RENAL HYPERTENSION IN RATS IMMUNIZED AGAINST ANGIOTENSIN I AND ANGIOTENSIN II*

By HELEN F. OATES, GORDON S. STOKES, BRIAN G. STOREY, ROBYN G. GLOVER, and BEVERLEY F. SNOW

(From the Cardio-Renal Unit, Medical Research Department, Kanematsu Memorial Institute, Sydney Hospital, Sydney, New South Wales 2000, Australia)

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Rat hypertension caused by constricting one renal artery, leaving the contralateral kidney untouched (two-kidney Goldblatt hypertension), is more characteristically accompanied by a rise in plasma renin activity than other forms of experimental renal hypertension (1). Also, it represents a close counterpart of clinical renovascular hypertension. Thus, it is of particular importance in investigating a possible role of the renin-angiotensin system in the pathogenesis of renal hypertension.

In this model, renin preinhibitor (2), angiotensin converting enzyme inhibitor (3), and a specific competitive inhibitor of angiotensin II (4), have each been shown to reduce blood pressure, so arguing in favor of an active contribution by angiotensin to the hypertensive state. Conversely, studies relying on angiotensin II (AII)[1] antibodies to neutralize endogenous angiotensin (5, 6), have provided good evidence that the direct pressor effect of circulating angiotensin is not essential for the development and maintenance of this type of hypertension. The issue thus remains unresolved.

It has been suggested that the precursor of AII, angiotensin I (AI), may exert a direct but weak action on AII vascular receptors (7). In addition, recent work has shown that AI has a significant central vasomotor action in the vertebral artery territory (8) and a marked, direct stimulatory effect on the adrenal medulla (9). Neither action is blocked nor even impaired by AII antibody. In view of these important findings, we felt that a study incorporating active immunization against AI was needed to complement data based on AII immunization alone (5) and to provide more valid immunologic evidence from which to evaluate the part played by the renin-angiotensin system in renal hypertension.

Materials and Methods

Animals.—Female Wistar rats weighing 240–290 g and kept on a standard laboratory diet were divided into three groups and immunized for 6–7 mo before renal artery constriction.

Immunization.—One group of 10 rats was immunized against AII by i.p. injection, at 3-wk

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[1] *Abbreviations used in this paper:* AI, angiotensin I; AII, angiotensin II.

intervals, of (Asn^1, Val^5)-angiotensin II (Hypertensin, Ciba Pharmaceutical Co., Summit, N. J.; 0.5 mg per rat) coupled to bovine serum albumin by glutaraldehyde condensation (10), and emulsified in Freund's complete adjuvant. A second group of 30 rats was similarly immunized against both AI and AII with (Ile^5)-angiotensin I (Schwarz Bio Research, Inc., Orangeburg, N. Y.), and (Asn^1, Val^5)-angiotensin II (0.2 mg of each peptide per rat). A control group of 30 rats was mock immunized with Freund's adjuvant according to the same time schedule.

Immunologic Evaluation.—

Antibody titers: Blood from each rat was sampled at 2–3-wk intervals. A leg vein was pierced under ether anesthesia, and blood collected by capillarity into a hematocrit tube containing EDTA (1 mg/ml). Serially diluted plasma (50 μl) was incubated with 40 pg (10,000 cpm) of [^{125}I]AII, or with the same amount of [^{125}I]AI, at pH 7.4 and 4°C for 24 h, and the titer that bound 50% of each iodinated peptide was determined.

Bioassay Rats: At the termination of the experiment, immune and control rats were anesthetized with Inactin (Promonta, Hamburg; 100 mg/kg i.p.), tracheotomized, vagotomized, and injected i.m. with atropine sulfate (1.5 mg/kg) and pentolinium tartrate (25 mg/kg). Two polyethylene catheters (PE 10) were ineserted into the right jugular vein, and the right common carotid artery was catheterized (PE50) and connected via a Statham pressure transducer (Statham Instruments, Inc., Los Angeles, Calif.) to a Sanborn polygraph (Sanborn Engineering Co., Park Ridge, Ill.) for continuous monitoring of arterial pressure. Using the venous catheters, dose-response curves were determined for AI and AII, and pressor responses to hog renin (General Biochemicals Div., Chagrin Falls, Ohio; 1 dog U/mg) and norepinephrine (Levophed, Winthrop Pharmaceuticals, New York) were recorded.

