Physiological and Immunopathological Consequences of Active Immunization of Spontaneously Hypertensive and Normotensive Rats Against Murine Renin

Jean-Baptiste Michel, Soumia Sayah, Catherine Guettier, Jurg Nussberger, Monique Philippe, Marie-Françoise Gonzalez, Claude Carelli, François-Xavier Galen, Joël Menard, and Pierre Corvol

Spontaneously hypertensive Okamoto-strain rats (SHR) and normotensive Wistar-Kyoto (WKY) rats were actively immunized with mouse renin to investigate the effect on blood pressure of blocking the renin-angiotensinogen reaction. Ten male SHR and 10 male WKY rats were immunized with purified mouse submandibular gland renin. Control rats were immunized with bovine serum albumin. Antirenin antibodies were produced by both SHR and WKY rats, but renin-immunized SHR had higher titers of circulating renin antibodies after three injections. The increase in renin antibody in renin-immunized SHR was associated with a significant drop in blood pressure (tail-cuff method) that became similar to that of the WKY control rats after four injections. The blockade by antirenin immunoglobulins of the renin-angiotensinogen reaction also decreased the blood pressure of normotensive rats. Perfusion of renin-immunized rats with mouse submandibular renin (10 μg) in vivo caused no increase in blood pressure. Perfusion of renin-immunized, salt-depleted SHR with converting enzyme inhibitor caused no further decrease in blood pressure but significantly decreased blood pressure in salt-depleted control rats. The presence of circulating renin antibodies was associated with low plasma renin activity (0.31 ± 0.23 ng angiotensin I [Ang I]/ml/hr). Plasma renin activity was unchanged in control animals (13.1 ± 3.9 ng Ang I/ml/hr in control SHR, 13.9 ± 3.2 ng Ang I/ml/hr in control WKY rats). Renin antibody–rich serum produced a dose-dependent inhibition of rat renin enzymatic activity in vitro. The chronic blockade of the renin-angiotensinogen reaction in renin-immunized SHR produced an almost-complete disappearance of Ang II (0.8 ± 0.7 fmol/ml; control SHR, 30.6 ± 15.7 fmol/ml) and a 50% reduction in urinary aldosterone. Renin immunization was never associated with a detectable loss of sodium after either 10 or 24 weeks. The glomerular filtration rate was not decreased 10 weeks after renin immunization, whereas blood pressure was significantly decreased, plasma renin activity was blocked, and renal plasma flow was increased. The ratio of left ventricular weight to body weight after 24 weeks was significantly below control levels in renin-immunized WKY rats and SHR. Histological examination of the kidney of renin-immunized SHR showed a chronic autoimmune interstitial nephritis characterized by the presence of immunoglobulins, mononuclear cell infiltration, and fibrosis around the juxtaglomerular apparatus. These experiments demonstrate that chronic specific blockade of renin decreases blood pressure in a genetic model of hypertension in which the renin-angiotensin system is not directly involved. It also offers a model for comparing chronic renin inhibition with other methods of blocking the renin-angiotensin system in the hypertensive rat. (Circulation 1990;81:1899–1910)
inhibitor peptides. However, the synthetic inhibitors designed so far mainly inhibit primate and human renin and are much less potent toward rat renin. They are also relatively short acting and must be administered intravenously, which is why they have been used in short-term experiments in primates and humans. The renin-angiotensin system may, alternatively, be inhibited by active immunization against renin. There have been reports on the effect of active immunization against renin in several species, particularly using hog renin in dogs and human renin in primates. However, interpretation of these early studies was limited by the use of unpurified renin, the absence of hormonal assays throughout the experiment, the lack of definition of renin species specificity, and the absence of immunological memory in these experimental studies. Michel et al recently reported that the active immunization of marmosets against pure human renin resulted in suppression of plasma renin activity that was associated with a chronic decrease in blood pressure and an organ-specific autoimmune disease of the kidney. We have now actively immunized spontaneously hypertensive rats of the Okamoto strain (SHR) against mouse submandibular gland renin to obtain a specific and chronic blockade of renin enzymatic activity in vivo. Blockade of the renin-angiotensin system by converting enzyme inhibitors decreases blood pressure in this genetic hypertensive strain to a level near to that of Wistar-Kyoto normotensive rats (WKY), demonstrating the role of the renin-angiotensin system in this model. But the specificity of this pharmacological approach is still questionable since converting enzyme inhibitors have other effects, such as preventing bradykinin degradation, which may lead to an increase in kinins and a decrease in blood pressure.

Methods

Biological Model

Two experimental protocols were used with murine renin immunization (R) of male SHR (S) and normotensive WKY rats (W). In the first experiment (1), the effect of active immunization of SHR against murine renin (RS 1) was compared with immunization of control SHR (AS 1) and WKY rats (AW 1) with bovine serum albumin. In the second experiment (2), the same protocol of renin immunization was used with two other groups of rats—SHR (RS 2) and WKY (RW 2).

Male SHR and WKY rats (7 weeks old and weighing 200 g) were purchased fromIFFA CREDO (Lyon, France) and allowed to acclimatize to the laboratory for 2 weeks. The SHR rats were assigned to one of two groups: 10 hypertensive animals were immunized with pure mouse renin (renin-immunized SHR) (RS 1); and the 10 remaining hypertensive (AS 1) and the 10 normotensive rats (AW 1) were given bovine serum albumin as a control immunogen. In the second experiment, 15 SHR (RS 2) and 15 WKY rats (RW 2) were immunized against renin. Fifteen SHR (AS2) and 15 WKY (AW 2) were immunized against bovine serum albumin.

