Role of Angiotensinogen in Blood Pressure Homeostasis

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SUMMARY The role of angiotensinogen in blood pressure control was assessed in normotensive rats by observing the changes resulting from inhibition by specific rat angiotensinogen antiserum. The antiserum decreased blood pressure in rats on normal sodium as well as sodium-free diets (respectively ΔBP = -30 ± 6 mm Hg and -42 ± 8 mm Hg). In binephrectomized sodium-replete rats, administration of antiserum did not reduce blood pressure, whereas in sodium-depleted animals it slightly decreased blood pressure by 11 ± 3 mm Hg. These results suggest that angiotensinogen participates in the regulation of blood pressure in normotensive rats, even in the sodium-replete state. (Hypertension 4: 185-189, 1982)

KEY WORDS • angiotensinogen antibodies • blood pressure • normotensive rats • sodium status

THE renin-angiotensin system plays an important role in regulating blood pressure; its role in normal homeostasis, hypertensive diseases, and edematous states continues to be defined by study of the use of effective blocking agents.

The initial step of the renin-angiotensin system, the action of renin on angiotensinogen, can be inhibited by specific antibodies, pepstatin and its derivatives, phospholipids, and substrate analogs. Angiotensinogen concentration is known to be a rate-limiting factor for angiotensin generation in human or rat plasma.

Treatment with estrogen or glucocorticoids causes a rise in angiotensinogen levels, which probably contributes to the increased incidence of high blood pressure observed in women receiving oral contraceptives and the hypertension associated with glucocorticoid excess.

In the present study, specific angiotensinogen antibodies obtained by immunizing rabbits against highly purified rat angiotensinogen have been used to inactivate the renin-angiotensin system in conscious rats on normal and sodium-free diets. This method of blockade has not been investigated previously and provides a useful comparison with other blocking methods.

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Production of Antiserum

Renin substrate (angiotensinogen) in binephrectomized rat plasma was purified in six successive steps: ammonium sulfate precipitation, followed by chromatography on blue dextran sepharose, DEAESephacel, hydroxylapatite, agarose-acrylamide, and then preparative isoelectric focusing. Purity was checked by sodium dodecyl sulfate gel electrophoresis and amino acid analysis, which demonstrated that the first 10 amino acids were exactly those of angiotensinogen I.

Antibodies to rat angiotensinogen were obtained in rabbits. They were injected intradermally with 50 µg of pure angiotensinogen mixed with complete Freund's adjuvant, and then after 8 weeks a booster injection of 50 µg of angiotensinogen mixed with incomplete Freund's adjuvant was administered at multiple intradermal sites. High titer antisera were obtained 3 weeks after the third injection. Binding of pure iodinated angiotensinogen was used to follow the successive titers of antibodies during the immunization procedure. Antisera selected for the experiments gave 50% binding of iodinated angiotensinogen (75 pg) at a final dilution of 1/70 000. Only a single immunoprecipitation line was observed when antiserum reacted with pure angiotensinogen and rat plasma in Ouchterlony gels.

Antibodies did not cross react with angiotensin I (AI), tetradecapeptide, or angiotensinogen of the human, dog, rabbit, monkey, or hog. Some antisera
were purified by ammonium sulfate precipitation and DEAE cellulose chromatography to isolate gamma globulin.14 In vitro and in vivo blockade experiments were performed either with gamma globulin or with antiserum.

In Vitro Experiments

Inhibitory properties of the gamma globulin fractions were determined by the in vitro inhibition of plasma renin activity (PRA). Increasing amounts of gamma globulin (0.08 to 1 mg) were preincubated with rat plasma (obtained from decapitated normal rats) for 1 hour at 4°C in phosphate buffer pH 7.5 in a total volume of 500 μl. The mixture was then incubated at 37°C for 2 hours, and the AI generated was measured by radioimmunoassay.6

