats were similarly anesthetized, and a pressure wire (RADI Medical Systems Type PGA 10) was inserted from the cardiac apex into the left ventricle for left ventricular pressure measurement. After completion of the hemodynamic measurement, rats were more deeply anesthetized, and the heart was excised. The atria were trimmed from the ventricles, and the right ventricle and left ventricle, including septum, were separated immediately and weighed. The left ventricle was rapidly frozen with liquid nitrogen and stored at −80°C until measurement of ATP, CrP, and RNA extraction. The skeletal muscle was also frozen for the measurement of RNA extraction.

Doppler Echocardiography

Trans thoracic echocardiography was performed13, 14 at the baseline and at 30 and 100 days after development of AR in the anesthetic state (ketamine and xylazine) by use of an echocardiographic machine with a 7.5-MHz transducer (SSD-2200, Aloka Corp). End-diastolic (LVDd) and end-systolic (LVDs) left ventricular dimension was measured. Fractional shortening (FS) was calculated as [(LVDd–LVDs)×100/LVDd] The cardiac output was calculated as [time velocity integral of right ventricular outflow velocity waveform]×[(π×(right ventricular outflow diameter/2)²)×heart rate]. Heart rate was determined from a simultaneously recorded ECG.

RNA Extraction and Northern Blot Hybridization

Total RNA was extracted from the left ventricle and skeletal muscle by use of the LiCl/urea method.15 The cDNA for rat UCP-2, atrial natriuretic peptide (ANP), and GAPDH were obtained by reverse transcription–polymerase chain reaction; 15 total RNA was extracted from the left ventricle and skeletal muscle including septum, were separated immediately and weighed. The left ventricle was rapidly frozen with liquid nitrogen and stored at −80°C until measurement of ATP, CrP, and RNA extraction. The skeletal muscle was also frozen for the measurement of RNA extraction.

Results

Blood Pressure and Heart Rate

No difference in systolic blood pressure was observed between untreated AR rats and sham-operated rats throughout the protocol. Diastolic blood pressure decreased after induction of AR and continued until the termination of protocol. In perindopril-treated AR rats, systolic and diastolic blood pressures markedly decreased after drug administration (Table 1). Heart rates were not different among the 3 groups throughout the protocol (at 100 days after operation: untreated AR, 357±16 bpm; perindopril-treated AR, 340±19 bpm; and sham, 336±17 bpm).

Echocardiography

There was no difference in LVDd among the 3 groups at baseline, but LVDd in untreated and perindopril-treated AR rats increased after induction of AR compared with that of sham-operated rats. In untreated AR rats, LVDd progressively dilated, but that in perindopril-treated AR rats did not dilate in the chronic phase. FS was reduced in untreated AR rats in the chronic phase of AR; however, no difference in FS was observed between perindopril-treated AR rats and sham-operated rats (Table 2). At 100 days after operation, cardiac output was lower in untreated AR rats than in perindopril-treated AR and sham-operated rats (Table 3).

Postmortem Organ Weights

The left ventricular wet weight–to–body weight ratio was higher in untreated rats than in perindopril-treated and sham-operated rats. The right ventricular wet weight–to–body weight ratio was higher in untreated rats than in sham-operated rats (Table 3).

Northern Blot Analysis for ANP and UCP-2 mRNA

Levels of ANP mRNA in the left ventricle of untreated AR rats were increased and reached 10.7-fold over that of sham-operated rats (Table 3).

**TABLE 1. Systolic and Diastolic Blood Pressure at Baseline and 100 Days After Operation**

<table>
<thead>
<tr>
<th></th>
<th>Systolic Blood Pressure (mm Hg)</th>
<th>Diastolic Blood Pressure (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>100 Days</td>
</tr>
<tr>
<td>Sham-operated rats</td>
<td>138±3</td>
<td>137±4</td>
</tr>
<tr>
<td>Untreated AR rats</td>
<td>139±3</td>
<td>138±2</td>
</tr>
<tr>
<td>Perindopril-treated AR rats</td>
<td>135±5</td>
<td>92±4†</td>
</tr>
</tbody>
</table>

All values are mean±SEM.

