Antirenin Immunization Versus Angiotensin Converting Enzyme Inhibition in Rats

Ming Lo, Claude Julien, Jean-Baptiste Michel, Madeleine Vincent, Catherine Cerutti, Celso E. Gomez-Sanchez, and Jean Sassard

The effects of specific active immunization against renin were compared with those of chronic angiotensin converting enzyme (ACE) inhibition. Male spontaneously hypertensive rats (SHR) were immunized (SHR-I) (n=10) against pure murine renin (four injections of 30 μg/kg s.c.) or received (SHR-P) (n=11) a converting enzyme inhibitor (perindopril, 2 mg/kg/day per os for 4 weeks). Sham-immunized SHR (SHR-S) (n=12) and normotensive Wistar-Kyoto (WKY-S) (n=12) rats served as controls. At 15 weeks of age, 24-hour average blood pressure was obtained in freely moving rats using intra-aortic pressure recording with computer analysis. Antirenin immunization induced high circulating titers of antibodies, a fall in plasma renin activity (~95%), and urinary excretion of mineralocorticoids. Perindopril abolished the pressor response to angiotensin I, whereas plasma ACE was only partly (~56%) decreased. It also increased plasma renin activity and did not alter the urinary excretion of steroids. Both immunization and perindopril allowed the blood pressure of SHR to return to the level of WKY-S rats and reduced the left ventricular weight. These decreases were associated with an elevated sympathetic nervous system activity as indicated by increases in the urinary excretion of catecholamines and their metabolites. It is concluded that, apart from an unaltered steroid synthesis, most of the cardiovascular effects of chronic ACE inhibition are similar to those of antirenin immunization, thus indicating that blockade of the circulating and renal renin-angiotensin system accounts for most of the effects of ACE inhibitors. (Hypertension 1990; 16:80-88)

In experimental models as well as in patients, inhibition of the renin-angiotensin system (RAS) has proven to be highly effective in reducing hypertension and much of the accompanying organic damage. Most of these data were obtained using angiotensin converting enzyme (ACE) inhibitors that, in addition to their role in inhibiting the RAS, could also act by altering other mechanisms such as kinins, prostaglandins, and the sympathetic nervous system (SNS). Therefore, it appeared of interest to compare, in chronic situations, the effects of ACE inhibitors with those of active immunization against murine renin, which decreases blood pressure in spontaneously hypertensive rats (SHR) due to a highly specific blockade of the renin substrate reaction in the plasma and the kidneys.

Therefore, in the present work, we studied in adult SHR the effects of active immunization against renin and those of chronic ACE inhibition on 1) blood pressure and its variability, 2) the cardiac baroreceptor reflex response, 3) the urinary excretion of mineralocorticoids, and 4) the possible adaptation of the SNS. To avoid any influence of stress or anesthesia, the present study was conducted in conscious, freely moving animals.

Methods

Experimental Animals and Protocol

Male SHR and normotensive Wistar-Kyoto (WKY) rats (IFFA-CREDO Les Oncins, Lyon, France) were used. They were housed in controlled conditions (temperature 21±1°C, humidity 60±10%, and a 12-hour light/dark cycle from 8:00 AM to 8:00 PM) and received a standard rat chow containing 0.3% sodium (Eleveage UAR, Villelmoison s/Orge, France) with tap water ad libitum. After 1 week of acclimatization in our animal house, the rats were separated into four groups: 10 SHR (SHR-I) were actively immunized against highly purified murine renin (30 μg/rat s.c.) at 7, 9, 11, and 13 weeks of age as previously described. The homogeneity of the enzyme was...
demonstrated by the presence of a single predominant protein band on sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and its specific activity was allowed to hydrolyze 9.5 μmol tetradecapeptide substrate per hour and per milligram of protein; 11 SHR (SHR-P) were orally treated with perindopril (2 mg/kg/24 hr), an ACE inhibitor, from 11 to 15 weeks of age; the concentration of perindopril in the drinking water was calculated every day on the basis of the water intake observed the day before. Twelve SHR (SHR-S), 12 WKY (WKY-S) rats, and the SHR-P group received Freund's adjuvant as a sham-immunization procedure. In all the rats, the body weight and the water intake were measured daily, and the systolic blood pressure was measured weekly by tail-cuff plethysmography (Narco Biosystems, Houston, Tex.) from 6 to 14 weeks. At 14 weeks of age, the rats were placed into individual metabolic cages for 1 day of habituation. On the second day, 24-hour urine specimens were collected into polyethylene vials containing 5 mg Na2EDTA and 1 mg Na2S2O5 for the determination of catecholamines and their metabolites. The third day, 24-hour urine specimens were collected without preservatives for urinary steroids, electrolytes, osmolality, urea, and creatinine. Urine specimens were centrifuged and stored at -80° C.