Mouse renin was extracted from the submandibular gland of male Swiss mice and purified to homogeneity by pepstatin affinity chromatography. Homogeneity of the enzyme was demonstrated by the presence of a single protein band on sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Groups RS and RW were given an initial injection of 10 µg renin in complete Freund's adjuvant. The second and third injections of 30 µg renin in incomplete Freund's adjuvant were given at 3-week intervals. Two similar injections were given 8 and 16 weeks later. All injections were given in the foot pad. The control SHR (AS 1 and 2) and control WKY rats (AW 1 and 2) had identical immunization protocols but were given bovine serum albumin (Sigma) instead of renin. Systolic blood pressure was recorded once a week in unanesthetized animals by the tail-cuff method (blood pressure recorder model 8005, W+W Electronic, Bagneux, France; 1 mm Hg=133 Pa). Blood samples were taken from the jugular vein under ether anesthesia. Ether anesthesia, which activates the renin-angiotensin system, was used for repeated anesthesia. Inactin was used as anesthesia for lengthy procedures, such as in clearance experiments. This type of anesthesia did not induce major renin-angiotensin or hemodynamic changes.

Blockade of the renin-angiotensinogen reaction in immunized and control animals was tested in vivo by recording the maximal changes in mean blood pressure via an arterial catheter under anesthesia after a bolus intravenous injection of a large excess of pure mouse submandibular gland renin (10 µg) and 10 µg angiotensin I (Ang I). To verify the absence of blood pressure effect of converting enzyme inhibition after renin immunization, 2 mg/kg body wt Lysinopril was infused into six sodium-depleted (1 week of low salt intake) control SHR and six sodium-depleted, renin-immunized animals.

In the second protocol (2), 10 renin-immunized rats (RS-RW) and 10 control rats (AS-AW) were placed in metabolic cages 1 week after the third injection of immunogen (week 10). The animals were acclimatized in individual metabolic cages for 1 day before starting a 24-hour urine collection at 10:00 AM. Food and water intakes, urinary volume, and urinary Na⁺ and K⁺ concentrations were measured daily for 4 successive days. The urinary concentrating ability of the kidneys was tested by keeping the rats in metabolic cages for 24 hours without any water.

Five rats from each group were then used for clearance experiments at week 10. Rats were anesthetized by intraperitoneal injection of Inactin (10 mg/100 g body wt) and placed on a heated table to keep the body temperature at 37°C. The right femoral artery, the right femoral vein, and the two ureters were catheterized with PE-10 catheters (Intramedic, Adam). The animals were perfused
throughout the experiments with a 0.9% NaCl solution containing 0.21 μCi/ml NaCl [14C]inulin (Molecules Marquées, Centre d’Etudes Nucléaires, Saclay, France), 1.67 μCi/ml [3H]p-aminohippuric acid (PAH; New England Nuclear, Boston, Massachusetts), and 2 mg/ml Na-p-aminohippurate at a rate of 100 μl/min. After an equilibration period of 1 hour, three consecutive 10-minute control clearance periods were completed. The animals used in the clearance study were killed. Kidneys were removed, weighed, fixed in Duboscq-Brazil solution, and frozen for histological examination. The remaining 10 animals in each group were again placed in metabolic cages on the 24th week and monitored as before (week 10).

In Vitro Experiments

The presence of renin antibodies was determined by the ability of rat plasma or kidney extract to bind iodinated mouse submandibular gland renin. The titer was defined as the dilution of plasma or extract able to bind 50% of the renin tracer. The ability of rat renin plasma antibodies to inhibit the enzymatic activity of exogenous rat renin in vitro was tested by incubating control rat plasma at 4°C for 48 hours with serial dilutions of renin-immunized rat plasma at pH 7.5.

Plasma renin activity was determined as the quantity of Ang I, measured by radioimmunoassay, generated by a 1-hour incubation (37°C) of 250 μl of rat plasma diluted 1:1 in phosphate buffer, pH 7.5.22 On one pooled sample, the effectiveness of the blockade was tested by measuring the quantity of Ang I generated after 180 minutes. Renal renin content was determined by measuring renin activity in the presence of excess rat substrate after extraction, as previously described.23 Protein was measured according to Lowry et al.24 Sodium was measured by an electrolyte Beckman analyzer. Enzymuria was checked by determining γ-glutamyl transferase (γ-GT), lactate dehydrogenase (LDH), and alkaline phosphatase in urine by colorimetry (test kits, Boehringer, Mannheim, FRG). Plasma urea was determined by urease hydrolysis and plasma creatinine by the method of Jaffe (urea and creatinine combination test, Boehringer, Mannheim, FRG). Urinary osmolarity was measured with a Fiske osmometer.

Plasma Ang II was measured as previously described.25 Briefly, a blood sample (2 ml) was taken from each animal under ether anesthesia and put into cold (4°C) tubes containing angiotensinase inhibitors. The samples were immediately centrifuged, and the plasma was quick-frozen in liquid nitrogen. Angiotensinogen II was extracted on a phenylisyl-silica cartridge (Bond Elut, Analytichem, Harbor City, California), eluted with methanol, isolated by high-performance liquid chromatography, and measured by radioimmunoassay. Plasma angiotensinogen (renin substrate) was determined by direct radioimmunoassay specific for rat angiotensinogen.26 Urinary free aldosterone was measured by radioimmunoassay after extraction with CH2Cl2.27 Urinary prostaglandin (PE)G, was measured by radioimmunoassay (kit, Amersham, UK) after solid-phase extraction (Amrep minicolumn, Amersham).