*P<0.05 vs sham-operated rats; †P<0.05 vs untreated AR rats.

**TABLE 2. Doppler Echocardiographic Parameters**

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>30 Days</th>
<th>100 Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>LV End-diastolic dimension, mm</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sham-operated rats</td>
<td>7.6±0</td>
<td>8.1±0</td>
<td>8.1±0</td>
</tr>
<tr>
<td>Untreated AR rats</td>
<td>7.7±0.3</td>
<td>9.8±0.2*</td>
<td>10.7±0.2*</td>
</tr>
<tr>
<td>Perindopril-treated AR rats</td>
<td>7.8±0.1</td>
<td>9.2±0.1*</td>
<td>9.2±0.2†</td>
</tr>
<tr>
<td>Fractional shortening, %</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sham-operated rats</td>
<td>36.0±0.8</td>
<td>35.2±1.1</td>
<td>38.3±1.1</td>
</tr>
<tr>
<td>Untreated AR rats</td>
<td>36.7±0.6</td>
<td>33.0±1.4</td>
<td>27.5±0.7*</td>
</tr>
<tr>
<td>Perindopril-treated AR rats</td>
<td>37.1±0.8</td>
<td>34.5±0.4</td>
<td>36.2±2.0†</td>
</tr>
</tbody>
</table>

All values are mean±SEM.

*P<0.05 vs sham-operated rats; †P<0.05 vs untreated AR rats.
operation (1.5-fold). No difference of the UCP-2 mRNA expression of skeletal muscle among the 3 groups was observed (Figure 2).

**ATP and CrP Level**

At 100 days after operation, although the ATP level was not different among the 3 groups (untreated AR, 20.5 ± 2.1 μmol/g dry tissue; perindopril-treated AR, 20.5 ± 2.2 μmol/g dry tissue; and sham-operated, 20.0 ± 1.3 μmol/g dry tissue), the CrP level was reduced in untreated AR rats (10.6 ± 0.7 μmol/g dry tissue) compared with perindopril-treated AR rats (15.9 ± 2.0 μmol/g dry tissue) and sham-operated rats (14.5 ± 1.8 μmol/g dry tissue) (Figure 3).

**Discussion**

The present study demonstrated that gradually progressive left ventricular dilation, increase in left ventricular end diastolic pressure, and decrease in cardiac output were manifested in the untreated AR rats. Left ventricular volume overload produced ventricular dilatation and diffuse impairment of left ventricular contraction. Consequently, this model does not represent all models of heart failure and is not parallel to heart failure caused by hypertension. In our AR model rats, increases in left ventricular and right ventricular weights were observed. Marked elevation of left ventricular end diastolic pressure probably augmented a pulmonary artery pressure, and the pressure overload to right ventricle may be a cause of right ventricular hypertrophy. Perindopril prevented cardiac remodeling and maintained cardiac performance in the chronic stage of AR. Simultaneously, CrP content was significantly reduced in untreated AR rats but not in perindopril-treated AR rats. In the failing heart, it has been clinically recognized that oxygen consumption is disproportionately high despite a decrease in CrP or the ratio of CrP to ATP indicating a reduction of myocardial energy efficiency. Our results demonstrate that perindopril maintained high-energy phosphate production at the chronic stage of AR.

We have reported that expression of UCP-2 was related to energy efficiency as a mechanism of heart failure in a rat model of AR. In the chronic phase of AR, the expression of UCP-2 was significantly increased in the heart, and the high-energy phosphate CrP decreased. In the present study, perindopril suppressed the UCP-2 mRNA expression and prevented reduction of CrP. Previous studies have demonstrated that ACE inhibitors significantly increase CrP levels in a model of acute left ventricular failure induced by an intracoronary injection of plastic microspheres and chronic heart failure induced by permanent left coronary artery ligation. These investigations indicated the relation between the renin-angiotensin system and myocardial energy efficiency. Although controversy continues regarding the details of myocardial energy metabolism, to our knowledge this is the first report that indicates a possible role of UCP-2 expression in renin-angiotensin–myocardial energy metabolism relationship.

