Role of Renin-Angiotensin System in Chronic Renal Hypertensive Rats

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SUMMARY The role of renin-angiotensin system has been examined in the maintenance of hypertension in acute and chronic two-kidney (36 weeks) and chronic one-kidney (12 weeks) Goldblatt hypertensive rats using three inhibitors of this system. The inhibitors used were URI-73A, a synthetic analog of lysophosphatidylethanolamine, which inhibits renin both in vivo and in vitro, SQ14,225, a potent converting enzyme inhibitor, and [Sar\(^1\), Thr\(^2\)] angiotensin II, an angiotensin II antagonist. When the inhibitors were administered in acute (high renin) hypertensive rats, they all lowered blood pressure significantly. However, in the chronic (low renin) hypertensive phase, both renin and converting enzyme inhibitors lowered blood pressure, whereas, Sar\(^1\), Thr\(^2\) failed to lower blood pressure. The renin inhibitor lowered plasma renin activity (PRA), and SQ14,225 and [Sar\(^1\), Thr\(^2\)] Ang II increased PRA. Further studies on water and electrolyte balance with one-kidney model hypertensive and uninephrectomized control rats showed no change in plasma volume. However, there was increased 24-hour urinary output and increased sodium excretion. This study indicates that in chronic renal hypertensive rats, blood pressure reduction is possible by either renin or converting enzyme inhibitor, but not by angiotensin antagonists. Since volume did not change either during the development or reversal of hypertension, volume did not appear to play a major role in the maintenance of hypertension.

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KEY WORDS • chronic hypertension • renin-angiotensin system • renin inhibitor • converting enzyme inhibitor • angiotensin antagonist • plasma volume

SINCE the discovery of the renin-angiotensin system there have been continued efforts to determine whether or not it is instrumental in maintaining blood pressure elevation in various forms of experimental or clinical hypertension. Interruption of the system by blocking renin, either with antibodies to renin\(^1,2\) or with a phospholipid renin inhibitor purified from kidney,\(^3,4\) lowered blood pressure in most instances in both short-(acute) and long-term (chronic) one- and two-kidney models of renal hypertensive rats. A major criticism of experiments involving either active or passive immunization to renin was the lack of homogeneity of renin used for antibody production. Use of the lipid renin inhibitor has been limited and it remains to be determined whether or not this compound has effects in vivo other than renin blockade.

Hosoki et al.\(^5\) reported the synthesis of a renin inhibitor, 2-[4-(4-chlorophenoxy) phenoxyacetylamino]-ethyl phosphonylphethanolamine (PE-104), which is an analog of the natural phospholipid renin inhibitor. PE-104 inhibits the reaction between renin and renin substrate in vitro and in vivo, and reduces the concentration of circulating angiotensin I in normotensive and renal hypertensive rats. It also lowered blood pressure in renal hypertensive rats when administered in a dose of 20 mg/kg/min.\(^5\) Turcotte et al.\(^6\) synthesized a large series of phospholipid analogs and examined their structure-activity relationship for inhibiting renin. Of the compounds tested, eicosatetraenyl (3-aminopropyl) phosphonate (URI-73A), which is an analog of lysophosphatidyl ethanolamine, was found to be the most potent inhibitor of renin both in vivo and in vitro.\(^6\)

In recent work\(^7\) utilizing specific angiotensin II inhibitors, the concept has been developed that in two-kidney hypertensive animals, the renin-angiotensin system plays a role in maintenance of hypertension but in the one-kidney model, where the contralateral kidney has been removed, it does not. The latter is generally believed to be more volume-dependent than renin-dependent.

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This concept is based mainly on observations that the plasma renin activity (PRA) in these animals is not elevated above that of normotensive animals and blockade of angiotensin II receptors using the competitive antagonist [Sar\(^1\), Ala\(^8\)] angiotensin II, with or without sodium depletion, did not produce a fall in blood pressure.\(^5\)\(^-\)\(^10\) We now have available a renin inhibitor (URI-73A),\(^4\) a converting enzyme inhibitor (SQ14,225),\(^11\) and angiotensin II antagonists\(^7\) to permit us to block each component of the renin-angiotensin system.