Standard Microscopy

Kidney, heart, and aorta samples were fixed in Duboscq-Brazil solution and snap-frozen in liquid nitrogen. Tissues were dehydrated, embedded in paraffin, and cut into 5–6-μm sections. Kidney sections were stained with Masson's trichrome. Immunostaining for renin was performed with rabbit antirat submandibular gland renin antibody28 on fixed, deparaffinized sections by the peroxidase-antiperoxidase technique (Biolyon PAP kit, Lyon, France) and on cryostat sections by indirect IgG using a fluorescein-labeled goat anti-rabbit immunoglobulin antibody (Institut Pasteur Production, Paris).

Direct immunofluorescence was performed on cryostat sections with fluoresceinated rabbit anti-rat IgG and anti-rat fibrinogen antisera (Nordic Tebu, The Netherlands). Slides were examined under a Leitz fluorescence microscope.

Statistical Methods

Values are given as mean±SD. One-way and two-way analysis of variance (ANOVA) were used for comparisons of blood pressure, body weight, and plasma parameters of control and renin-immunized animals.

Results

Effect of Renin Immunization on Blood Pressure and Hormonal Parameters

Protocol 1. Immunization of SHR with mouse submandibular gland renin was followed quickly by the appearance of renin antibodies in the plasma (Figure 1A). The titer of circulating antibodies reached a plateau of more than 1:10,000 dilution after the third injection. This high titer was obtained on week 9 and was associated with a significant decrease in blood pressure (Figure 1B), which was not significantly different from that of normotensive control rats after the fourth boost. At the same time, plasma renin activity decreased markedly, whereas it did not change in control SHR (Figure 1C). There was no significant difference in plasma renin activities, sampled under ether anesthesia, between control SHR and control WKY rats. No Ang I was produced in vitro after incubation of plasma from renin-immunized SHR for as long as 180 minutes at 37°C, pH 7.5, whereas the production of Ang I was linear with incubation time in control rats. A large excess of submandibular gland renin (20×10⁻³ Goldblatt units) generated no Ang I in the SHR plasma containing blocking antibodies; thus, plasma angiotensinogen could not be measured by the indirect method.28 The presence of antibodies inhibiting rat renin in plasma from renin-immunized SHR was further tested by their ability to block Ang I production in plasma from control rats (Figure 2). The specificity of the renin-angiotensinogen blockade by
FIGURE 1. Plots of effect of active immunization against murine renin in spontaneously hypertensive rats (SHR). Arrows indicate the times of immunization and booster injections. Panel a: Time-dependent appearance of mouse submandibular gland renin antibodies in the plasma of renin-immunized SHR. At the time of death, these immunoglobulins were identified by enzyme-linked immunoassay as predominant IgG_2a isotypes— IgM and IgA isotypes were undetectable (plasma protein antisera, Nordic Immunology, The Netherlands). Panel b: Changes in the systolic blood pressure in renin-immunized SHR (○—○) compared with hypertensive (●—●) and normotensive control rats (►—►). Systolic blood pressure in renin-immunized SHR did not significantly differ from that of Wistar-Kyoto control rats after the 16th week of the experiment (Dunnett’s test: t<1.5, p=NS). Panel c: Change in plasma renin activity in renin-immunized SHR (■) and control SHR (■).
active renin immunization was also tested in vivo by studying the effect of injecting renin-immunized SHR, control SHR, and normotensive control rats with exogenous ANG I or mouse submandibular gland renin (Table 1). Large excesses of renin and Ang I produced similar changes in mean arterial blood pressure in nonimmunized animals. The renin-induced change in the arterial blood pressure of immunized animals was significantly less than that in control animals \( (p<0.001) \), whereas Ang I was still able to increase blood pressure by 106±23 mm Hg \( (p<0.001) \) (two-way ANOVA, \( F=62.8, p<0.001 \)). Furthermore, converting enzyme inhibitor significantly decreased blood pressure in anesthetized sodium-depleted control SHR, whereas it did not significantly change the blood pressure of sodium-depleted, renin-immunized SHR (Table 2).

The hormonal effects of chronic immunization against renin were evaluated at 18 weeks (Table 3). Plasma Ang II was almost undetectable, but there was still a significant positive linear correlation between plasma renin activity and plasma Ang II \( (r=0.97, p<0.001) \). Plasma angiotensinogen (direct assay) was significantly increased by chronic renin immunization. Urinary aldosterone fell by ~50%. Plasma urea was significantly higher in renin-immunized rats than in control rats \( (p<0.001) \). There were no differences in the plasma protein or plasma sodium levels in control and renin-immunized SHR. At the time of death (week 24), kidney extracts of renin-immunized SHR contained antirenin antibodies, and the renal renin content was significantly increased, despite the presence of renin antibodies (Table 4).