In Vivo Experiments

General Procedure

In male Wistar rats weighing 250–300 g anesthetized with sodium pentobarbital (Nembutal, 50 mg/kg i.p.), a PE 10 carotid catheter was implanted and exteriorized at the back of the neck and brought out to extend through the top of individual cages. The catheter was sealed with a three-way stopcock and the animals allowed to recover for at least 4 hours prior to the experiment. Arterial blood pressure was measured continuously through the carotid cannula with a Hewlett-Packard transducer (78205 A). After baseline pressure recordings for 15 minutes, 0.4 ml of undiluted antiserum was injected as a single bolus through the carotid catheter, and the pressure response was measured every 30 seconds for up to 45 minutes. Control rats were injected with 0.4 ml of non-immune undiluted rabbit serum. Additional control experiments were performed in two rats maintained on the sodium-free diet described below, using 0.4 ml of undiluted serum from a rabbit immunized against rat albumin (specific antiserum RAR a/Alb-Nordic. Immunological Laboratories, Tilburg, The Netherlands).

To check that angiotensin pressor effects in rats treated with angiotensinogen antibody remained unaffected, the following protocol was performed: eight conscious rats on normal sodium intake were injected with a 50 ng bolus of angiotensin I (1-Asp-5-Ile-AI, Schwarz-Mann) at 0 time and 5 minutes (control period). From 14 to 20 minutes, three rats received the same bolus of AI four times at 2-minute intervals (experimental period). An identical schedule of AI injections was performed in three rats injected with 0.4 ml of normal rabbit serum and in two rats injected with 0.4 ml of angiotensinogen antisemur.

Experimental Groups

Test and control animals were maintained on the following diets: 1) normal sodium intake (Group 1) with free access to tap water and a regular diet (sodium content: 170 mEq/kg of food); and 2) sodium-free intake (Group 2) with free access to distilled water and sodium-free food for 15 days. At 2 days before the experiments, these animals were injected twice subcutaneously with furosemide (20 mg/kg/day).

In addition to these animals, others were maintained either on the normal (Group 3) or sodium-free (Group 4) diet but were binephrectomized under Nembutal anesthesia 24 to 30 hours before antiserum injections. Furosemide (20 mg/kg/day) was injected 48 hours and 24 hours before binephrectomy in Group 4.

All animals in Groups 1 through 4 received unpurified antibodies. The purified antiangiotensinogen gamma globulin (15 mg/rat) was used in two normal rats and one binephrectomized rat maintained on the normal sodium diet and two normal rats prepared with the sodium-free regimen. On the night before the experiments, animals were placed in individual metabolic cages for 12 hours for urinary electrolyte determination (Klina flame photometer-Beckman). Water and food were withheld overnight. Plasma renin activity (PRA) and plasma renin substrate (PRS) were determined by radioimmunoassay of generated AI before administration of angiotensinogen antiserum or non-immune serum.8

Statistical Analysis

Results are expressed as means ± SEM, and statistical significance was determined by the use of paired or unpaired Student’s t test.

Results

In Vitro Experiments

The inhibitory effect of antiangiotensinogen gamma globulin on PRA (0.7 ng/AI/ml/hr) is shown on figure 1. A complete inhibition was obtained with 720 ng of the gamma globulin preparation.

In Vivo Experiments

Characteristics of the experimental groups are summarized in table 1. Groups 1 and 2 showed similar initial blood pressure levels. The binephrectomized rats (Groups 3 and 4) had a significantly lower blood pressure than rats of Groups 1 and 2 (p < 0.001). As expected, the renin-angiotensin system was affected by sodium balance and binephrectomy. PRA was increased in Group 2 (p < 0.001). Binephrectomy (Groups 3 and 4) enhanced PRS whereas PRA was undetectable.

Administration of non-immune rabbit serum to the four control rats of Groups 1 and 2 did not induce any change in the blood pressure. The four control rats in binephrectomized Groups 3 and 4 showed a small increase in blood pressure after injection of 0.4 ml of non-immune serum, slightly higher in salt-restricted (8–10 mm Hg) than in normal rats (5–6 mm Hg). No change was observed in the blood pressure of the two sodium-deficient rats injected with rat albumin antiserum.
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NORMAL Na+ INTAKE

FREE INTAKE

Blood pressure lowering effect of 0.4 ml of angiotensinogen specific antiserum in normal rats (●—●) and binephrectomized rats (■—■) maintained on normal Na+ intake (left) and Na+ free intake (right).

