Plasma Immunoreactive Endothelin-1 in Experimental Malignant Hypertension

Masakazu Kohno, Koh-ichi Murakawa, Takeshi Horio, Koji Yokokawa, Kenichi Yasunari, Toshiki Fukui, and Tadanao Takeda

We measured plasma concentrations of immunoreactive endothelin-1 (irET-1) in the prehypertensive and hypertensive phases in spontaneously hypertensive rats (SHR) and in malignant hypertension caused by deoxycorticosterone acetate (DOCA)-salt administration in SHR. We also measured concentrations of this peptide in another model of malignant hypertension, the two-kidney, one clip (2K1C) renovascular hypertensive rats chronically given caffeine. Plasma irET-1 concentrations in young (6-week-old) and mature (18-week-old) SHR did not differ from those of age-matched Wistar-Kyoto (WKY) rats. Four weeks of treatment with DOCA-salt increased blood pressure, blood urea nitrogen, serum creatinine, and plasma irET-1 in SHR but not in WKY rats. Eight weeks of DOCA-salt treatment further increased these values in SHR. Plasma irET-1 concentrations were not increased in the 2K1C rats. Six weeks of caffeine administration increased blood pressure, blood urea nitrogen, serum creatinine, plasma renin activity, and plasma irET-1 in the 2K1C rats but not in the sham-operated rats. High-performance liquid chromatographic profiles of plasma extracts pooled from these rats with malignant hypertension showed that a major component of irET-1 eluted in the position of synthetic ET-1(1-21). Furthermore, acute hypertension induced by angiotensin II or phenylephrine did not affect the plasma irET-1 concentration in rats. The results suggested that the plasma ET-1 concentration is increased in rat models of malignant hypertension and that the high blood pressure itself is not the main factor involved in the increase of plasma ET-1.

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The role of the vascular endothelium in the regulation of vascular tonus has been the focus of much interest. Endothelial cells release endothelium-derived relaxing factors (EDRF) that are short-lived in response to vasoactive agents such as bradykinin and acetylcholine,1-3 and one EDRF has been identified as nitric oxide or a related substance.4 Several previous studies have described a protease-sensitive vasoconstrictor activity in supernatants of cultured endothelial cells.5-7 Yanagisawa et al8 isolated endothelin, a 21-residue vasoconstrictive peptide derived from the endothelium from the culture supernatant of porcine aortic endothelial cells, determined its amino acid sequence, and cloned the peptide precursor. This peptide has potent, long-lasting vasoconstrictor activity8,9 and causes a prolonged elevation in blood pressure and marked reduction in renal blood flow.10-12

Cultured porcine, bovine, and human endothelial cells13-19 and porcine aortic strips with an intact endothelium20 release immunoreactive endothelin-1 (irET-1) in a time-dependent way. irET-1 is present in rat21 and human22,23 plasma, and its levels are high in patients with acute myocardial infarction24,25 or uremia26,27 and in hypertensive patients with severe hypertension or renal involvement.28

To assess any changes in plasma irET-1 concentrations in experimental malignant hypertension, we measured these concentrations in two experimental models of malignant hypertension: malignant hypertension caused by deoxycorticosterone acetate–salt (DOCA-salt) in spontaneously hypertensive rats (SHR)29,30 and renovascular hypertension, caused by the two-kidney, one clip (2K1C) method, in rats that were given caffeine long-term.31 In addition, we measured plasma irET-1 concentrations in young and mature SHR and in age-matched Wistar-Kyoto (WKY) rats. We also characterized ET-1 immunoreactivity in pooled plasma from these experimental groups using high-performance liquid chromatography (HPLC) coupled with a radioimmunoassay.
Methods

Plasma Immunoreactive Endothelin-1 Concentration in Young and Mature Spontaneously Hypertensive Rats

Male 5-week-old SHR and WKY rats and male 17-week-old SHR and WKY rats (Shizuoka Lab, Shizuoka, Japan) were housed in groups of eight rats each in a room with controlled temperature (25°C), humidity (50–60%), and lighting (14-hour light/10-hour dark cycle). All rats were given tap water ad libitum and ordinary rat food containing 0.39% sodium and 0.98% potassium (Clea Japan Inc., Tokyo). After 1 week of habituation, rats were anesthetized by an intraperitoneal injection of 40 mg/kg thiamylal sodium, and blood samples were immediately withdrawn from the abdominal aorta.