In the present experiments, these compounds were given to both short-term (acute) and long-term (chronic) one- and two-kidney renal hypertensive rats. These models were selected so that the effect of inhibitors can be studied both at the high- and low-renin phase of experimental renal hypertension. Furthermore, blockade of the renin-angiotensin system could be achieved more effectively by inhibiting enzymes, such as renin or converting enzyme, rather than antagonizing a product, i.e., angiotensin II, the agonist especially at the receptor site. By inhibiting enzymes, the catalytic formation of product, which is an agonist, i.e., angiotensin II, could be prevented.

Methods

Preparation of Hypertensive Rats

Two-kidney model, Sprague-Dawley male rats (150–175 g) were made hypertensive by placing a 0.2 mm silver clip on the left renal artery under ether anesthesia with the other kidney left untouched. Rats were first treated with inhibitors 6 weeks after clipping, when the PRA was elevated, and then again 36 weeks after clipping when PRA was low.

One-kidney model, Sprague-Dawley rats (150–175 g) were made hypertensive by placing a silver clip (0.2 mm) on the left renal artery as above and the right kidney was removed at the same time. In this model rats were treated with inhibitors 12 weeks after clipping.

Determination of Renin Activity

Plasma renin activity was determined by the method as described by Menard and Catt\(^14\) which is a modification of a micromethod of renin determination described by Boucher et al.\(^13\) The modification involves measurement of angiotensin I formed at the end of incubation by radioimmunoassay. Renin substrate was prepared using plasma from male rats 48 hours after bilateral nephrectomy.\(^12\) Blood samples (0.4 ml) for renin assay were drawn as described previously\(^18\) under light ether anesthesia 1 day prior to treatment, at the end of the treatment period, and 3 days after treatment.

Administration of Inhibitors

The phospholipid analog (URI 73A) used in this study as an inhibitor of renin was dissolved in sodium deoxycholate (3 mg/ml) and administered intramuscularly (0.2 ml) for 8 days in a dose of 25 mg/kg/24 hours.

The converting enzyme inhibitor SQ14,225 has been reported to be a potent orally active converting enzyme inhibitor (CEI) and has lowered blood pressure in experimental hypertensive rats.\(^11\) This was administered by intramuscular injection in saline (0.2 ml). Rats with two-kidney hypertension were given 6 mg/kg/24 hours and those with one-kidney hypertension required a higher dosage of 20 mg/kg for significant blood pressure response.

As a competitive antagonist of angiotensin II,\(^7\) [Sar\(^1\), Thr\(^8\)] angiotensin II has been used extensively to study the role of renin-angiotensin system in experimental animals.\(^1\) We administered [Sar\(^1\), Thr\(^8\)] angiotensin II subcutaneously (0.2 ml) in oil (100 μg/8 hr). This route was found to be effective in blocking angiotensin II and lowering blood pressure in acute two-kidney hypertensive rats.\(^14\)

In chronic hypertensive rats, the same group of rats was treated with all three inhibitors separately. The time interval between switching from one inhibitor to the other was 5 days. In three chronic hypertensive rats an Alzet osmotic pump (Alza) was implanted subcutaneously to infuse Sar\(^1\), Thr\(^8\) continuously for 5 days at a dose of 500 ng/hr.

Determination of Blood Pressure

Indirect blood pressure was determined by using the tail-cuff method as described by Friedman and Freed\(^18\) obtained from Narco Biosystems. For each rat a mean of five consecutive readings (systolic) were used. Blood pressure was determined by a trained technician, during approximately the same time of the day, who was unaware of any drug treatment.

Determination of Plasma Volume

Plasma volume was measured in control and treated rats by using albumin tagged with \(^{131}\)I; 5 μCi of \(^{131}\)I-albumin was injected intravenously through the femoral vein. Ten minutes after the injection, a blood sample was drawn, centrifuged, and radioactivity of plasma was determined against a standard in a gamma counter. This time interval is adequate to permit equilibration of albumin distribution in the rat.\(^16\)

Determination of Total Body Water

Rats were killed, weighed on a precision balance, and an incision was made from the abdomen up to the chest. They were freeze dried for 15 days and kept in a desiccator over P\(_2\)O\(_5\) for a further 7 days until constant weight was obtained.