**Blood Pressure Recording in Conscious Rats**

At 15 weeks of age, a floating polyethylene catheter (PE-20) was inserted under halothane anesthesia into the lower abdominal aorta for direct blood pressure measurement, and another catheter was brought into the left jugular vein for intravenous injections. After a 2-day recovery period, the rats were placed into individual recording cages, and the aortic catheter was connected to a blood pressure transducer (Statham P23 ID, Gould, Inc., Cleveland, Ohio) via a rotating swivel that allowed the rats to move freely. The systolic (SBP) and diastolic (DBP) blood pressure and heart rate were measured using our previously described technique. The blood pressure signal was digitized by a computer (Motorola Microsystems, Tempe, Ariz.) and processed off-line by a work-station (Sun Microsystems, Mountain View, Calif.). Immediately after connection to the blood pressure transducer (i.e., between 4:00 and 6:00 PM), the blood pressure response to an angiotensin I bolus (160 ng/kg i.v.) was measured to check the efficiency of ACE blockade. The rats were then allowed an overnight habituation period, and SBP, DBP, and heart rate were continuously recorded by beat for the next 24 hours. The next day, baroreceptor reflex sensitivity (msec/mm Hg) was measured between 8:00 and 9:00 AM to avoid the influence of circadian rhythm, using an adaptation of the method described by Smyth et al. For that purpose, each rat received two intravenous injections of phenylephrine (1.5–3 μg/kg) and two injections of nitroglycerin (200 μg/kg). As previously described, the best relation between the drug-induced changes in SBP and heart period (msec=60,000/heart rate) was computed and used as an index of baroreceptor reflex sensitivity when its significance reached the p<0.001 level. During the afternoon, the role of the SNS in blood pressure control was assessed by means of an intravenous injection of hexamethonium bromide (25 mg/kg i.v., Sigma Chemical Co., St. Louis, Mo.). Forty-eight hours later, the rats were killed between 9:00 and 11:00 AM by decapitation, and blood was drawn into heparinized tubes at 4° C for the measurement of the RAS parameters. The left ventricle and the kidneys were removed and weighed.

**Analytical Procedures**

The titer of renin antibodies was defined as the antiserum dilution able to bind 50% of pure iodinated murine renin used as a tracer. The plasma renin activity (PRA) was measured by a radioimmunoassay for generation of angiotensin I after incubation at pH 7.4 so as to avoid any dissociation between renin and renin antibodies. ACE activity was evaluated according to the method of Cushman and Cheung. Free urinary catecholamines (dopamine, norepinephrine, and epinephrine), their respective methoxylated derivatives (3-methoxytyramine, normetanephrine, and metanephrine), and total 3-methoxy-4-hydroxyphenylglycol were measured by high-performance liquid chromatography with electrochemical detection. Free urinary corticosterone, aldosterone, deoxycorticosterone (DOC), and 19-nor-deoxycorticosterone (19-nor-DOC) were measured by specific radioimmunoassays. Urinary sodium was measured by flame photometry (II meter); urea and creatinine by enzymatic colorimetric assays (Boehringer Ingelheim, Mannheim, FRG).

**Statistical Analysis**

Variability of blood pressure and heart rate was calculated as the standard deviation of the recorded values. Results are expressed as mean±SEM. Statistical analysis used one-way analysis of variance followed by a multiple comparisons test using the method of contrasts. Values of p<0.05 were considered statistically significant.