Plasma Immunoreactive Endothelin-1 in Deoxycorticosterone Acetate–Salt Spontaneously Hypertensive Rats

Male 8-week-old SHR and WKY rats were divided into the following six groups, each comprising seven rats. Groups of DOCA-salt SHR and WKY rats were treated with DOCA and also given 1% NaCl drinking water ad libitum as described previously. DOCA (Sigma Chemical Co., St. Louis, Mo.) was administered once a week by subcutaneous injection (2 ml/kg of a suspension containing, per milliliter of water, 50 mg DOCA, 10.5 mg methylcellulose, 3 mg carboxymethyl cellulose, 1 mg polysorbate 80, and 15 mg NaCl). Groups of salt-treated SHR and WKY rats were given 1% NaCl drinking water ad libitum and subcutaneously injected once a week for DOCA. Control group SHR and WKY rats were given water ad libitum and subcutaneously injected once a week with the vehicle for DOCA and NaCl. All groups received ordinary rat food containing 0.39% sodium and 0.98% potassium. At 12 and 16 weeks of age, rats were anesthetized, and blood samples were taken as described above.

Plasma Immunoreactive Endothelin-1 Concentration in Two-Kidney, One Clip Renovascular Hypertensive Rats Given Caffeine Long-term

Male 10-week-old Wistar rats weighing 220–250 g (n=24) were housed in a room with controlled temperature and humidity and given ordinary rat food as described above. After 1 week of habituation, rats were anesthetized with thiamylal sodium (50 mg/kg i.p.). An incision was made in the left abdomen, the left renal artery was isolated, and a silver clip (0.25 mm gap) was placed around the left renal artery. In some rats the clip was then removed (sham-operated rats), and in the others the clip was left on the renal artery (2K1C rats).

Twelve 2K1C rats were randomly assigned to receive either 0.1% caffeine in their drinking water (2K1C caffeine group, n=6) or drinking water without caffeine (2K1C no-caffeine group, n=6). In addition, 12 sham-operated rats were randomly assigned to receive either 0.1% caffeine in their drinking water (sham-caffeine group, n=6) or drinking water without caffeine (sham-control group, n=6). Treatment with caffeine began immediately after surgery. The procedures were done as reported by Ohnishi et al. In the 6-week observation period, systolic blood pressure was measured several times. Rats were anesthetized by an intraperitoneal injection of 40 mg/kg thiamylal sodium, and blood samples were collected as described above.

Short-term Effects of Angiotensin II and Phenylephrine on Plasma Immunoreactive Endothelin-1 Concentration

We examined here whether the plasma irET-1 concentration was affected by vasoconstrictors given short-term to anesthetized rats. Male 20-week-old Wistar rats (n=12) weighing 350–450 g were anesthetized. Catheters were implanted in the left carotid artery and the left jugular vein for blood sampling and for administration of angiotensin II or phenylephrine, respectively. After a 20-minute equilibration period, arterial blood pressure was measured and arterial blood was collected before and 1, 3, and 10 minutes after an intravenous administration of 1 μg angiotensin II (n=4) or 5 μg phenylephrine (n=4). The blood removed (1 ml) was replaced with an equivalent volume of saline. Control rats (n=4) underwent sham operations, and samples were taken in the same way.

Analytical Methods

Systolic blood pressure was measured by the tail-cuff method with an electrophysymomanometer (model RS-100, Riken Kihatsu Co., Tokyo). Five readings were averaged for each rat.

Mean arterial pressure was measured by connection of the catheter inserted into the left carotid artery to a pressure transducer (model TP-400T, Nihon Kohden, Tokyo) as described previously.

Blood urea nitrogen (BUN) and serum creatinine were measured by a routine automatic method. Plasma renin activity (PRA) was measured by radioimmunoassay.