**Protocol 2.** The efficiency of renin immunization in blocking the renin-angiotensin reaction in SHR prompted an examination of the effect of this procedure on normotensive rats and its consequences for sodium handling in both normotensive and hypertensive animals. The quality and duration of the immunological response to renin immunization of the two strains differed. The identical renin immunization protocol blocked the renin substrate reaction to a lesser extent in WKY rats (RW) than in SHR (RS), and the tendency for escape from immunization was more pronounced in RW than in RS. For example, 1 week after the third boost, the plasma renin activity was 0.211±0.02 ng Ang I/ml/hr in renin-immunized SHR and 0.40±0.21 ng Ang I/ml/hr in renin-immunized WKY rats, whereas the renin antibody titers were 1:88,000±1:19,300 and 1:86,000±1:21,000, respectively. Two weeks later, plasma renin activity was 0.31±0.23 ng Ang I/ml/hr in renin-immunized SHR and 2.34±2.4 ng Ang I/ml/hr in renin-immunized WKY rats, whereas the renin antibody titers were 1:55,000±1:31,500 and 1:44,000±1:14,700, respectively two-way ANOVA: time effect on plasma renin activity \( 1 \text{ vs. 3 weeks after boost}, F=5.67, p=0.023 \); strain effect [SHR vs. WKY rats], \( F=6.77, p=0.013 \); interaction: \( F=4.56, p=0.04 \). The effects of renin-immunization on normotensive and hypertensive rats are shown in Figure 3. Renin immunization decreased the absolute values of blood pressure in both strains \( F=368, p<0.0001 \), but the hypotensive effect was less marked in the normotensive rats than in the hypertensive strain \( F=120, p<0.0001 \).

**Sodium Excretion and Renal Function (10th Week)**

Salt homeostasis proteinuria, enzymuria, renal urinary concentrating ability, and renal clearances were

![Figure 2](image-url)
measured after the third boost to determine whether
the significant decreases in blood pressure in the two
strains were mainly due to the blockade of renin-
substrate reaction or to salt loss due to autoimmune
nephrropathy. There was no difference between the
salt excretion of renin-immunized rats and control
rats of either strain (SHR or WKY) \( (F=0.41, p=NS) \).
The cumulative sodium balances (salt intake minus
salt excretion during 4 days) of renin-immunized and
control animals were also similar \( (F=1.6, p=NS) \)
(Table 5). Urinary excretion of proteins was not
modified by strain or by renin immunization. Daily
excretions of \( \gamma \)-GT and LDH were significantly lower
in renin-immunized SHR than in control SHR and
were not affected by renin immunization in WKY
rats. Alkaline phosphatases were undetectable in all
four groups.

There was no difference between strains and with
immunization in the ability of the kidney to concen-
trate urine during 24 hours of water deprivation.
Plasma creatinine was slightly increased in the renin-
immunized groups.

There were both immunization and strain effects
on renal plasma flow, but there was no interaction
between them (two-way ANOVA). Renal plasma
flow, evaluated by PAH clearance, was significantly
higher in renin-immunized rats than in control rats
\( (F=7.5, p<0.01) \) and higher in WKY rats than in
SHR \( (F=12.5, p<0.001) \) (Table 5) without interac-
tion. The glomerular filtration rate, measured by
inulin clearance, was not altered by renin immuniza-
tion \( (F=0.05, p=0.8) \) and was similar in both strains
\( (F=3.8, p=0.06) \). Urinary PGE\(_2\) excretion was
significantly higher in SHR than in WKY rats
\( (F=35.66, p<0.001) \). Renin immunization was also associated
with a significant decrease in urinary PGE\(_2\) excretion
\( (F=9.56, p<0.005) \).

**Sodium Excretion and Renal Function (24th Week)**

Like at the 10th week, there was no difference in
sodium excretion and cumulative sodium balance (6
days) between renin-immunized and control rats
\( (F=0.11, p=NS) \) of normotensive and hypertensive
strains at the end of the experimental period (Table
6). In contrast to the 10th week, blood urea nitrogen
and plasma creatinine values were significantly elev-
ated in renin-immunized animals.

**Organ Weights**

The mean body weight of the WKY rats was
slightly higher than that of the SHR throughout the
experimental period as well as at the time of death
\( (p<0.001) \). There was no difference in body weight
between renin-immunized and control rats \( (F=1.7,
p=NS) \) of either strain.

At the 10th week, there was no significant differ-
ence in kidney weights \( (F=1.8, p=NS) \), but the ratio
of left ventricular weight to body weight was already
significantly lower in renin-immunized rats \( (F=5.5,
p<0.05) \). At the 24th week, the left ventricular weight
of renin-immunized animals (Table 6) was signifi-
cantly lower than that of control rats \( (F=112.6,
p<0.001) \). The ratio of left ventricular weight to body
weight of renin-immunized SHR was not significantly
different from that of normotensive control rats.
There was an interaction between strain and immu-
nonization on this parameter \( (F=15, p<0.001) \); renin-
immunization decreased the ratio of left ventricular
weight to body weight in SHR more than in WKY
rats. The kidney weight of renin-immunized animals
was also lower than that of control rats \( (F=28.7,
p<0.001) \); this effect was most marked in renin-
immunized SHR \( (F=6.6, p<0.02) \).