**Results**

**Body Weight, Water Intake, and Renal Function**

The body weight of SHR-S, which was lower than that of WKY-S rats, was not affected by the treatments (Table 1). According to previous observations, perindopril increased the water intake and correspondingly the urinary volume but not the urinary sodium excretion. Because this increased water intake did not appear in SHR-S receiving the same dose of perindopril by gavage (personal observation), it may not be related to the pharmacological properties of the drug but rather to its taste. SHR-I exhibited a significant increase in the plasma urea concentration, which may reflect a developing renal impairment due to autoimmune glomerular disease. However, as the creatinine clearance remained unaf-
TABLE 1. Body Weight, Water Intake, and Renal Function

<table>
<thead>
<tr>
<th>Variables</th>
<th>SHR-S (n=12)</th>
<th>SHR-I (n=10)</th>
<th>SHR-P (n=11)</th>
<th>WKY-S (n=12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>290±6*</td>
<td>283±6*</td>
<td>284±4*</td>
<td>327±5</td>
</tr>
<tr>
<td>Water intake (ml/24 hr)</td>
<td>31.9±1.2</td>
<td>33.7±1.7†</td>
<td>48.3±3.4‡</td>
<td>36.9±1.3</td>
</tr>
<tr>
<td>Urinary volume (ml/24 hr)</td>
<td>13.5±0.9</td>
<td>12.8±0.5f</td>
<td>27.7±2.4‡</td>
<td>14.0±1.2</td>
</tr>
<tr>
<td>Sodium excretion (mmol/24 hr)</td>
<td>1.47±0.12</td>
<td>1.22±0.13</td>
<td>1.38±0.12</td>
<td>1.39±0.11</td>
</tr>
<tr>
<td>Plasma urea (µmol/ml)</td>
<td>8.3±0.8</td>
<td>12.2±1.2*‡†</td>
<td>6.3±0.3</td>
<td>7.0±0.3</td>
</tr>
<tr>
<td>Creatinine clearance (ml/min)</td>
<td>2.0±0.1</td>
<td>2.0±0.2</td>
<td>2.1±0.1</td>
<td>1.8±0.1</td>
</tr>
</tbody>
</table>

Values are mean±SEM. SHR-S, sham-immunized spontaneously hypertensive rats (SHR); SHR-I, immunized SHR; SHR-P, perindopril-treated SHR; WKY-S, sham-immunized Wistar-Kyoto rats. *p<0.05 vs. WKY-S; †p<0.05 vs. SHR-S.

fected, the possible alterations of the kidney function should be modest.

Renin-Angiotensin System and Urinary Steroids

SHR-S exhibited a lower ACE activity than WKY-S rats, but both their PRA and their blood pressure response to angiotensin I did not differ (Table 2). In SHR-I, the efficiency of antirenin immunization was demonstrated by a high titer of antirenin antibodies (from 1/32,000 to 1/59,000) and by an almost suppressed PRA. The blockade of ACE in SHR-P was evidenced by a decreased plasma ACE activity (-56%) and a threefold increase in PRA. More importantly, the blood pressure response to angiotensin I was abolished in SHR-P, whereas it did not differ between SHR-I and SHR-S. SHR-S differed from WKY-S rats by a decreased aldosterone and an increased DOC urinary excretion. Chronic ACE inhibition increased the urinary excretion of corticosterone, whereas immunization significantly decreased urinary corticosterone, aldosterone, and DOC excretions.

Indirect Systolic Blood Pressure Evolution During Antirenin Immunization and Angiotensin Converting Enzyme Inhibition

As shown by Figure 1, SBP, as measured by tail-cuff plethysmography, rapidly increased with age in SHR-S. In SHR-I, the first two immunizations delayed the SBP increase and the third one induced a pronounced decrease in SBP. In SHR-P, SBP rapidly decreased after starting perindopril administration. At 14 weeks of age, indirect SBP of SHR-I and SHR-P were identical and did not differ from that of WKY-S rats.