Plasma Immunoreactive Endothelin-1 Measurement

Blood was drawn immediately into siliconized disposable glass tubes chilled on ice and containing aprotinin (500 kallikrein inactivator units/ml) and ethylenediaminetetraacetic acid (1 mg/ml). Plasma was separated by centrifugation for 10 minutes at 4°C and was immediately frozen and stored at −80°C for several days.

irET-1 was extracted from plasma as previously reported. Briefly, plasma was diluted with 4% acetic acid. After centrifugation, the supernatant was pumped at the rate of 1 ml/min through a Sep-Pak C18 cartridge (Waters Chromatography Division, Millipore Corp., Milford, Mass.). After the cartridge was washed with distilled water, the absorbed peptides were eluted with 86% ethanol in...
Whether the plasma irET-1 concentration was examined. The irET-1 concentrations in plasma obtained from the carotid artery of conscious and almost unrestrained Wistar rats were not significantly different from the ET-1 values obtained from samples from the abdominal aorta after anesthesia by an intraperitoneal injection of 40 mg/kg thiamylal sodium (2.1±0.3 versus 2.0±0.4 pg/ml).

**Statistical Analysis**

Statistical analysis was done by analysis of variance followed by Scheffe’s test for multiple comparisons. The correlation between the plasma irET-1 concentration and blood pressure, BUN, or serum creatinine was examined by linear regression analysis. Values were expressed as mean±SD.

### Results

**Plasma Immunoreactive Endothelin-1 Concentration in Young and Mature Spontaneously Hypertensive Rats**

Mean body weight, mean systolic blood pressure, and mean plasma irET-1 concentrations in young (6-week-old) and mature (18-week-old) SHR and age-matched WKY rats are shown in Table 1. The mean body weight was not different between SHR and WKY rats at either 6 weeks or 18 weeks of age. The mean systolic blood pressure in the 6-week-old SHR was insignificantly higher than in the age-matched WKY rats, but the mean systolic blood pressure in the 18-week-old SHR was significantly higher than in the age-matched WKY rats. Plasma irET-1 concentrations were not different between SHR and WKY rats at either age.

**Plasma Immunoreactive Endothelin-1 Concentration in Deoxycorticosterone Acetate–Salt Spontaneously Hypertensive Rats**

Mean body weight, mean systolic blood pressure, BUN, serum creatinine concentrations, and plasma irET-1 concentrations in SHR and WKY rats treated with DOCA and salt for 4 weeks and 8 weeks; SHR and WKY rats treated with salt for 4 weeks and 8 weeks; and the corresponding SHR and WKY rat controls are shown in Tables 2 and 3. The mean body weight in SHR treated with DOCA and salt for 8 weeks was significantly lower than in the other groups. The mean systolic blood pressure...
TABLE 2. Effects of Treatment for 4 Weeks With Deoxycorticosterone Acetate–Salt or Salt on Body Weight, Systolic Blood Pressure, and Levels of Blood Urea Nitrogen, Serum Creatinine, and Plasma Immunoreactive Endothelin-1 Concentrations in Spontaneously Hypertensive Rats and Wistar-Kyoto Rats

<table>
<thead>
<tr>
<th>Measurements</th>
<th>WKY</th>
<th>SHR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control (n)</td>
<td>Salt (n)</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>247±14</td>
<td>239±21</td>
<td>224±14</td>
</tr>
<tr>
<td>Systolic BP (mm Hg)</td>
<td>130±8</td>
<td>132±10</td>
</tr>
<tr>
<td>BUN (mg/dl)</td>
<td>19±2</td>
<td>18±3</td>
</tr>
<tr>
<td>Serum creatinine (mg/dl)</td>
<td>0.5±0.1</td>
<td>0.6±0.1</td>
</tr>
<tr>
<td>Plasma irET-1 (pg/ml)</td>
<td>1.9±0.6</td>
<td>2.1±0.4</td>
</tr>
</tbody>
</table>

WKY, Wistar-Kyoto rats; SHR, spontaneously hypertensive rats; DOCA, deoxycorticosterone acetate; BP, blood pressure; BUN, blood urea nitrogen; irET-1, immunoreactive endothelin-1.

*<p<0.05 compared with control WKY rats.
†<p<0.05 compared with control SHR.
‡<p<0.05 compared with salt WKY rats.
§<p<0.05 compared with salt SHR.