Induction of Renovascular Hypertension.—Under ether anesthesia, a silver clip was applied to the left renal artery of each immune and control rat (11), the contralateral kidney being untouched. This method, used in our laboratory in previous studies involving 120 rats, has consistently produced hypertension in 50% of rats, the mean postoperative systolic blood pressure of these being 165 ± 9 (SE) mm Hg, as opposed to 125 ± 1 preoperatively. The clips used in our work (0.27 mm i.d.) were wider than those (0.20 mm i.d.) recently reported to induce more predictable hypertension (5, 12).

Blood Pressure Determination.—The systolic blood pressure of each rat was measured twice a week for 3 wk before and 6 wk after renal artery constriction, by the method of Eide, (5), employing a photoelectric cell and a proximal cuff on the tail of the conscious, prewarmed rat.

RESULTS

After 7 mo of immunization, 27 of the rats immunized against AI and AII, and all 10 of those immunized against AII only, were considered to be immune on the basis of sustained antibody titers. This was subsequently confirmed by bioassay. Those immunized against equal parts of AI and AII were found to have developed antibodies with antiangiotensin titers 6–15 times greater for AI than for AII and were thus predominantly AI-immune. There were 29 rats in the mock-immunized control group.

Blood pressures of all rats were measured twice per week for 3 wk before renal artery constriction. The mean preoperative systolic blood pressure of the control group was 125 ± 1 (SE), that of the (AI + AII)-immune group was 126 ± 1, and that of the AII-immune group 125 ± 2. Thus, sustained AI and/or AII immunity did not alter blood pressure ($P > 0.4$ in each case).

Development of Hypertension.—From the control group of 29 rats, 14 (48%) developed stable hypertension (>150 mm Hg) within 17 days after application

of the renal artery clip. Corresponding figures for the (AI + AII)-immune group were 14 out of 27 (52%), and for the AII-immune group, 6 out of 10 (60%). The mean systolic blood pressure of those developing hypertension in each group is plotted against time in Fig. 1. All groups reached comparable levels of hypertension, mean blood pressures during the period of study, from day 17 to 6 wk after operation, being 165 ± 4 (SE) for control rats, 167 ± 6 for the (AI + AII)-immune rats, and 167 ± 9 for the AII-immune rats. Thus, neither AII immunity, nor combined AI and AII immunity, changed the