Metabolism Studies

Both one-kidney hypertensive and uninephrectomized control rats were placed in metabolism cages for measurement of urinary output and Na and K excretion over a 24-hour period before and during ad-
Table 1. Course of Development of Hypertension and Parallel Changes in Plasma Renin Activity (PRA)

<table>
<thead>
<tr>
<th>Rat Model</th>
<th>Control</th>
<th>6 weeks</th>
<th>12 weeks</th>
<th>36 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Two-kidney model (n = 12)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PRA (ng Ang I/ml/hr)</td>
<td>100 ± 10</td>
<td>545 ± 11*</td>
<td>260 ± 9*</td>
<td>57 ± 2*</td>
</tr>
<tr>
<td>Blood pressure (mm Hg)</td>
<td>115 ± 7</td>
<td>175 ± 10*</td>
<td>190 ± 11*</td>
<td>210 ± 5*</td>
</tr>
<tr>
<td>One-kidney model (n = 12)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PRA (ng Ang I/ml/hr)</td>
<td>62 ± 8</td>
<td>57 ± 5</td>
<td>36 ± 3†</td>
<td></td>
</tr>
<tr>
<td>Blood pressure (mm Hg)</td>
<td>100 ± 5</td>
<td>200 ± 10*</td>
<td>230 ± 12*</td>
<td></td>
</tr>
</tbody>
</table>

*Significantly higher than corresponding control (p < 0.01).
†Significantly lower than control.

Administration of converting enzyme inhibitor SQ14,225.

Statistical Analysis

Data were expressed as mean ± SEM. Paired t tests were used for the data obtained from rats before and after drug treatment and the comparison between normal and hypertensive group means were made using Student's t test.

Results

Development of Hypertension

The course of development of hypertension and parallel change in plasma renin activity in both two-kidney and one-kidney hypertensive rats is summarized in Table 1. The control values differ between the two groups since the one-kidney model rats are uninephrectomized and, therefore, are expected to have lower PRA.

The blood pressure of two-kidney hypertensive rats rose significantly 5 weeks after clipping (175 mm Hg vs 115 mm Hg) along with a very significant increase in PRA (545 ng/ml/hr vs 100 ng/ml/hr). In the same rats the blood pressure remained elevated 12 weeks after clipping but PRA had fallen to approximately one-half that observed at 5 weeks, but still remained significantly elevated compared to the control period. After 34 weeks of hypertension blood pressure remained elevated, but PRA was now lower than during the control period (57 ng/ml/hr vs 100 ng/ml/hr). These data show that in the two-kidney hypertensive animals, the initial phase (5–12 weeks after clipping) of hypertension was accompanied by an increase in PRA followed by a phase of hypertension with low plasma renin activity.

In contrast, in the one-kidney model of hypertension 5 weeks after clipping, the blood pressure rose significantly, but PRA was not significantly different from the control period and fell below normal after 12 weeks of hypertension (Table 1).

Determination of Plasma Volume

The changes in plasma volume in both one- and two-kidney hypertensive animals are summarized in Table 2. The plasma volumes were determined during the high-renin phase and the low-renin phase in the two-kidney hypertensive rats. As the rats grew older, the absolute values of plasma volume increased, but when normalized based on body weight, no significant differences were observed between any groups.

Effect of Inhibitors on Two-Kidney Model

High-Renin Phase (Acute)

Eight hypertensive (two-kidney) and eight sham-operated control animals were treated separately with renin inhibitor (URI-73A), converting enzyme inhibitor (SQ14,225) and angiotensin II antagonist ([Sar¹, Thr⁸] angiotensin II) during the high-renin phases of hypertension. The data are shown in Figure 1. All three antagonists of renin-angiotensin system significantly lowered blood pressure in the hypertensive rats during this phase. The blood pressure in normotensive rats was not altered by any of these antagonists.

Table 2. Plasma Volume During Development and Maintenance of Renal Hypertension in Rats

<table>
<thead>
<tr>
<th>Rat</th>
<th>Plasma volume (ml/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Two kidney</td>
<td></td>
</tr>
<tr>
<td>Control (n = 8)</td>
<td>42 ± 5</td>
</tr>
<tr>
<td>6 weeks (n = 10)</td>
<td>39 ± 3</td>
</tr>
<tr>
<td>30 weeks (n = 8)</td>
<td>45 ± 6</td>
</tr>
<tr>
<td>One kidney</td>
<td></td>
</tr>
<tr>
<td>Control (n = 8)</td>
<td>44 ± 3</td>
</tr>
<tr>
<td>12 weeks (n = 8)</td>
<td>41 ± 2</td>
</tr>
</tbody>
</table>
FIGURE 1. Effect of inhibitors of renin angiotensin system on two-kidney (high-renin) hypertensive rats 6 weeks following clipping.