Plasma Immunoreactive Endothelin-1 Concentration in the Two-Kidney, One Clip Renovascular Hypertensive Rats Given Caffeine Long-term

Table 4 shows the mean body weight, mean systolic blood pressure, BUN, serum creatinine, PRA, and plasma irET-1 concentrations in the 2K1C caffeine and no-cafeine groups and in the sham-cafeine and sham-control groups. The mean body weight in the 2K1C caffeine group was significantly lower than in the three other groups. The mean systolic blood pressure in the 2K1C no-cafeine group was significantly higher than in the sham-cafeine and sham-control groups, and this value in the 2K1C caffeine group was markedly higher than in the three other groups. BUN and serum creatinine levels in the 2K1C caffeine group was slightly but significantly higher than the 2K1C no-cafeine group, but this difference was not ob-

TABLE 3. Effects of Treatment for 8 Weeks With Deoxycorticosterone Acetate–Salt or Salt on Body Weight, Systolic Blood Pressure, and Levels of Blood Urea Nitrogen, Serum Creatinine, and Plasma Immunoreactive Endothelin-1 Concentrations in Spontaneously Hypertensive Rats and Wistar-Kyoto Rats

<table>
<thead>
<tr>
<th>Measurements</th>
<th>WKY</th>
<th>SHR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control (n)</td>
<td>Salt (n)</td>
</tr>
<tr>
<td>Rats (n)</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>295±9</td>
<td>296±12</td>
</tr>
<tr>
<td>Systolic BP (mm Hg)</td>
<td>139±6</td>
<td>142±10</td>
</tr>
<tr>
<td>BUN (mg/dl)</td>
<td>19±1</td>
<td>19±3</td>
</tr>
<tr>
<td>Serum creatinine (mg/dl)</td>
<td>0.5±0.1</td>
<td>0.5±0.2</td>
</tr>
<tr>
<td>Plasma irET-1 (pg/ml)</td>
<td>2.3±0.4</td>
<td>2.2±0.5</td>
</tr>
</tbody>
</table>

WKY, Wistar-Kyoto rats; SHR, spontaneously hypertensive rats; DOCA, deoxycorticosterone acetate; BP, blood pressure; BUN, blood urea nitrogen; irET-1, immunoreactive endothelin-1.

*<p<0.05 compared with control WKY rats.
†<p<0.05 compared with control SHR.
‡<p<0.05 compared with salt WKY rats.
§<p<0.05 compared with salt SHR.
served between the sham-caffeine and sham-control groups. PRA was six times higher in the 2K1C caffeine group and 2.5 times higher in the 2K1C caffeine group compared with the sham-control group. The plasma irET-1 concentration in the 2K1C caffeine group was significantly higher than in the three other groups, but this value in the 2K1C no-caffeine group was not different from that in the sham-caffeine and sham-control groups.

**Reverse-Phase HPLC of Immunoreactive Endothelin-1 in Extracts of Rat Plasma**

Reverse-phase HPLC profiles of irET-1 in plasma extracts pooled from DOCA-salt SHR, the 2K1C rats given caffeine, SHR, and WKY rats are shown in Figure 2. Two components of ET-1 immunoreactivity were observed: there was a major component with irET-1 eluted in the position of standard ET-1(1-21) and a minor component eluted earlier than ET-1. The elution profile observed in the two experimental models of malignant hypertension was essentially the same as those in SHR and WKY rats.

**Effects of Angiotensin II and Phenylephrine Given Short-term on Plasma Immunoreactive Endothelin-1 Concentration**

Table 5 shows the changes in the mean blood pressure and plasma irET-1 concentration after an injection of 1 μg angiotensin II or 5 μg phenylephrine. The administration of angiotensin II or phenylephrine caused a transient increase in arterial pressure but did not affect the plasma irET-1 concentration.