Blood Pressure and Heart Rate Values and Their Variability During 24-Hour Recording

Because the different groups of SHR exhibited similar blood pressure and heart rate increases during the nighttime (active rats) when compared with the daytime (resting rats), the values recorded beat-to-beat for 24 hours were averaged, and the spontaneous variability was expressed as their standard deviation (Table 3). SHR-S exhibited significantly higher SBP and DBP than WKY-S rats. Both immunization against renin and ACE inhibition significantly decreased blood pressure to levels that were not significantly different from that of WKY-S rats. However, SHR-I exhibited higher blood pressure than SHR-P. Heart rate did not significantly differ between SHR-S and either SHR-I or SHR-P. The variability of SBP and heart rate was similar in SHR-S and WKY-S rats (12±0.8 and 10±0.4 mm Hg for SBP; 36±2 and 38±2 beats/min for heart rate in SHR-S and WKY-S rats, respectively). It was unal-

TABLE 2. Renin-Angiotensin System Parameters and Urinary Steroids

<table>
<thead>
<tr>
<th>Variables</th>
<th>SHR-S (n=12)</th>
<th>SHR-I (n=10)</th>
<th>SHR-P (n=11)</th>
<th>WKY-S (n=12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ΔSBP-AI (mm Hg)</td>
<td>57.4±5.3</td>
<td>51.2±6.1*</td>
<td>0.7±0.6‡</td>
<td>50.3±3.8</td>
</tr>
<tr>
<td>Plasma</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PRA (ng Ang I/ml/hr)</td>
<td>4.60±1.22</td>
<td>0.23±0.02‡‡</td>
<td>11.09±2.38‡†</td>
<td>5.79±1.77</td>
</tr>
<tr>
<td>ACE (nmol/ml)</td>
<td>40.8±4.5‡</td>
<td>44.0±2.5‡</td>
<td>18.0±3.0‡</td>
<td>62.6±9.3</td>
</tr>
<tr>
<td>Urinary</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B (ng/24 hr)</td>
<td>730±48</td>
<td>557±37‡†</td>
<td>918±55‡†</td>
<td>647±36</td>
</tr>
<tr>
<td>Aldo (ng/24 hr)</td>
<td>8.4±0.9‡</td>
<td>1.3±0.2‡‡</td>
<td>6.4±0.9‡</td>
<td>14.5±1.0</td>
</tr>
<tr>
<td>DOC (ng/24 hr)</td>
<td>0.9±0.1†</td>
<td>0.6±0.1*†‡</td>
<td>1.0±0.1‡</td>
<td>0.5±0.1</td>
</tr>
<tr>
<td>19-nor-DOC (ng/24 hr)</td>
<td>7.6±1.1</td>
<td>8.2±1.3</td>
<td>7.4±0.4</td>
<td>9.0±0.8</td>
</tr>
</tbody>
</table>

Values are mean±SEM. SHR-S, sham-immunized spontaneously hypertensive rats (SHR); SHR-I, immunized SHR; SHR-P, perindopril-treated SHR; WKY-S, sham-immunized Wistar-Kyoto rats; ΔSBP-Ang I, systolic blood pressure response to angiotensin I; PRA, plasma renin activity; ACE, angiotensin converting enzyme; B, corticosterone; Aldo, aldosterone; DOC, deoxycorticosterone; 19-nor-DOC, 19-nor-deoxycorticosterone. *p<0.05 vs. SHR-P; †p<0.05 vs. SHR-S; ‡p<0.05 vs. WKY-S.
tered after either immunization or ACE inhibition. Left ventricular weight was significantly higher in SHR-S than in WKY-S rats. When compared with SHR-S, it was significantly decreased in SHR-I and even more in SHR-P. In addition, when considering the four groups of rats, the ventricular weight was found positively related \( r=0.71, n=45, p<0.001 \) to the SBP level observed as a mean during the 24 hours. The kidney weight did not differ between SHR-S and WKY-S rats and was not changed by either immunization or ACE inhibition. It is noteworthy that indirect SBP values were in good accordance with the 24-hour average direct values, used as a reference, in SHR-I, SHR-P, and WKY-S rats. However, in SHR-S indirect values were, as a mean, 34 mm Hg above those given by the aortic catheter.