Figure 1. In vitro plasma renin activity (PRA) inhibition by increasing amounts of antiangiotensinogen gamma globulin (Control PRA = 0.70 ng angiotensin I/ml/hr).

Rats with kidneys in situ (six rats of Group 1 and seven rats of Group 2) showed a substantial and significant fall in blood pressure following antiserum administration (p < 0.01). This decrease generally occurred within 10 minutes after antiserum injection (43, 21, 35, 35, 41, 7 mm Hg in Group 1 rats and 42, 28, 59, 35, 46, 72, 11 mm Hg in Group 2 rats). The duration of hypotension was 31 ± 8 minutes in Group 1 animals and was not influenced by sodium status (37 ± 8 minutes in Group 2).

Binephrectomized rats maintained on normal sodium intake (Group 3) showed a small non-significant increase in blood pressure following antiserum injection similar to that observed in binephrectomized rats injected with 0.4 ml of non-immune serum. In the sodium-depleted binephrectomized rats (Group 4), a small decrease in blood pressure was observed within 11 minutes after antibody administration. Figure 2 summarizes results concerning the maximum fall in blood pressure in all these groups.

Animals receiving purified gamma globulin behaved like rats receiving unpurified antibodies. Figure 3 shows the results of a typical experiment in two normal sodium-replete rats receiving either unpurified antibodies or purified gamma globulin.

In experiments investigating the site of interruption of the renin-angiotensin system, the two successive injections of AI made during the control period in

<table>
<thead>
<tr>
<th>Na+ intake</th>
<th>Blood pressure (mm Hg)</th>
<th>Plasma renin activity (ngAI/ml/hr)</th>
<th>Plasma renin substrate (ngAI/ml)</th>
<th>U_{Na}V (mEq/hr)</th>
<th>U_{K}V (mEq/hr)</th>
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</thead>
<tbody>
<tr>
<td>Normal rats</td>
<td></td>
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<tr>
<td>Normal Na+ intake (Group 1)</td>
<td>129 ± 2 (n = 10)</td>
<td>1.18 ± 0.18 (n = 5)</td>
<td>966 ± 62 (n = 5)</td>
<td>29.87 ± 3.6 (n = 10)</td>
<td>35.27 ± 2.7 (n = 10)</td>
</tr>
<tr>
<td>Na+-free intake + furosemide (Group 2)</td>
<td>133 ± 2* (n = 11)</td>
<td>9.62 ± 0.9† (n = 6)</td>
<td>883 ± 74* (n = 6)</td>
<td>0.18 ± 0.02† (n = 11)</td>
<td>25.6 ± 8.7* (n = 11)</td>
</tr>
<tr>
<td>Binephrectomized rats</td>
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<tr>
<td>Normal Na+ intake (Group 3)</td>
<td>106 ± 3† (n = 10)</td>
<td>0 (n = 5)</td>
<td>3755 ± 205† (n = 5)</td>
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<td>—</td>
</tr>
<tr>
<td>Na+ free intake + furosemide (Group 4)</td>
<td>85 ± 4‡ (n = 10)</td>
<td>0 (n = 5)</td>
<td>4430 ± 522‡ (n = 5)</td>
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</table>

From all groups, four rats served as subsequent controls for the action of angiotensinogen antiserum on blood pressure. AI = angiotensin I; U_{Na}V = urinary sodium excretion; U_{K}V = urinary potassium excretion, n = number of animals. Values are means ± SEM.

Furosemide was given subcutaneously at a dose of 20 mg/kg/day, 48 and 24 hours before antibody experiment. *NS. †p < 0.001 compared to Group 1. ‡p < 0.001 compared to Group 1 or 2. §p < 0.001 compared to Group 3.
Antibodies against AII have been injected in rats and have induced a fall in the blood pressure of normotensive sodium-replete rats and in the two-kidney, one clip model of experimental hypertension. Gamma globulin directed against pure pulmonary converting enzyme also decreased blood pressure of normotensive conscious rats and of two-kidney, one clip hypertensive rats. Intravenous injection of antisera against pure dog renin caused a prompt decrease in arterial blood pressure of conscious normotensive dogs maintained on a low sodium diet and in uninephrectomized animals with renovascular hypertension but did not change blood pressure of normotensive sodium-replete dogs. On the basis of these results, immunologic blockade of either AII, renin, or converting enzyme leads to the conclusion that these three elements of the renin-angiotensin system contribute importantly to the control of blood pressure under the circumstances of the experiments.