**Discussion**

We showed here that irET-1 is present in plasma from rats subjected to one of two kinds of experimental malignant hypertension as well as SHR and WKY rats and that a major component of ET-1 immunoreactivity eluted in the position of synthetic ET-1(1-21), with a minor component eluted earlier than ET-1(1-21). Saito et al. have shown that ET-1 immunoreactivity is detected in plasma from Wistar rats and that two components of ET-1 immunoreactivity eluted in the position of big ET-1 and ET-1. ET-1 and big ET-1 are concomitantly released from cultured human and bovine endothelial cells17,18 and

### Table 4: Body Weight, Systolic Blood Pressure, Plasma Renin Activity, Blood Urea Nitrogen, Serum Creatinine, and Plasma Immunoreactive Endothelin-1 Concentration in Two-Kidney, One Clip Renovascular Hypertensive Rats Given Caffeine for Six Weeks

<table>
<thead>
<tr>
<th>Measurements</th>
<th>2K1C rats Caffeine</th>
<th>2K1C rats No caffeine</th>
<th>Sham-operated Caffeine</th>
<th>Sham-operated No caffeine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rats (n)</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>290±12**††</td>
<td>306±9</td>
<td>308±5</td>
<td>314±7</td>
</tr>
<tr>
<td>Systolic BP (mm Hg)</td>
<td>210±9*††</td>
<td>174±10††</td>
<td>131±8</td>
<td>126±5</td>
</tr>
<tr>
<td>PRA (ng Ang I · ml⁻¹· hr⁻¹)</td>
<td>14.0±0.9*††</td>
<td>6.5±1.0††</td>
<td>2.6±0.4</td>
<td>2.4±0.3</td>
</tr>
<tr>
<td>BUN (mg/dl)</td>
<td>31±2*††</td>
<td>21±2††</td>
<td>18±2</td>
<td>17±1</td>
</tr>
<tr>
<td>Serum creatinine (mg/dl)</td>
<td>1.0±0.1*††</td>
<td>0.6±0.1</td>
<td>0.4±0.1</td>
<td>0.4±0.1</td>
</tr>
<tr>
<td>Plasma irET-1 (pg/ml)</td>
<td>3.9±0.4*††</td>
<td>2.3±0.2</td>
<td>2.2±0.2</td>
<td>2.0±0.2</td>
</tr>
</tbody>
</table>

2K1C, two-kidney, one clip; BP, blood pressure; PRA, plasma renin activity; Ang I, angiotensin I; BUN, blood urea nitrogen; irET-1, immunoreactive endothelin-1.

* † ‡ p<0.05 compared with the 2K1C no-caffeine group.

† ‡ p<0.05 compared with sham-operated caffeine group.

‡ p<0.05 compared with sham-operated no-caffeine group.
Figure 2. Histograms show reverse-phase high-performance liquid chromatography profiles of immunoreactive endothelin-1 in extracts of pooled plasma from rats with one of two kinds of malignant hypertension, deoxycorticosterone acetate (DOCA)-salt spontaneously hypertensive rats (SHR), two-kidney, one clip (2K-1C) renovascular rats given caffeine, SHR, and Wistar-Kyoto (WKY) rats. Samples were treated with use of a Sep-Pak C$_{18}$ cartridge as described in text. Elution positions of standard ET-1, ET-2, ET-3, and big ET-1 (porcine, 1–39) are shown by arrows.

Pretincer aortic strips. Furthermore, Ando et al$^{23}$ and we$^{28}$ have shown that irET-1 in human plasma is in one of two forms, one corresponding to ET-1 and the other to big ET-1. Therefore, the minor component eluted earlier than ET-1 may be rat big ET-1.

We showed that the irET-1 concentration was not increased in either the prehypertensive phase or the hypertensive phase in SHR compared with WKY rats. In addition, the plasma irET-1 concentration was not increased in the 2K1C renovascular hypertensive rats. It is unlikely, therefore, that ET-1 is released in large amounts into the circulation in SHR or in the 2K1C renovascular hypertensive rats. However, the possibility remains that ET-1 is involved in the hypertension of SHR, because vasocontractile sensitivity to ET-1 is increased in the renal$^{35}$ and mesenteric$^{9}$ arteries and the aorta$^{36}$ isolated from SHR as compared with those from WKY rats.