Low-Renin Phase (Chronic)

The effects of all three inhibitors, administered in succession as described before, on the blood pressure of eight two-kidney hypertensive rats 36 weeks following clipping, are shown in figure 2. A gradual reduction in blood pressure was observed when the renin inhibitor, URI-73A, was administered in a dose of 25 mg/kg/24 hours. The maximum blood pressure lowering effect (50 mm Hg; \( p < 0.01 \)) was noted 8 days after administration and after withdrawal of inhibitor the blood pressure rose to pretreatment level.

The converting enzyme inhibitor, SQ14,225, middle panel of figure 2, also produced a significant reduction in blood pressure (68 mm Hg; \( p < 0.01 \)), and the blood pressure remained low as long as the treatment was continued (3 days). Upon withdrawal, the blood pressure promptly rose to pretreatment level.

In marked contrast, the angiotensin antagonist [Sar\(^1\), Thr\(^8\)] angiotensin II, shown on the right panel of figure 2, did not lower the blood pressure during this phase; in fact, there was a slight increase in blood pressure (10 mm Hg). The dose of [Sar\(^1\), Thr\(^8\)] angiotensin II (100 \( \mu \)g/kg/8 hrs) administered was sufficient to completely block the effect of exogenous injection of 50, 100, 200 and 360 ng of angiotensin II. The above effect of exogenous angiotensin II was tested separately in a pilot experiment by cannulating the carotid artery of normal and hypertensive rats (three each), and injecting angiotensin II intravenously while recording the blood pressure. Furthermore, continuous infusion of [Sar\(^1\), Thr\(^8\)] angiotensin II by implanting an osmotic pump (Alzet) in a dose of 500 ng/hr for 5 days also failed to lower blood pressure.

Effect of Blockers on Plasma Renin Activity

The plasma renin activity of low-renin two-kidney renal hypertensive rats (36 weeks after clipping) after administration of antagonists is summarized in Table 3. As shown, a significant reduction in PRA was noted after treatment with the renin inhibitor URI 73A, along with a reduction in blood pressure. However, a sevenfold increase in PRA was noted in the same group of rats after treatment with the converting enzyme inhibitor SQ14,225, along with a drop in blood pressure. The angiotensin II antagonist [Sar\(^1\), Thr\(^8\)] angiotensin II did not significantly alter blood pressure, but it increased PRA by about fivefold.

Effect of Blockers on One-Kidney Models

The role of the renin-angiotensin system has been examined in one-kidney hypertension (12 weeks after clipping) by blocking each component of renin-angiotensin system individually and then an electrolyte and volume metabolism study was done during SQ14,225 administration to attempt to better understand the underlying mechanism for the blood pressure lowering effect.

Effect of Inhibitors

The effect of renin, converting enzyme, and angiotensin II inhibitors in one-kidney chronic hypertensive rats (12 weeks after clipping) are sum-

Table 3. Effect of Inhibitors of Renin-Angiotensin System on Plasma Renin Activity (PRA) of Two-Kidney Hypertensive Rats*

<table>
<thead>
<tr>
<th>PRA (ng Ang I/ml/hr)</th>
<th>URI 73A</th>
<th>SQ14,225</th>
<th>[Sar(^1), Thr(^8)] Ang II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before</td>
<td>After</td>
<td>Before</td>
<td>After</td>
</tr>
<tr>
<td>Two-kidney model</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(n = 8)</td>
<td>57 ± 2</td>
<td>30 ± 2*</td>
<td>36 ± 3</td>
</tr>
<tr>
<td>Average change in blood pressure</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-50 mm Hg</td>
<td>-68 mm Hg</td>
<td>+10 mm Hg</td>
<td></td>
</tr>
</tbody>
</table>

*Effect of inhibitors on PRA of hypertensive rats (36 weeks after clipping) has been determined in the same group of rats before and after administration of each inhibitor. Data expressed as mean ± SEM. The time interval between each inhibitor was 5 days.