**Cardiac Baroreceptor Reflex Sensitivity**

As shown by Figure 2, the cardiac baroreceptor reflex sensitivity measured after phenylephrine injection was significantly lower in SHR-S than in WKY-S rats. Immunization against renin slightly and ACE inhibition significantly increased baroreceptor reflex sensitivity in SHR to reach values that did not differ from that of WKY-S rats. Cardiac baroreceptor reflex sensitivity was assessed functionally and biochemically. As shown by Figure 3, the absolute decrease in SBP induced by hexamethonium did not differ between SHR-S and WKY-S rats and was significantly decreased in SHR-I and even more in SHR-P. The absolute SBP response to phenylephrine injection \( (3 \mu g/kg \text{ i.v.}) \) used for baroreceptor reflex sensitivity determination was higher in SHR-S than in WKY-S rats and was significantly decreased in SHR-I and even more in SHR-P. When expressed as percentage changes from the initial blood pressure level, these variations did not differ between the different groups. However, the absolute responses and, to a lesser extent, the percentage changes induced by hexamethonium and phenylephrine were significantly correlated in the four groups of rats.

When considering the urinary excretion of catecholamines and methoxylated metabolites (Figure 4), SHR-S differed from WKY-S rats by a slight increase in urinary norepinephrine associated with a decrease in blood pressure did not differ between SHR-S and WKY-S rats and was not significantly enhanced by either immunization or ACE inhibition.

### Sympathetic Nervous System Activity

The SNS activity, which could counterbalance that of the RAS, was assessed functionally and biochemically. As shown by Figure 3, the absolute decrease in SBP induced by hexamethonium did not differ between SHR-S and WKY-S rats and was significantly decreased in SHR-I and even more in SHR-P. The absolute SBP response to phenylephrine injection \( (3 \mu g/kg \text{ i.v.}) \) used for baroreceptor reflex sensitivity determination was higher in SHR-S than in WKY-S rats and was significantly decreased in SHR-I and SHR-P compared with SHR-S. When expressed as percentage changes from the initial blood pressure level, these variations did not differ between the different groups. However, the absolute responses and, to a lesser extent, the percentage changes induced by hexamethonium and phenylephrine were significantly correlated in the four groups of rats.

When considering the urinary excretion of catecholamines and methoxylated metabolites (Figure 4), SHR-S differed from WKY-S rats by a slight increase in urinary norepinephrine associated with a decrease

### Table 3. Cardiovascular Parameters

<table>
<thead>
<tr>
<th>Variables</th>
<th>SHR-S ( (n=12) )</th>
<th>SHR-I ( (n=10) )</th>
<th>SHR-P ( (n=11) )</th>
<th>WKY-S ( (n=12) )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Indirect SBP (mm Hg)</td>
<td>245±5*</td>
<td>173±2†</td>
<td>162±4‡</td>
<td>170±4</td>
</tr>
<tr>
<td>24-hour average</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SBP (mm Hg)</td>
<td>211±6*</td>
<td>173±7‡</td>
<td>153±2†</td>
<td>163±3</td>
</tr>
<tr>
<td>DBP (mm Hg)</td>
<td>140±4*</td>
<td>112±6†</td>
<td>98±3‡</td>
<td>108±3</td>
</tr>
<tr>
<td>HR (beats/min)</td>
<td>311±6</td>
<td>298±8*‡</td>
<td>324±5</td>
<td>321±3</td>
</tr>
<tr>
<td>Left ventricular weight (mg/100 g body wt)</td>
<td>321±8*</td>
<td>261±3*‡</td>
<td>250±4†</td>
<td>249±3</td>
</tr>
</tbody>
</table>

Values are mean±SEM. SHR-S, sham-immunized spontaneously hypertensive rats (SHR); SHR-I, immunized SHR; SHR-P, perindopril-treated SHR; WKY-S, sham-immunized Wistar-Kyoto rats; SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate. *\( p<0.05 \) vs. WKY-S; †\( p<0.05 \) vs. SHR-S; ‡\( p<0.05 \) vs. SHR-P.
in normetanephrine leading to a significant decrease in the normetanephrine/norepinephrine ratio (1.94±0.14 in SHR-S versus 2.89±0.24 in WKY-S rats, p<0.01). In addition, SHR-S exhibited a higher dopamine excretion than WKY-S rats. In SHR-I, the urinary excretion of normetanephrine was higher than in SHR-S. In SHR-P, a pronounced increase in both urinary normetanephrine and 3-methoxy-4-hydroxyphenylglycol excretions was observed and was associated with a profound decrease in the excretion of dopamine. The normetanephrine/norepinephrine ratio was slightly enhanced in SHR-I (2.57±0.57, p=NS) and significantly elevated in SHR-P (3.74±0.35, p<0.001) when compared with SHR-S (1.94±0.14). Finally, there was a significant inverse relation between 24-hour average SBP and urinary normetanephrine in the three groups of SHR (r=-0.50, n=33, p<0.01).