Fukunaga et al examined a serial expression of the UCP-2 and UCP-3 mRNA in the heart of genetic stroke-prone spontaneously hypertensive rats and demonstrated that the tendencies of these expression did not change, or rather decrease, with the concomitant development of hypertension (at 6 weeks: UCP-2, 1.6-fold; UCP-3, 3.6-fold; at 15 weeks: UCP-2, 1.4-fold; UCP-3, 2.4-fold). Their model rats did not develop heart failure despite a development of hypertension. In our AR model rats, perindopril decreased UCP-2 expression in association with an improvement of heart failure, despite a reduction of blood pressure. Consequently, an alteration in blood pressure itself may not play an important role in the regulation of the UCP mRNA expression, and we may be able to reject a possibility of the UCP-2 suppression by blood pressure reduction. Thus, the expression of UCP-2 may be regulated by the heart function, not by an alteration in blood pressure.

In conclusion, perindopril improved cardiac remodeling and performance and prevented decrease in the high-energy phosphate at the chronic phase of AR. The suppression of UCP-2 mRNA expression may play an important role in improvement of myocardial energy efficiency of the failing heart.

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**TABLE 3. Cardiac Output, Left Ventricular End-diastolic Pressure, and Postmortem Heart Weights at 100 Days After Operation**

<table>
<thead>
<tr>
<th>CO (mL/min)</th>
<th>LVEDP (mm Hg)</th>
<th>LV/BW (mg/g)</th>
<th>RV/BW (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham-operated rats</td>
<td>154 ± 6</td>
<td>10 ± 1</td>
<td>1.75 ± 0.06</td>
</tr>
<tr>
<td>Untreated AR rats</td>
<td>116 ± 5*</td>
<td>20 ± 3*</td>
<td>2.59 ± 0.09*</td>
</tr>
<tr>
<td>Perindopril-treated AR rats</td>
<td>152 ± 7†</td>
<td>11 ± 2†</td>
<td>1.91 ± 0.07†</td>
</tr>
</tbody>
</table>

All values are mean ± SEM. CO indicates cardiac output; LVEDP, left ventricular end-diastolic pressure; LV, left ventricle; BW, body weight; and RV, right ventricle.

*P < 0.05 vs sham-operated rats; †P < 0.05 vs untreated AR rats.

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**Figure 1.** Comparison of ANP mRNA extraction among 3 groups. The ANP mRNA extraction of the left ventricle markedly increased in untreated AR rats. Sham indicates sham-operated rats; AR, untreated AR rats; and AR + PE, perindopril-treated AR rats. *P < 0.05 vs sham-operated rats; †P < 0.05 vs untreated AR rats.
Perspectives

Although further studies are necessary to examine the link between the administration of ACE inhibitors and myocardial energy production in various heart conditions, including pressure overload, the present study demonstrated that perindopril improved cardiac performance and myocardial energy efficiency in the chronic stage of AR and that suppression of UCP-2 expression may play an important role in this response. Varin et al. demonstrated the decreased oxidative stress after perindopril administration in heart failure of left coronary artery ligation rats, and opposed the normalized production of NO or the changes in oxidant status secondary to the changes in blood flow by ACE inhibition as its possible mechanisms. Additionally, Echtay et al. examined the interaction of superoxide with UCP families and demonstrated the relation of the concentration of reactive oxygen species inside mitochondria with the UCP expression. Based on these reports, we speculated that perindopril suppressed UCP-2 mRNA expression by reducing the concentration of reactive oxygen species. Because we did not measure the concentration of reactive oxygen species and controversy concerning the antioxidant effects of ACE inhibitors still exists, details of this linkage should be further examined. In regard to myocardial UCP regulation, β-stimulants, thyroid hormone, or exercise has been reported. Thus, it is possible that the alteration in neurohumoral activity, excepting renin-angiotensin system induced by the administration of perindopril, suppresses the UCP-2 expression. The regulating mechanism of myocardial UCP family expression is still unknown, but it may be a new and important clue in pathophysiological elucidation and treatment of heart failure.

References


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