\( \dagger p < 0.01 \).
RENIN-ANGIOTENSIN SYSTEM AND CHRONIC HYPERTENSION/Sen et al.

36 ± 3) rats were treated with SQ14,225. The increase in PRA in normal rats was not accompanied by a significant reduction in blood pressure (9 mm Hg), whereas in the hypertensive group a significant reduction (68 mm Hg) in blood pressure was noted. In con-

Effect on Plasma Renin Activity

A significant increase in PRA was noted when both normotensive (183 ± 68 ng/ml/hr vs 46 ± 2 ng/ml/hr) and hypertensive (377 ng/ml/hr ± 110 vs

Figure 2. Effect of inhibitors of renin-angiotensin system on blood pressure of two-kidney Goldblatt chronic hypertensive rats 36 weeks after clipping. Left panel: Effect of renin inhibitor (URI-73A) 25 mg/kg/24 hrs. Middle panel: Effect of converting enzyme inhibitor SQ14,225 20 mg/kg/24 hrs. Right panel: Effect of angiotensin II antagonist [Sar\(^1\), Thr\(^2\)] ang II, 100 mg/kg/8 hrs.

Figure 3. Effect of renin-angiotensin system inhibitors on blood pressure of one-kidney chronic hypertensive rats 12 weeks after clipping. Open columns = hypertensive controls; cross-hatched columns = treated with inhibitors. Asterisks = statistically significant (p < 0.01). The dose of inhibitors was same as in figure 2.

marized in figure 3. The effects are similar to those observed with two-kidney chronic (low-renin) hypertensive rats. Renin inhibitor significantly lowered blood pressure (210 mm Hg to 160 ± 5 mm Hg; \(p < 0.01\)) when injected intramuscularly in a dose of 25 mg/kg/day. In two out of eight rats a higher dose (35 mg/kg) was necessary to obtain significant blood pressure lowering effect. After withdrawal of drug therapy, the blood pressure rose to pretreatment level.

The effect of converting enzyme inhibitor (SQ14,225), summarized in figure 3, middle panel, caused very significant (210 mm Hg to 140 mm Hg; \(p < 0.001\)) reduction in blood pressure when injected intramuscularly in a dose of 20 mg/kg. However, a much higher dosage of SQ14,225 was necessary to lower blood pressure in the one-kidney (chronic) model compared to the two-kidney model (6 mg vs 20 mg/kg). When treatment was discontinued, blood pressure did not return to pretreatment level even 72 hours after withdrawal. A typical response is shown in figure 4. The blood pressure fell from 240 mm Hg to 140 mm Hg after SQ14,225 therapy (20 mg/kg), but upon withdrawal, the blood pressure only rose to 165 mm Hg after 3 days.

Administration of [Sar\(^1\), Thr\(^2\)] angiotensin II did not alter blood pressure when administered either by subcutaneous injection in oil (100 \(\mu\)g/kg/8 hrs) (fig. 3, right panel) or continuous infusion by implantation of an osmotic pump (500 ng/hr for 5 days).
Effect of CEI on Water and Electrolyte Metabolism

Effect on Plasma Volume

The effect of SQ14,225 on plasma volume and electrolyte excretion is summarized in Table 4. First, no significant difference in plasma volume was noted between the uninephrectomized and one-kidney model hypertensive groups (44 ± 3 ml/kg vs 41 ± 2 ml/kg; p = NS). Second, the reduction of blood pressure in one-kidney chronic hypertensive rats by SQ14,225 was not accompanied by a change in plasma volume. The plasma volume before treatment was 41 ± 2 ml/kg and after 3 days treatment was 42 ± 2.4 (p = NS) when the blood pressure fell from 212 ± 5 to 132 ± 5 (p < 0.001). Also, in control animals, no significant change in plasma volume was noted (44 ± 3 ml/kg vs 41.0 ± 2) alone with slight, but not significant, reduction in blood pressure (111 ± 7 mm Hg vs 102 ± 5 mm Hg; p < 0.2).