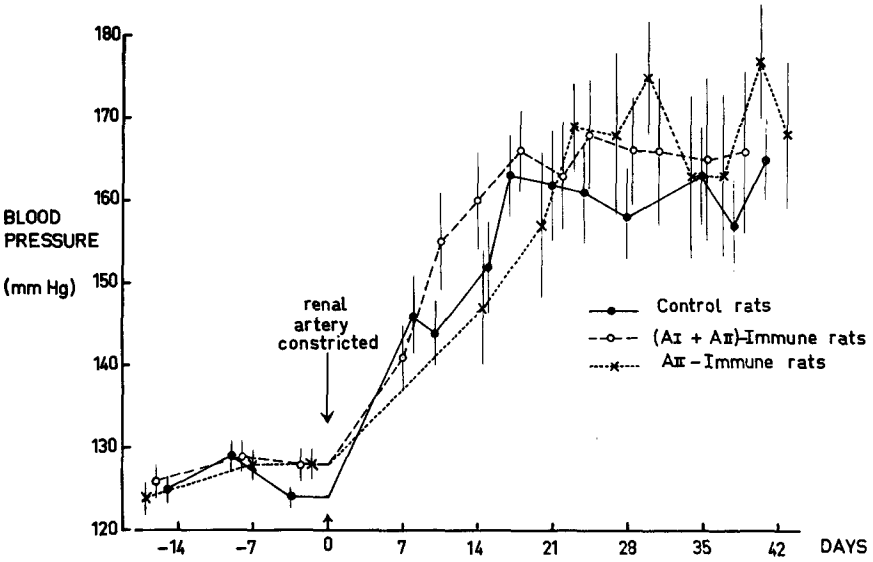

FIG. 1. Preoperative and postoperative systolic blood pressures of 14 control mock-immunized rats (●), 14 (angiotensin I + II)-immune rats (○), and 6 angiotensin II-immune rats (×) that became hypertensive after left renal artery constriction. Vertical bars indicate standard errors of means.

severity of hypertension from that observed in the control group ($P > 0.8$ for each). The mean postoperative systolic blood pressure of rats failing to develop hypertension was 131 ± 1 (SE) in the control group, 129 ± 1 in the (AI + AII)-immune group, and 132 ± 3 in the AII-immune group.

Despite the fact that half of the rats in each group did not exhibit hypertension (>150 mm Hg) during the 6 wk after left renal artery constriction, postmortem examination revealed the left kidney to be reduced in size and the right kidney hypertrophied. From the 29 nonimmune control rats, those that developed hypertension had a ratio of left kidney weight:right kidney weight of 0.51 ± 0.08 (SE), and those failing to become hypertensive 0.59 ± 0.08. The last two groups were not significantly different from each other ($P > 0.4$), but both were highly significantly different from the ratio of 0.99 ± 0.01 found in

normal unoperated female rats of the same age ($P < 0.001$ in each case). In the angiotensin-immune groups, the hypertensive rats had a left : right kidney weight ratio of 0.56 ± 0.08, and those failing to become hypertensive, 0.60 ± 0.09 (differences from corresponding nonimmune groups, $P > 0.6$). As in the control group, this difference was not significant ($P > 0.7$).

Immunologic Evaluation.—The efficacy of angiotensin immunization was tested in two ways: (*a*) by regularly sampling the blood of all rats, throughout the period of study, for in vitro radioimmunological determination of plasma antibody concentration, expressed as antibody titer; and (*b*) by injecting the rats i.v. with angiotensin, renin, and norepinephrine, to determine in vivo their pressor sensitivity to synthetic and endogenous angiotensin.

(*a*) In the group of rats immunized against both angiotensin I and II, the mean plasma anti-AI titer before renal artery constriction was $\frac{1}{29,000} \pm \frac{1}{4,000}$ (SE), and the anti-AII titer $\frac{1}{2,000} \pm \frac{1}{700}$. Immunization was continued every 2–3 wk postoperatively, so that titers remained fairly constant. The mean values for this group over the entire postoperative period were: anti-AI antibody $\frac{1}{25,000} \pm \frac{1}{4,600}$ and anti-AII $\frac{1}{4,000} \pm \frac{1}{2,000}$. In the case of the AII immunized group, the mean anti-AII titer for the postoperative period was $\frac{1}{13,000} \pm \frac{1}{4,000}$, but titers waned between immunizations to values as low as $\frac{1}{7,000} \pm \frac{1}{500}$. No AI cross-reactivity was detectable. High titers of antibody against AII were more difficult to elicit and maintain than were those against AI.

In the (AI + AII)-immune group, during the first 17 postoperative days, the 14 rats that developed hypertension proved to have somewhat higher antibody titers than did the 13 remaining normotensive. Mean anti-AI titers were $\frac{1}{32,000} \pm \frac{1}{6,900}$ (SE) and $\frac{1}{17,000} \pm \frac{1}{5,000}$ for hypertensives and normotensives, respectively ($0.05 < P < 0.1$). The difference between their anti-AII titers was not significant ($P > 0.2$). Similarly, in the AII-immune group, during this period, the mean anti-AII titer of the rats that became hypertensive was $\frac{1}{18,000} \pm \frac{1}{5,600}$ (SE), as opposed to $\frac{1}{6,000} \pm \frac{1}{1,700}$ in those remaining ($0.05 < P < 0.1$).