<table>
<thead>
<tr>
<th>Table 3. Effect of Renin Immunization on Systolic Blood Pressure and Hormonal Parameters (18th Week) (Protocol 1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control SHR ( (n=10) )</td>
</tr>
<tr>
<td>-----------------------------------------------</td>
</tr>
<tr>
<td><strong>Systolic blood pressure (mm Hg)</strong> 186±10</td>
</tr>
<tr>
<td><strong>Plasma renin activity (ng Ang I/ml/hr)</strong> 14.31±3.92</td>
</tr>
<tr>
<td><strong>Plasma Ang II (pmol/ml)</strong> 30.55±15.67</td>
</tr>
<tr>
<td><strong>Plasma angiotensinogen (pmol/ml)</strong> 1.03±10</td>
</tr>
<tr>
<td><strong>Urinary aldosterone (ng/24 hr)</strong> 4.33±1.03</td>
</tr>
<tr>
<td><strong>Plasma urea (mg/ml)</strong> 0.36±0.03</td>
</tr>
<tr>
<td><strong>Plasma proteins (mg/ml)</strong> 68.1±3.2</td>
</tr>
<tr>
<td><strong>Plasma sodium (mEq/ml)</strong> 141±2</td>
</tr>
</tbody>
</table>

SHR, spontaneously hypertensive rats; Ang I and II, angiotensin I and II.
TABLE 4. Renal Renin Content and Renal Renin Antibodies in Renin-Immunized and Control Spontaneously Hypertensive Rats (Protocol 1)

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Renin-immunized SHR</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kidney weight (mg)</td>
<td>1,331±76</td>
<td>1,055±111</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Renal renin content (μg Ang I/mg protein)</td>
<td>2.64±1.13</td>
<td>8.16±4.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Antirenin titer (50% binding of iodinated renin)</td>
<td>0</td>
<td>1:1,850±1:550</td>
<td></td>
</tr>
</tbody>
</table>

SHR, spontaneously hypertensive rats; Ang I, angiotensin I.

Renal Histology

Controls. The kidneys of control SHR showed only occasional, mild concentric hyperplasia of arterioles and interlobular arteries (Figure 4A). Fixed deparaffinized sections immunostained for renin showed a few positive cells in the juxtaglomerular apparatus in about 10% of glomeruli. Antifibrinogen staining was negative in frozen sections. Immunostaining with anti-IgG antibody was negative in almost all controls.

Immunized rats. The myocardium and arterial walls of the thoracic aorta and the truncular renal artery of renin-immunized animals were microscopically normal and without detectable autoimmune disease.

Kidneys were examined at the 10th and 24th weeks of the experiment. The kidneys were macroscopically normal at the 10th week. The most striking histological feature was the presence of mononuclear, mainly monocytes or macrophages, cell infiltration around the juxtaglomerular apparatus and at the distal part of the preglomerular afferent arteriole. There was no vascular necrosis. Small foci of interstitial fibrosis with early tubular atrophy and numerous mononuclear inflammatory cells were regularly distributed under the capsule.

After 24 weeks, the surface of each kidney appeared macroscopically granular due to strips of cell-poor fibrosis centered on the interlobular arteries and associated with atrophic changes in the cortical tubules. The glomerular tufts in the fibrous areas were slightly retracted within the urinary space.

The perivascular infiltrates were still present but contained mostly lymphocytes. The fibrotic lesions involved less than one third of the renal cortex, and the medulla was normal.

Immunostaining of fixed, deparaffinized sections for renin demonstrated hyperplasia of renin-positive cells in nearly 25% of glomerular sections, which extended up the afferent arteriole to the interlobular artery. The degree of hyperplasia correlated roughly with the intensity of perivascular infiltrates and fibrosis (Figure 4C). Direct immunofluorescence on frozen sections revealed large extracellular deposits of IgG in the juxtaglomerular apparatus area and arteriolar walls of all glomeruli (Figure 4D). The IgG deposits were more abundant by the 24th week. Bowman's capsules and the tubular basement membranes in the vicinity of positive arterioles contained a few granular IgG deposits. Antirenin staining on frozen sections showed weak vascular positive responses in the same area as the IgG staining.

Discussion

Blockade of the Renin-Angiotensin Reaction

The enzymatic and immunological species specificity of renin must be kept in mind in all attempts to inhibit the enzymatic reaction with antibodies or synthetic renin inhibitors. Rat models of hypertension, particularly genetic models such as SHR of the Okamoto strain, provide accessible tools for studying the potential and limitations of chronic blockade of the renin-substrate reaction. The activity of the circulating and intrarenal renin-angiotensin system in this strain has been well documented. Circulating renin activity, renal renin content, and plasma aldosterone32 are slightly lower in mature SHR than in normotensive control rats. Nevertheless, although the signal (renin-angiotensin system) appears to be "normal" in mature SHR, the effector (smooth muscle cells) seems to be functional and structurally abnormal.33,34 The renin-angiotensin system is functional in spontaneously hypertensive animals, as in normotensive animals, and thus participates in maintaining the level of "high" or "normal" blood pres-
**TABLE 5. Cumulative Sodium Balance, Renal Mass, and Function**