**Discussion**

The present work was devoted to comparing in conscious, freely moving SHR the effects of a chronic RAS inhibition by 1) active antirenin immunization, which selectively blocks the circulating and renal RAS and 2) ACE inhibition, which may be less specific, as it has been reported to interfere with other neurohumoral systems such as kinins and prostaglandins.1-3 Our results demonstrate that active immunization against renin induced potent cardiovascular effects that are similar to those of

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**Figure 3.** Bar graphs showing hexamethonium- and phenylephrine-induced changes (upper panels) in systolic blood pressure (ΔSBP) expressed as absolute or as percentage changes from the initial blood pressure level, and plots showing relations (lower panels) between systolic blood pressure changes induced by hexamethonium (ΔSBP-HEXA) and phenylephrine (ΔSBP-PHE) in sham-immunized (SHR-S), antirenin immunized (SHR-I), and perindopril-treated (SHR-P) spontaneously hypertensive rats (SHR) and sham-immunized Wistar-Kyoto (WKY) (WKY-S) rats. *p<0.05 vs. SHR-S; †p<0.05 vs. WKY-S; ‡p<0.05 vs. SHR-P.

**Figure 4.** Bar graphs showing the free urinary catecholamines norepinephrine (NE), epinephrine (E), and dopamine (DA) and their respective methoxylated derivatives normetanephrine (NMN), metanephrine (MN), 3-methoxytyramine (MT) and total 3-methoxy-4-hydroxyphenylglycol (MHPG) in sham-immunized (SHR-S), antirenin immunized (SHR-I), and perindopril-treated (SHR-P) spontaneously hypertensive rats (SHR) and sham-immunized Wistar-Kyoto (WKY-S) rats. *p<0.05 vs. SHR-S; †p<0.05 vs. WKY-S; ‡p<0.05 vs. SHR-P.
ACE inhibition with the exception of urinary mineralocorticoids, which were decreased after immunization but not after ACE inhibition. This finding indicates that most of the antihypertensive properties of ACE inhibitors depends on the blockade of circulating and renal RAS.

The study was conducted using the protocol of active immunization developed by one of us. Pure murine renin, which is close to rat renin, was used, and high titers of antirenin antibodies were obtained in all the animals. As a consequence, PRA was reduced 20-fold in all the rats but one, in which the PRA remained in the lower normal range while its blood pressure was reduced. Plasma ACE activity, which was lower in SHR-S than in WKY-S rats, was not changed by immunization, suggesting that there is no feedback inhibition of plasma ACE by angiotensin I concentration. Because no relation could be disclosed between the antibodies titer and PRA or SBP or the left ventricular weight, it can be suggested that the RAS was almost totally blocked by the immunization procedure. ACE inhibition was achieved using perindopril. Its structural formula is devoid of -SH function, and we have previously shown that, at the doses of 1 or 2 mg/kg/day per os, perindopril exhibits potent and long-lasting antihypertensive properties in SHR. Higher doses (10 mg/kg/day) have been reported to produce more pronounced effects in stroke-prone SHR. However, as the blood pressure of SHR-P was slightly lower than that of WKY-S rats, it can be hypothesized that all of the contribution of the RAS to blood pressure elevation in SHR-S was blocked in our experiment. The efficiency of ACE inhibition was demonstrated in SHR-P by the absence of pressor response to angiotensin I injection. Interestingly, plasma ACE activity was only partly decreased (−56% from SHR-S values). This discrepancy cannot be explained by a longer duration of blockade of tissue ACE than of plasma ACE as angiotensin I was injected at the end of the afternoon and blood was drawn at the end of the morning (i.e., closer to the time [nighttime] during which the rats drank water containing perindopril). Therefore, it seems more likely that plasma ACE values, which have been shown to depend on the substrate used for their determination, are a questionable index of tissue ACE blockade.