In Fig. 2, the maximal postoperative systolic blood pressure of each (AI + AII)-immunized rat that became hypertensive is plotted against its plasma anti-AI antibody titer at the time of the peak pressure reading. It is evident that the severity of hypertension was virtually uninfluenced by anti-AI antibodies (correlation coefficient = 0.17).

(*b*) Table I summarizes the pressor sensitivity of angiotensin-immunized and control mock-immunized rats to i.v. injected AI, AII, hog renin, and norepinephrine. Six hypertensive rats from each group (classed as nonimmune, predominantly AI-immune, and AII-immune, on the basis of their antibody titers) were set up as bioassay preparations to test in vivo the degree and specificity of their immunity. Six nonimmune rats that did not become hypertensive were also included. A 3–4-point log dose-response plot was first determined for both

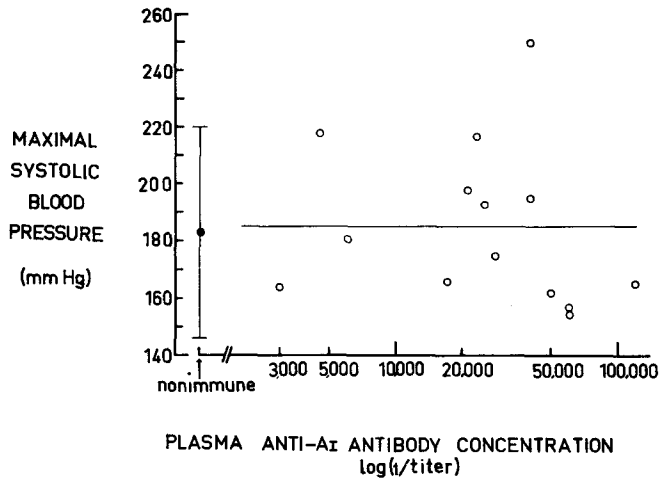

FIG. 2. The maximal systolic blood pressures of 14 predominantly AI-immune rats (open circles) that became hypertensive after unilateral renal artery constriction, are plotted against their anti-AI antibody titers at the times of peak pressure. Note the lack of correlation. The mean of the maximal pressures in the control group (closed circle) was 183 mm Hg (±2 SD indicated by vertical bars), as compared to 185 in the immune group.

AI and AII, and the doses required to give a systemic pressor response of 15 mm Hg were calculated by solving the linear regression equations. Pressure increments resulting from paired injections of 0.05 U of hog renin, and 30 ng of norepinephrine were then recorded.

From examination of Table I, it is clear that, for nonimmune clipped rats, there was no significant difference between those that were normotensive, and those that were hypertensive, with respect to responsiveness to the pressor agents tested ($P > 0.3$ in each case). On the other hand, much greater doses of AI and AII were required to elicit a pressure rise of 15 mm Hg in each of the angiotensin-immunized groups than in control rats with hypertension of comparable severity. In addition, the pressor response to 0.05 U of hog-renin, averaging 40.8 ± 3.2 (SE) mm Hg in the nonimmune hypertensive rats, was virtually abolished in both the predominantly AI-immune and the AII-immune groups ($P < 0.001$), despite the fact that the mean antibody titers of both groups were considerably lower at the time of bioassay than they had been during the crucial period of developing hypertension (60% and 44%, respectively). The response to 30 ng of norepinephrine was not significantly different in either immune group from that seen in the nonimmune hypertensive rats ($P > 0.2$ for each).

The predominantly AI-immune group (Table I, column 3) was found to be more resistant to the pressor effects of both endogenous angiotensin, liberated by injected renin, and synthetic (Ile^5)-angiotensin I, than was the AII immunized group (column 4), as may have been anticipated from the differing specificities and titers of their antibodies.