<table>
<thead>
<tr>
<th>Control rats (SHR/WKY)</th>
<th>Renin-immunized rats (C/R)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Body weight (g)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WKY 361±48</td>
<td>375±29</td>
<td>NS</td>
</tr>
<tr>
<td>SHR 314±16</td>
<td>339±29</td>
<td></td>
</tr>
<tr>
<td><strong>Ratio of left ventricular to body weight (mg/g)</strong></td>
<td>&lt;0.05</td>
<td></td>
</tr>
<tr>
<td>WKY 2.52±0.36</td>
<td>2.19±0.11</td>
<td></td>
</tr>
<tr>
<td>SHR 3.06±0.26</td>
<td>2.79±0.27</td>
<td></td>
</tr>
<tr>
<td><strong>Kidney weight (mg)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WKY 1,160±60</td>
<td>1,130±85</td>
<td>NS</td>
</tr>
<tr>
<td>SHR 1,065±105</td>
<td>1,205±95</td>
<td></td>
</tr>
<tr>
<td><strong>PAH clearance (ml/min/g kidney)</strong></td>
<td>&lt;0.01</td>
<td></td>
</tr>
<tr>
<td>WKY 3.29±1.12</td>
<td>4.72±2.06</td>
<td></td>
</tr>
<tr>
<td>SHR 2.35±0.82</td>
<td>3.10±0.73</td>
<td></td>
</tr>
<tr>
<td><strong>Inulin clearance (ml/min/g kidney)</strong></td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>WKY 0.84±0.32</td>
<td>0.90±0.35</td>
<td>NS</td>
</tr>
<tr>
<td>SHR 0.73±0.21</td>
<td>0.65±0.20</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Plasma creatinine (µmol/l)</strong></td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>WKY 34.1±5.9</td>
<td>36.7±4.3</td>
<td>NS</td>
</tr>
<tr>
<td>SHR 38.6±5.6</td>
<td>43.7±4.6</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Urinary osmolarity (µosm/ml)</strong></td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>WKY 1,661±639</td>
<td>1,322±317</td>
<td>NS</td>
</tr>
<tr>
<td>SHR 1,565±327</td>
<td>1,449±231</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Plasma sodium (µeq/ml)</strong></td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>WKY 140±4.22</td>
<td>140±2.8</td>
<td>NS</td>
</tr>
<tr>
<td>SHR 141±1.24</td>
<td>141±2</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Urinary aldosterone (µg/24 hr)</strong></td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>WKY 4.89±2.10</td>
<td>2.86±0.78</td>
<td></td>
</tr>
<tr>
<td>SHR 5.4±1.46</td>
<td>3.28±1.2</td>
<td></td>
</tr>
<tr>
<td><strong>Cumulative sodium balance (µmol/4 days)</strong></td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>WKY +5,229±853</td>
<td>+4,747±902</td>
<td>NS</td>
</tr>
<tr>
<td>SHR +4,226±827</td>
<td>+3,918±538</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Urinary prostaglandin E2 (pg/µmol creatinine)</strong></td>
<td>&lt;0.005</td>
<td></td>
</tr>
<tr>
<td>WKY 452±302</td>
<td>322±202</td>
<td>NS</td>
</tr>
<tr>
<td>SHR 1,069±389</td>
<td>689±186</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td><strong>Proteinuria (mg/24 hr)</strong></td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>WKY 99.9±9</td>
<td>28.4±5.3</td>
<td>NS</td>
</tr>
<tr>
<td>SHR 31.2±7.2</td>
<td>32.0±8.6</td>
<td>NS</td>
</tr>
<tr>
<td><strong>γ-GT (µ units/24 hr)</strong></td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>WKY 42±25</td>
<td>53±27</td>
<td>NS</td>
</tr>
<tr>
<td>SHR 103±66</td>
<td>30±18</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>LDH (µ units/24 hr)</strong></td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>WKY 113±31</td>
<td>116±46</td>
<td>NS</td>
</tr>
<tr>
<td>SHR 199±67</td>
<td>138±56</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

n=5 per group.

C, control rats; R, renin-immunized rats; WKY, Wistar-Kyoto rats; SHR, spontaneously hypertensive rats; PAH, [14C]α-aminohippuric acid; γ-GT, γ-glutamyl transferase; LDH, lactate dehydrogenase.

**TABLE 6. Body Left Ventricular and Kidney Weights of Renin-Immunized and Control Animals at Death (24th Week) by Two-Way Analysis of Variance**

<table>
<thead>
<tr>
<th>Control rats (SHR/WKY)</th>
<th>Renin-immunized rats (C/R)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Plasma creatinine (µmol/l)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WKY 37.9±1.57</td>
<td>41.2±3.26</td>
<td>NS</td>
</tr>
<tr>
<td>SHR 36.1±2.4</td>
<td>40.0±6.1</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td><strong>Cumulative sodium balance (µmol/6 days)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WKY +4,363±3,303</td>
<td>+6,546±4,652</td>
<td>NS</td>
</tr>
<tr>
<td>SHR +4,074±1,047</td>
<td>+5,551±1,923</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Body weight (g)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WKY 412±37</td>
<td>411±25.1</td>
<td>NS</td>
</tr>
<tr>
<td>SHR 361±21</td>
<td>354±20</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Left ventricular weight (mg)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WKY 1,028±104</td>
<td>857±44</td>
<td>NS</td>
</tr>
<tr>
<td>SHR 1,079±68</td>
<td>829±39</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Ratio of left ventricular to body weight (mg/g)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WKY 2.45±0.006</td>
<td>2.13±0.16</td>
<td>NS</td>
</tr>
<tr>
<td>SHR 2.99±0.16</td>
<td>2.34±0.08</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Kidney weight (mg)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WKY 1,577±200</td>
<td>1,231±87</td>
<td>NS</td>
</tr>
<tr>
<td>SHR 1,700±114</td>
<td>1,092±119</td>
<td>NS</td>
</tr>
</tbody>
</table>

n=10 per group.

C, control rats; R, renin-immunized rats; WKY, Wistar-Kyoto rats; SHR, spontaneously hypertensive rats.