When total body water was measured in hypertensive and control groups, no significant difference was noted. In hypertensives and controls, the total body water was 671.5 ± 12 ml/kg and 646 ± 5.0 ml/kg (p < 0.2), respectively. Other reports have shown similar amounts of total body water in rats (670 ml; range 633–697) of this weight group.

Effects on Water and Electrolytes

The data on the changes in water intake, output and electrolyte excretion during CEI treatment are shown in Table 4. In normal animals, a slight increase in water intake (34 ± 2 ml vs 40 ± 3 ml) and a significant increase in urinary output (21 ± 1 vs 25 ± 2; p < 0.05) were observed during administration of CEI. Total urinary sodium and potassium excretions were not altered during drug therapy (Na — 3460 ± 168 vs 3118 ± 290 μEq/24 hrs; p < 0.4, and K — 3850 ± 94 vs 3710 ± 321 μEq/24 hrs). There were no significant changes in plasma volume, blood pressure, or electrolyte excretion in the treatment groups compared to control groups.

Table 4. Effect of CEI on Control (Uninephrectomized) and One-Kidney Hypertensive Rats on Water and Electrolytes Metabolism*

<table>
<thead>
<tr>
<th></th>
<th>Control (n = 8)</th>
<th>Hypertensive control (n = 8)</th>
<th>Treated with SQ14,225 (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood pressure (mm Hg)</td>
<td>111 ± 7</td>
<td>102 ± 5</td>
<td>212 ± 4</td>
</tr>
<tr>
<td>Plasmavolume (ml/24 hrs)</td>
<td>44 ± 3</td>
<td>41.0 ± 2</td>
<td>41 ± 2</td>
</tr>
<tr>
<td>Water intake (ml/24 hrs)</td>
<td>34.4 ± 2</td>
<td>40 ± 3</td>
<td>41 ± 1.7</td>
</tr>
<tr>
<td>Urinary output (ml/24 hrs)</td>
<td>20.5 ± 1</td>
<td>24.7 ± 2</td>
<td>18.5 ± 4</td>
</tr>
<tr>
<td>Urinary Na (μEq/24 hrs)</td>
<td>3460 ± 168</td>
<td>3118 ± 290</td>
<td>2935 ± 240</td>
</tr>
<tr>
<td>Urinary K (μEq/24 hrs)</td>
<td>3850 ± 97</td>
<td>3710 ± 231</td>
<td>NS</td>
</tr>
</tbody>
</table>

*Data are expressed as mean ± SEM. The data on water intake, urinary output, urinary Na and K depict mean of readings for 3 days of control period and 3 days during drug therapy. Data were analyzed using paired t test.
was no significant change in food intake. The data shown in table 4 are expressed as the mean for 3 consecutive days before treatment and 3 days during treatment. During each 3-day period, the data were not significantly different between Days 1, 2 and 3.

In the hypertensive group (table 4), SQ14,225 therapy caused a significant increase in water intake (41 ± 2 ml vs 57 ± 7 ml; p < 0.05) and significant increase in 24-hour urinary output (18.5 ± 4 ml vs 41 ± 5; p < 0.01). An increase in 24-hour Na excretion (2935 ± 240 μEq/24 hrs vs 3576 ± 337; p < 0.05) and no significant change in K excretion (3400 ± 275 vs 3446 ± 575 μEq/24 hrs). The sodium intake in both hypertensive and normal control groups were not different. Each rat consumed 20 ± 2 g of powdered rat chow per day containing 0.204 mEq of Na/g.

Converting enzyme inhibitor* has been shown to prevent the degradation of bradykinin (kininase II). To study whether the blood pressure lowering effect of SQ14,225 was due to accumulation of bradykinin, a group of three hypertensive rats (two kidney) was treated with SQ14,225 (20 mg/kg, intramuscularly) and a significant reduction of arterial pressure was noted 3 hours later (249 ± 14 mm Hg to 86 ± 6 mm Hg). Five days later, when the blood pressure rose to 220 ± 10 mm Hg, the same group of rats was treated with 10,000 units of Trasylol (a kinin inactivator) and 30 minutes later, SQ14,225 was administered and a normalization of blood pressure was again noted (210 ± 10 mm Hg vs 128 ± 5 mm Hg; p < 0.001). This suggested that in the absence of Trasylol, a greater blood pressure lowering effect was obtained. Bradykinin alone probably cannot be solely responsible for the blood pressure lowering effect of SQ14,225, for normalization of blood pressure was possible in Trasylol-treated rats when the effect of bradykinin would be expected to be minimal or absent.