Moreover, use of AII antagonists, or converting enzyme inhibitors, or renin inhibitors in other experiments have led to the same conclusion and have emphasized the role of sodium balance and the renin-angiotensin system in the maintenance of blood pressure.

In our present experiments, a new tool has been made available, since purification of rat angiotensinogen has led to the production of specific rat angiotensinogen antibodies. The gamma globulin fraction isolated from the antisera neutralized in vitro the biological property of rat renin substrate, i.e., the ability to generate AII through the enzymatic action of renin.

The in vivo results of the present study show that angiotensinogen antibody injection evokes a hypotensive response in the normal conscious rat maintained on normal sodium diet and provides evidence that the renin-angiotensin system, through angiotensinogen, participates in blood pressure homeostasis in this situation. As anticipated, the hypotensive effect of angiotensinogen antibodies was more pronounced in the sodium-depleted state than in the normal sodium state. A very limited fall in blood pressure is also observed in binephrectomized animals after sodium depletion and fits well with the hypotensive effect of other blockers of the renin-angiotensin system in binephrectomized animals.

It is unlikely that the blood pressure reduction observed in our experiments resulted from a nonspecific effect; we cite six factors supporting this argument. 1) In vitro results showed inhibition of the enzymatic action of renin on angiotensinogen. 2) No side effects were observed during the injection, especially no cutaneous nor respiratory phenomena that would suggest an anaphylactoid reaction. The hypotension was delayed and progressive, and the recovery of blood pressure was also a progressive phenomenon. All these observations suggest a physiological process and not a pathological response. By comparison, an intravenous injection of 10 to 50 μg/kg of histamine causes an immediate fall in rat blood pressure followed immediately by a rise above the basal value.

**Discussion**

The passive transfer of renin or AII antibodies has been used previously to inhibit the renin-angiotensin system in vivo and to elucidate its role in physiological and pathological circumstances. Many experiments performed with antibodies raised against impure haptens have been criticized due to the possible occurrence of nonspecific effects, but during the past several years, purification of hog, human, and dog renin as well as rabbit converting enzyme has been realized and has led to the preparation of specific antibodies.

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3) The injection of non-immune rabbit serum was devoid of any effect on blood pressure. 4) Binephrectomized rats maintained on normal sodium diet, in which renin levels are not detectable, did not respond to antibody injection. 5) The injection of purified gamma globulin gave the same response curve as the injection of antiserum. 6) Albumin antiserum injection did not change the blood pressure of sodium-deficient rats.

The blood pressure decrease observed in our experiments is not explained by a change in vascular reactivity to AI nor by a change in converting enzyme activity. Indeed, injections of 50 ng of AI following angiotensinogen antiserum administration always had a pressor effect, which demonstrates both the continued conversion of AI to All and the continued vascular response to these peptides. Furthermore, the pressor effect of AI was slightly increased, which could be explained in terms of the increase in free All receptors caused by the angiotensinogen antibody. The profound fall in pressure in nonnephrectomized rats contrasts with the short-term duration but could be explained in different ways. The immediate inactivation of angiotensinogen could be followed by its rapid production, requiring continuous infusion of large amounts of antibody for neutralization of the biological properties. The duration of hypotension is also relatively short when All antibodies are injected, suggesting that compensatory physiological responses can rapidly elevate the blockade.

Our results with angiotensinogen antisera suggest that the renin-angiotensin system is a normal mechanism for maintaining blood pressure in normotensive rats. The system is more important in the sodium-depleted state than in the normal sodium state. These observations are in full agreement with results obtained with other blockers of the renin-angiotensin system, but reemphasize the importance of angiotensinogen in the system.

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