There was a significant interaction (more pronounced effect in SHR) in kidney weight (F=6.54, p=0.014) and ratio of left ventricular to body weight (F=15, p<0.001) (Protocol 2).

hypertrophy15,16 in this model of genetic hypertension and in normotensive control animals.35

The present study shows clearly that rats respond to immunization with mouse submandibular gland renin by producing high-titer antibodies that bind to and inhibit endogenously produced renin. Rat renin is closely related, although not identical, to mouse submandibular gland renin in that the two proteins have 81% amino acid identity.36 Antibodies generated in the rat against mouse renin inhibited the catalytic activity of rat renin both in vivo and in vitro. In vitro, the antibodies from renin-immunized rats blocked the renin-substrate reaction in the plasma of control rats in a dose-dependent manner. In vivo, blockade of the renin-angiotensinogen reaction was demonstrated by a marked decrease in plasma renin activity in renin-immunized animals, which remained close to the limit of sensitivity of the Ang I radioimmunoassay. Intravenous infusion of high doses of renin did not increase blood pressure in renin-immunized animals. Moreover, administration of converting enzyme inhibitor significantly decreased
blood pressure in sodium-depleted control SHR but not in sodium-depleted, renin-immunized SHR. This result confirmed that the renin-angiotensin system was blocked in renin-immunized rats because the blockade of an other level of the angiotensin system did not induce a further decrease in blood pressure.

The consequences of inhibiting circulating renin activity were also as might be expected: The plasma level of Ang II was very low, and the urinary aldosterone excretion rate was decreased by 50%. The extent of the role of renin-angiotensin system in aldosterone secretion had already been addressed in studies on dogs immunized with hog renin,37-39 which showed a decrease in secondary hyperaldosteronism associated with antiren antibodies. Despite the chronic blockade of the renin system, as shown by the extremely low level of Ang II, the plasma aldosterone level remained 50% that of normal rats. Similar results were also obtained in renin-immunized marmosets.12

The renal renin content of renin-immunized rats was elevated, as confirmed by immunostaining for renin. This finding is in agreement with previous studies40-42 in which the phenomenon of myoepithelial cell recruitment during active immunization of renovascular hypertensive dogs with hog renin was first described. These data are readily correlated with the suppression of the negative feedback effect of Ang II on renin secretion.

Chronic inhibition of the renin-substrate by anti-renin immunoglobulins was associated with a significant decrease in blood pressure in both hypertensive and normotensive animals. The decrease in blood pressure of SHR was quite remarkable because the blood pressure of renin-immunized SHR, as measured by two methods, was in the range of that of the normotensive WKY control rats. The result of inhibition in WKY rats was also interesting in that it was shown that the blockade of the renin-angiotensin system also chronically decreases blood pressure in a normotensive rat strain. These effects on blood pressure were substantiated by the considerable decrease in cardiac mass in each strain.

The dissociation between the fall in plasma renin activity and the decrease in blood pressure with time is not readily explained. Plasma renin activity fell rapidly to the limit of detection just after the third immunization, whereas blood pressure decreased...
more slowly in both hypertensive and normotensive strains. This early phenomenon could not be explained by sodium loss because the sodium balances during the first and second experimental periods were essentially the same. This phenomenon might be due to a progressive reversal of the arterial lesions induced by hypertension and the chronic sustained effect of Ang II on blood pressure.

Salt homeostasis was measured during the phase of decreasing blood pressure and at the end of the experimental period to support the contention that salt homeostasis was maintained and that the observed decrease in blood pressure was due to inhibition of the renin-angiotensinogen reaction rather than to salt loss due to autoimmune nephropathy. There was a significant difference between salt excretion of SHR and WKY rats. This phenomenon has been reported and is probably the result of hypertension-induced natriuresis, according to Guyton. During the two times of measurement, sodium balance was positive in renin-immunized animals and similar to what was observed in control animals. Total body sodium or plasma volume had not been measured. Nevertheless, the plasma protein concentration was similar in renin-immunized SHR and control rats. These data support the finding that renin-immunization did not significantly change the plasma volume.

The glomerular function is assessed by insulin clearance and proteinuria. Clearly, renin immunization neither decreased filtration rate nor increased proteinuria. Therefore, the glomerular function seems to be conserved in renin-immunized animals. Direct assessment of tubular function by micropuncture has not been performed in the present study. Nevertheless, enzymuria and renal ability to concentrate urine were used as indirect assessments of the tubular function. Increase in enzymuria seems to be an indicator of proximal tubule disease. γ-GT and LDH were higher only in control SHR, demonstrating that hypertension rather than renin immunization was associated with an increase in enzymuria. Alkaline phosphatase was undetectable in the different groups. Urine concentrating ability was not decreased in renin-immunized animals, showing that collecting duct function is preserved. These data demonstrate that the limited autoimmune nephropathy observed in renin-immunized animals does not induce predominant deterioration of tubular function. Moreover, the autoimmune disease was histologically limited at the 10th week and could not account for the observed decrease in blood pressure.

To exclude the role of a secondary activation of renal vasodilator hormones due to nephritis in the hypotensive response, urinary PGE$_2$ was measured by radioimmunoassay. Data reported in Table 5 clearly show that renin immunization decreases the urinary excretion of PGE$_2$. These results could be related to the suppression of an Ang II-induced ionic stimulation in prostaglandin production. Therefore, a secondary activation of renal vasodilator prostaglandin could not account for the observed decrease in blood pressure.