The plasma irET-1 concentration was significantly increased in these two experimental models of malignant hypertension. Results of experiments with animals suggest that ET-1 infused intravenously acts on the renal circulation, reducing renal blood flow$^{10–12}$ and sodium excretion.$^{10}$ Kon et al$^{37}$ have shown that anti-ET-1 antibody injected into the renal artery ameliorates the vasoconstriction of kidneys after ischemia. Furthermore, Shibouta et al$^{38}$ have shown that the monoclonal antibody to ET-1 dose-dependently inhibits the decrease in renal excretory function in a model in rats of ischemic acute renal failure caused by the occlusion of both renal arteries. They also report that the renal ET-1 content

Table 5. Changes in Mean Blood Pressure and Plasma Immunoreactive Endothelin-1 Concentration After Injection of 1 μg Angiotensin II or 5 μg Phenylephrine

<table>
<thead>
<tr>
<th></th>
<th>Mean blood pressure (mm Hg)</th>
<th>Plasma irET-1 (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>1 min</td>
</tr>
<tr>
<td>Ang II (1 μg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>96±3</td>
<td>150±*</td>
</tr>
<tr>
<td>PHE (5 μg)</td>
<td>101±5</td>
<td>167±*</td>
</tr>
<tr>
<td>Sham operation</td>
<td>101±7</td>
<td>107±3</td>
</tr>
</tbody>
</table>

irET-1, immunoreactive endothelin-1; Ang II, angiotensin II; PHE, phenylephrine. *p<0.05 compared with sham-operated rats.
as well as the plasma ET-1 concentration increases in this model and that the increase in plasma ET-1 precedes an elevation in BUN. In DOCA-salt SHR, plasma irET-1 concentrations were correlated with BUN, serum creatinine, and blood pressure levels. These observations suggest that this peptide may contribute, in part, to a deterioration in renal function and hemodynamics or to the progression of hypertension in rats with malignant hypertension.

An increase in the plasma ET-1 concentration may induce vascular smooth muscle cells to proliferate, thereby contributing to the progression of hypertension, because ET-1 causes a rapid and transient increase in the c-fos and c-myc messenger RNA (mRNA) levels and stimulates the DNA synthesis in such cells. However, the pathophysiological significance of the increased plasma ET-1 concentration in experimental malignant hypertension is not known.

These are several possible explanations for the increased levels of plasma irET-1 in rats with malignant hypertension. One possibility is that an increase in the transmural pressure creates stress across the endothelial cells, activating the production or release of ET-1, or both. The shear stress that would occur as the transmural pressure increases might stimulate the expression of ET-1 mRNA in polygonal endothelial cells and also increase the release of irET-1. However, acute hypertension induced by the administration of angiotensin II or phenylephrine did not increase the plasma irET-1 concentration in rats. Moreover, the elevation of blood pressure in SHR and chronic hypertension in the 2K1C rats failed to increase the ET-1 values. A further increase in blood pressure caused by salt intake in SHR also did not affect the plasma irET-1 concentration. Therefore, it seems unlikely that high blood pressure itself is the main factor involved in the increase of plasma irET-1 in rats with malignant hypertension.

Another explanation is that the release or production of ET-1 is stimulated in response to the activation of vascular coagulation. Thrombin or transforming growth factor β stimulates the release or production of ET-1. Furthermore, plasma irET-1 concentrations are high in acute myocardial infarction, and ET-1 values are correlated with the concentration of thrombin-antithrombin III complex. Histological examination of the kidneys of DOCA-salt SHR and the 2K1C rats given caffeine showed wall thickening and obstruction of the small arteries with hemorrhage or fibrinoid necrosis in the vascular walls. These findings may be evidence for this second possibility.

A third possible explanation is that ET-1 or its degradation products may circulate because of the impaired renal function. However, in a model of ischemic acute renal failure, the plasma ET-1 concentration increased before BUN did. Furthermore, the plasma ET-1 concentration does not increase in spite of severe azotemia 20 hours after reperfusion in this model. Therefore, the third possibility seems unlikely.

Further investigation is required to clarify whether ET-1 or big ET-1 acts as a circulating hormone and to find which contributes more to the pathophysiology of malignant hypertension.

References


**KEY WORDS** • malignant hypertension • renovascular hypertension • endothelin • spontaneously hypertensive rats
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