Discussion

It has generally been concluded that the renin-angiotensin system plays a minor or insignificant role in the maintenance of elevated pressure during the chronic (normal or low-renin) phase of experimental renal hypertension (either one- or two-kidney model). This conclusion is based on the following observations: 1) the PRA in such animals is the same as that found in normal animals, and 2) the lack of response of chronic renal hypertensive animals to blockade of angiotensin II by either anti-angiotensin II antibody or angiotensin II antagonists such as [Sar1, Thr1] angiotensin II or [Sar1, Ala1] angiotensin II. In contrast to the latter negative observation, the reduction of blood pressure in chronic renal hypertensive animals by inhibiting renin with crude anti-renin antibody was discounted since the response could have been due to the presence of antibodies to unknown renin-related factors.

In the present experiments, we have prevented the formation of angiotensin II by inhibition of renin with a phospholipid analog* and by inhibition of the converting enzyme with a peptide analog.2 With both enzyme inhibitors, the blood pressure fell significantly but when using the renin inhibitor, PRA also fell, while with the converting enzyme inhibitor the PRA rose. However, the attempt to lower blood pressure by inhibition of angiotensin using [Sar1, Thr1] angiotensin II failed. At the present time, we can only speculate about the explanation for these findings. The phospholipid analog is known to inhibit* the enzyme renin in vitro which would result in the reduction in angiotensin I formation, but it is not yet known whether or not this compound has any additional actions in vivo. Similarly, SQ14,225 would inhibit conversion of angiotensin I to angiotensin II and also reduce the rate of destruction of bradykinin. The role of the possible increase in circulating bradykinin with SQ14,225 in blood pressure reduction is not known, but is believed to be minor since this inhibitor has little, if any, blood pressure lowering activity in normotensive animals and, in addition, the blood pressure lowering effect could not be prevented by pretreating the hypertensive rats with up to 10,000 units of Trasylol, a kallikrein inactivator.

The failure of [Sar1, Thr1] angiotensin II to lower blood pressure in low-renin animals is not surprising for it has been observed by others in experiments. It, however, the reason for this failure, if inhibition of the two enzymes leading to the formation of angiotensin II reduces blood pressure, must be considered unknown. It may be that in vivo the angiotensin analog cannot effectively compete with endogenous natural peptide. It should be emphasized that this peptide analog is a competitive antagonist and therefore its effect can be overcome by increasing amounts of the agonist while the other two compounds are enzyme inhibitors which can prevent formation of the agonist.

One major difference on the effect of SQ14,225 observed between the two- and one-kidney models is that the blood pressure of one-kidney hypertensive rats did not rebound to pretreatment levels even after 3 days.

These differences in responses to SQ14,225 in the one-kidney model initiated further studies on salt and water excretion. The increase in water intake has been shown by Lehr et al.30 using SQ20,881, another converting enzyme inhibitor. The increase in urinary output along with increase in Na excretion might suggest a relationship between sodium ion and hypertension; however, further studies on such parameters in two-kidney models and with renin blocker (such as phospholipid) are essential. A similar increase in urinary Na after SQ14,225 has been reported by Bengis et al.31

Several investigators4,8 have speculated that volume expansion could be a contributory factor in the maintenance of low-renin renal hypertension. To the best of our knowledge, our data are the first where plasma volume was actually determined in such animals and no expansion in plasma volume was noted either at the chronic state of hypertension or after lowering pressure by SQ14,225. Of course, any change
in plasma volume occurring during the earlier phase of this type of hypertension may have been missed. This suggested that perhaps changes in volume alone are probably not major factors in the maintenance of chronic hypertension of renal origin. Since there are evidences that hypertension is associated with sodium retention, perhaps sodium itself may be playing an important role in the maintenance of hypertension.

In conclusion, the reduction of blood pressure in chronic renal hypertensive rats by compounds expected to inhibit either renin or converting enzyme would seem to support the previous observations made by using anti-renin antibodies. These positive observations warrant further studies to evaluate the role of the renin-angiotensin system in this experimental disease than previously expected from the negative findings obtained using only angiotensin II analogs.

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