The different groups of SHR were compared with one group of normotensive WKY control rats. All the animals received Freund's adjuvant according to the schedule used for active antirenin immunization, to avoid the influence of pain and of unspecific enhancement of immunological response. Blood pressure was measured in conscious rats using either an indirect plethysmographic technique after preheating or our direct computerized method. Plethysmography indicated that, 3 weeks after the third antirenin immunization or 3 weeks after starting the perindopril treatment, SBP of SHR was at the same level as WKY-S rats. Using the direct measurement, it was confirmed that 24-hour average blood pressure had returned to normal after RAS blockade. Interestingly, the SBP values given by the indirect and the direct techniques were quite similar in SHR-I, SHR-P, and WKY-S rats, whereas in SHR-S indirect plethysmographic values were 34 mm Hg higher than those directly recorded. This suggests that the blood pressure responses to preheating or to the accompanying stress are higher in SHR-S than in WKY-S rats, as previously shown by Ferrari et al and that such a difference disappears after RAS inhibition. The 24-hour spontaneous variability of blood pressure and heart rate did not differ between SHR-S and WKY-S rats and was not modified by the treatments. This finding accords well with our previous observation that blood pressure variability is controlled by the SNS but not by the RAS. Finally, the perindopril-induced decrease in blood pressure was associated with a nonsignificant increase in heart rate, as previously described, and antirenin immunization induced a slight bradycardia.

The cardiac baroreceptor reflex sensitivity measured after phenylephrine injection was lower in SHR-S than in WKY-S rats, which is in accordance with other reports in SHR as well as in genetically hypertensive rats of the Lyon strain. After immunization against renin, cardiac baroreceptor reflex sensitivity was not significantly increased but was no longer different from that of WKY-S rats. ACE inhibition induced its previously described significant increase in cardiac baroreceptor reflex sensitivity measured after blood pressure elevations. When measured after nitroglycerin injections, the cardiac baroreceptor reflex sensitivity did not differ between SHR-S and WKY-S rats and was unaltered by RAS blockade. These data suggest that ACE inhibitors, more than antirenin immunization, reduce the generation of angiotensin II within the central nervous system, where it specifically depresses cardiac baroreceptor reflex responses to increases, but not to decreases, in blood pressure. However, it has been recently shown that brain ACE was not consistently decreased by most of the ACE inhibitors. In addition, antirenin immunization also tended to increase the baroreceptor reflex sensitivity. Therefore, the differences observed between SHR-I and SHR-P could be due to the lower SBP of SHR-P, which allows in the measurement of the baroreceptor reflex sensitivity a greater proportion of the linear steep part of the relation between SBP and heart period to be studied.

When considering the 24-hour urinary excretion of steroids, SHR-S differed from WKY-S rats by an elevated DOC associated with a decreased aldosterone excretion, which is similar to what we previously reported in genetically hypertensive rats of the Lyon strain. The effects of the two methods used for blocking the RAS were strikingly different. ACE inhibition increased the urinary excretion of corticosterone and did not change that of aldosterone, DOC, and 19-nor-DOC, whereas immunization against renin significantly decreased the urinary
excretion of all the steroids studied, with the exception of 19-nor-DOC. Such a lack of significant effect of ACE inhibition on urinary steroids has already been reported by others. The reduction in urinary steroids exhibited by SHR-I cannot be related to a possible renal impairment as the urinary excretion of 19-nor-DOC was found to be normal in SHR-I. Therefore, the most likely explanation for the differences observed between SHR-P and SHR-I is that oral ACE inhibition does not block angiotensin II formation throughout the 24-hour period, whereas antirenin immunization induces a permanent blockade. This hypothesis is supported by the finding of persisting plasma levels of angiotensin II in ACE-inhibited rats which can maintain the adrenal synthesis, whereas after immunization plasma angiotensin II is undetectable.