TABLE I

Pressor Sensitivity of Hypertensive, Angiotensin-Immune Rats, Compared to that of Normotensive and Hypertensive, Nonimmune Rats* (Means ± SE)*

Normotensive nonimmune	Hypertensive nonimmune	Hypertensive AI-immune	Hypertensive AII-immune
Blood pressure (BP) (*mm Hg*)			
135 ± 2	171 ± 7	172 ± 8	172 ± 10
Angiotensin dose (*ng i.v.*) causing 15 mm Hg BP rise			
AI 2.8 ± 0.2	2.9 ± 0.3	754 ± 252	223 ± 110
AII 2.5 ± 0.2	2.5 ± 0.3	102 ± 42	411 ± 245
BP response to 0.05 units of hog renin (*mm Hg*)			
45 ± 4	41 ± 3	1.7 ± 0.9 (Negligible)	4.2 ± 1.2
BP response to 30 ng of norepinephrine (*mm Hg*)			
33 ± 1	33 ± 3	29 ± 2	28 ± 3
Plasma antiangiotensin antibody titers at the time of bioassay			
AI Nil	Nil	1/19,000 ± 1/4,900	Nil
AII Nil	Nil	1/1,000 ± 1/300	1/8,000 ± 1/1,600

* Six rats from each of the four groups were studied 6–8 wk after renal artery constriction, and had been classed as nonimmune, predominantly AI-immune, or AII-immune, on the basis of plasma antibody titers against (Ile^5)-angiotensin I (AI), and (Asn^1, Val^5)-angiotensin II (AII), determined by radioimmunoassay.

DISCUSSION

It is reasonable to suppose that, if pressor activity of angiotensin were primarily involved in the etiology of renovascular hypertension caused by unilateral renal artery constriction, then effective immunization against angiotensin should prevent such hypertension (5, 13). It has been demonstrated that, only 3 wk after induction of unilateral renal ischemia, the ischemic kidney ceases to release into renal vein blood the potent vasopressor agent demonstrable in arterial blood during the acute stage of hypertension (14). It is possible that hypertension may then persist independent of the causative agent (11). Thus, in the present study, as in the AII immunization study of Eide (5), angiotensin immunity was established before application of the renal artery

clip, and antiangiotensin antibody titers of the rats were maintained at peak levels postoperatively, thoughout the early stage of the hypertension.

To test in vivo the adequacy of their immunity, angiotensin-immunized and mock-immunized control rats were challenged with large doses of AI, AII, and hog renin. None of the rats in either the predominantly AI-immune group or the AII-immune group responded at all to doses of AI or AII less than 55 ng, which was about 50 times the dose required to give a detectable pressor response in nonimmune rats with hypertension of comparable severity. One hypertensive rat with an anti-AI antibody titer of $\frac{1}{25,000}$ at the time of bioassay (as compared to the mean titer of $\frac{1}{32,000}$ attained by the 14 AI-immune rats that became hypertensive), required 2,100 ng of AI to elicit a pressor response. Thus, despite the fact that antibody titers had fallen considerably by the time of bioassay, our rats (summarized in Table I), were at least as effectively immune as were those of Eide (5), which required an average angiotensin dose of 490 ng to produce a definite pressor effect. The lack of responsiveness of the immune animals was not due to a nonspecific refractoriness, because they all responded normally to norepinephrine. Furthermore, the injection of hog renin ensured that the antibodies raised by immunization of the rats against synthetic angiotensins I and II were also able to neutralize endogenous angiotensin liberated from rat renin substrate by the injected renin.

For several reasons, listed below, it occurred to us that AII immunization might not completely block endogenous activity of the renin-angiotensin system. We therefore felt that it would be important to try combined AI and AII immunization before ruling out, on the basis of immunological evidence, participation of a direct pressor effect of circulating angiotensin in renal hypertension. The following recent findings cast doubt upon the adequacy of AII immunization alone: (*a*) The catecholamine-releasing effect of AII is blocked by AII antibody, whereas adrenal medullary responses to AI remain unchanged (9). (*b*) The hypertensive effect of vertebral artery infusion of AI in dogs is virtually unimpaired by administration of AII antibody, while that of AII is abolished (8). (*c*) AI may have direct activity on AII receptors amounting to 4% of that of AII (7), and, in addition, may be partly converted to AII, intramurally, at extrapulmonary sites (7), thus minimizing exposure of AII to circulating antibodies. (*d*) Concentrations of AI in renal venous blood are reported to be 45 times greater than those of AII and in arterial blood, 24 times greater (15). The circulating concentration of AI, relative to that of AII, thus appears to be high enough to consider AI as an effector hormone (15).