In the present study, as expected, renal plasma flow was lower in SHR than in WKY rats. Blockade of renin by immunization significantly increased renal plasma flow in the two strains (43% in SHR and 32% in WKY) without significantly changing the glomerular filtration rate. Therefore, the maintenance of sodium homeostasis in renin-immunized animals could be the net result of an increase in renal plasma flow, a decrease in perfusion pressure, and a suppression of the tubular effect of Ang II. Thus, sodium loss, plasma volume reduction, glomerular disease, tubular toxicity, and increased prostaglandin production are not major factors in the observed decrease in the blood pressure of renin-immunized animals. Nevertheless, the progressive appearance of autoimmune nephropathy could, together with the blockade of extrarenal and intrarenal renin activity and probably with the decrease in blood pressure, partially explain the progressive decrease in renal function (increase in blood urea nitrogen and creatinine). This decrease in renal function was more rapid in SHR than in WKY rats, perhaps because of the greater immunological response and the blockade of the renin-angiotensin system, leading to greater autoimmune nephropathy in SHR.

**Immunological Response to Renin**

Active immunization of rats with mouse submandibular gland renin was associated with an autoimmune disease in the kidney. A similar result was obtained after active immunization of marmosets against human renin. The immunization protocol used for this study on rats induced an immunological memory and a kidney autoimmune disease that was less developed than that in marmosets. The lesions were limited, producing only focal amputation of nephrons in the renal cortex with no general destruction of the renal organization. This may be because mouse submandibular gland renin is more heterologous for rats than human renin is for the marmoset, producing less recognition of self-renin by self-immunoglobulins in rats and hence less immediate toxicity than in marmosets.

The autoimmune disease was restricted to the kidney in rats, as in marmosets. The heart, walls of the aorta, and renal arteries appeared normal and free of cellular infiltration, suggesting that the autoimmune disease involved the major sites of production, storage, and release of renin.

That rat kidney autoimmune disease appeared to have two components—immunoglobulins colocalized with renin and interstitial periarteriolar cellular infiltration—indicates that both humoral (B lymphocytes) and cellular (T lymphocytes) components of the immune response were stimulated by the immunization protocol. Only the immunoglobulins were significant in terms of inhibition of the renin-angiotensinogen reaction, but the cellular immune
response probably plays a major role in the immunological memory and thus prolongs inhibition.

The immunological blockade of renin appears to be strain dependent; it was more heterogeneous in WKY rats than in SHR. This could be because the WKY strain is a more outbred strain than SHR, so the immune response in WKY rats was more heterogeneous than that in SHR, or it could be because the two strains differ in their genetic determination of the immune response.

Conclusions

Active immunization of SHR against murine renin offers an in vivo model of chronic inhibition of the renin-substrate reaction. It can, therefore, be compared with other methods of inhibiting the renin-angiotensin system, particularly the inhibition of converting enzyme and the blockade of renin-substrate reaction by synthetic inhibitors. This model may be also considered a pathological model of renin autoimmune disease. It could provide an approach to understanding the roles of humoral and cellular immune responses in renin autoimmune renal arteriolitis.

Active immunization against murine renin in SHR also shows the limits of such a manipulation of two in vivo biologically active systems—the renin-angiotensin and the immunological systems. Both systems have clearly different kinetics. The renin-angiotensin system is a physiologically adaptive system with a short response time, which remains constant in different circumstances of stimulation, therefore allowing maintenance of the blood pressure within a range compatible with renal function. The immune system has a long response time, which can produce a constant blockade of a hormonal system but cannot be rapidly reversed. In addition to these physiological limitations, the model shows the potential risk associated with the immunological blockade of a regulated endogenous enzymatic activity in vivo. Such an approach may be appropriate when the endogenous antigen is not constantly present, as with gonadotrophic hormones, but is less suitable if the endogenous antigen is constantly present, as with renin, and its levels of biosynthesis, storage, and secretion increase in relation to its inhibition. It is therefore difficult to imagine how the autoimmune risk could be sufficiently controlled by using a different immunization protocol or synthetic epitopes to permit the development of this immunological approach in humans.

Acknowledgments

The authors wish to acknowledge the technical assistance of Mrs. Danièle Gentic, Irene Laboulandine, and Liliane Marville and to thank Mrs. Nicole Braure and Annie Boisquillon for preparing the manuscript.

References


16. Pfeffer JM, Pfeffer MA, Mirsky I, Braunwald E: Regression of left ventricular hypertrophy and prevention of left ventricular dysfunction by captopril in the spontaneously hypertensive rat. Proc Natl Acad Sci USA 1982;79:3310–3314


34. Muhanyi MJ, Hansen PK, Aalkjaer C: Direct evidence that the greater contractility of resistance vessels in SHR is associated with a narrowed lumen, a thickened media and an increased number of smooth muscle cell layers. *Circ Res* 1978;43:854–864

**KEY WORDS** • angiotensin II • hypertension • blood pressure • autoimmune disease
Physiological and immunopathological consequences of active immunization of spontaneously hypertensive and normotensive rats against murine renin.
J B Michel, S Sayah, C Guettier, J Nussberger, M Philippe, M F Gonzalez, C Carelli, F X Galen, J Menard and P Corvol

Circulation, 1990;81:1899-1910
doi: 10.1161/01.CIR.81.6.1899

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