To assess the SNS activity in conscious rats, we measured the urinary excretion of catecholamines and their methoxylated metabolites, which are an index of the overall sympathetic drive during the urine collection period. In accordance with others, we observed that adult SHR-S did not exhibit a higher SNS activity than WKY-S rats. Even more, the urinary excretion of normetanephrine that we have shown to be a close index of the norepinephrine release in the cardiovascular system was lower in SHR-S than in WKY-S rats. Inhibition of the RAS was associated with increases in urinary normetanephrine, 3-methoxy-4-hydroxyphenylglycol, and the normetanephrine/norepinephrine ratio, which were more pronounced in SHR-P than in SHR-I. These data indicate that, despite the suppressed facilitatory action of angiotensin II on norepinephrine release, there is an elevated SNS activity that may be induced by and tend to oppose the blood pressure decrease as suggested by the significant inverse relation found between the SBP level and urinary normetanephrine. In addition, a striking decrease in the urinary excretion of dopamine was observed in SHR-P. Because urinary free dopamine is mainly of renal origin, where it exerts natriuretic properties, such a decrease could be compensatory for the sodium loss that is induced by ACE inhibition. The absolute SBP responses to the acute suppression of SNS activity induced by hexamethonium were reduced in SHR-I and SHR-P compared with SHR-S. When these responses were expressed as percentage changes from the preinjection SBP level, no difference could be discerned between SHR-I, SHR-P, and SHR-S. This indicates that the degree of SNS control of blood pressure is the same in the three groups of rats. However, if the SBP level was the major determinant of the responses to acute manipulations of the SNS, one would have expected an enhancement of the pressor effects of phenylephrine. On the contrary, the absolute but not the relative SBP increases induced by phenylephrine were reduced in SHR-I and SHR-P compared with SHR-S. In addition, because the SBP responses to hexamethonium and phenylephrine were significantly correlated, even when they were expressed as percentages, it can be proposed that they reflect decreases in the contractile mass of the whole cardiovascular system. This hypothesis is strengthened by the reduction in left ventricular weight observed in SHR-I and SHR-P, which correlated with the SBP level and could be explained by the blockade of the growth factor-like action of angiotensin II.

Therefore, it appears that both specific antirenin immunization and ACE inhibition exert similar and potent actions on blood pressure and SNS. The slight differences observed between these two procedures are difficult to interpret as one cannot define precisely a dose–response curve for active immunization. However, it must be emphasized that we used a nearly maximal effective dose of perindopril and that antirenin immunization may also produce a nearly maximal effect as suggested by the lack of relation between the titer of circulating antibodies and the blood pressure effects. In the present study, we have no data that allow a direct exclusion of the hypothesis that the antihypertensive effect of ACE inhibition could have involved not only the suppression of the circulating and renal RAS but also that of tissue RAS or could have enhanced vasodilatory mechanisms such as bradykinin or prostaglandins. However, these mechanisms remain speculative. There is no evidence showing that chronic ACE inhibition actually enhances the plasma concentration or the urinary excretion of bradykinin. In addition, the lack of a long-acting competitive inhibitor of bradykinin makes it impossible to address this issue in chronic situations. The involvement of vasodilatory prostaglandins such as prostacyclin remains also questionable. Indomethacin, which blocks the cyclooxygenase controlling the synthesis of all the primary prostaglandins, does not significantly modify the antihypertensive properties of captopril and we found (unpublished observations made with C. Richer) that a chronic oral treatment with perindopril (3 mg/kg/24 hours for 1 week) did not alter the urinary excretion of the stable metabolites of prostacyclin and thromboxane in SHR. Finally, the recent demonstration by Levens et al that a thromboxane synthase inhibitor enhances the action of ACE inhibitors, although it does not potentiate that of a renin inhibitor, suggests that if ACE inhibition differs from direct renin inhibition it will lead to an increased synthesis of vasoconstrictor thromboxane, which is unlikely to enhance the antihypertensive properties of ACE inhibitors.

In conclusion, the present study demonstrates that, in chronic situations, the cardiovascular effects of ACE inhibitors are basically identical to those of an active immunization against renin in freely moving SHR. Therefore, even if ACE inhibitors do not suppress continuously circulating angiotensin II, as suggested by a maintained steroids synthesis, even if one cannot totally exclude their interference with bradykinin or prostaglandins, it can be concluded that the chronic antihypertensive properties of ACE inhi-
bition are mainly due to the functional blockade of circulating and renal RAS.

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