From examination of Table I, it is clear that rats, shown in vitro to have produced antibodies directed predominantly against AI, inactivated AI in vivo more effectively than they did AII. On the other hand, rats immunized against AII inactivated injected AI less readily than they did AII, consistent with the in vitro specificity of their antibodies,[2] but also consistent with residual activity

[2] For immunization, antibody assay, and i.v. injection, the AI used was (Ile^5)-angiotensin I, and the AII, (Asn^1, Val^5)-angiotensin II.

of AI, as mentioned above (*a–c*). It should be pointed out that the anti-AII titers of the group immunized against AI and AII were four times greater during the time of developing hypertension than they were at the time of bioassay, so that this group was indeed effectively immune to both AI and AII. Results reported here thus establish that sustained immunity to both AI and AII neither changes the preoperative blood pressure of rats from that of control mock-immunized rats nor alters the incidence or severity of hypertension induced by unilateral renal artery constriction.

There is much evidence indicative of increased reactivity of blood vessels to norepinephrine and angiotensin in hypertensive animals (16, 17) and man (18, 19). The pressor effect of small quantities of free endogenous angiotensin, escaping neutralization by circulating antibodies, could be increased by such augmented vascular reactivity. Thus, it was of interest to note that, in the present study, there was no significant difference between normotensive rats and hypertensive rats (whether angiotensin immune or nonimmune), with respect to pressor sensitivity to norepinephrine. Nor was there any significant difference between the pressor responsiveness of normotensive and hypertensive nonimmune rats, to angiotensins I and II. This is in contrast to results of Christlieb et al. (20), who found that rats made hypertensive by unilateral renal artery clipping showed a significantly subnormal responsiveness to angiotensin. Perhaps the explanation for the differing results lies in the fact that our control rats had been subjected to renal artery constriction, but had not become hypertensive, whereas, in the study of Christlieb, normal rats served as controls. Our rats were also ganglion blocked before bioassay to minimize interference from homeostatic blood pressure control mechanisms.

The evidence from our experiments thus makes it extremely difficult to sustain any major role for circulating AI, or AII, in initiating that form of renovascular hypertension induced in rats by unilateral renal artery constriction without contralateral nephrectomy. The results in no way conflict with those of Carretero et al. (21), for these authors studied the accelerated or malignant phase of hypertension produced in rats by coarctation of the aorta. Our results also rule out involvement of AII produced from circulating AI by conversion within arteriolar walls, close to receptor sites (7, 22), for AI immunity would block such a mechanism where AII immunity might fail. Moreover, although the renin-angiotensin system has been shown to possess sufficient time response and gain characteristics to participate significantly in the normal regulation of arterial pressure (23), it is clear that a normal pressure is maintained in the conscious rat, despite sustained immunity to both forms of angiotensin.

SUMMARY

Rats, actively immunized against angiotensin I (AI) and angiotensin II (AII), were subjected to unilateral renal artery constriction to determine whether the resulting hypertension, which may still ensue in the animal immunized against AII, could be prevented by such combined immunity.

Sustained immunity to both AI and AII neither changed preoperative blood pressures of the rats from those of control mock-immunized rats nor altered the incidence or severity of renal clip hypertension. Vascular hyperresponsiveness to small quantities of free angiotensin could not be invoked to explain the hypertension, for there was no significant difference between mock-immunized hypertensive animals, and those remaining normotensive, regarding pressor sensitivity to intravenous AI, AII, renin, and norepinephrine. (AI + AII)-immunized hypertensive rats required AI doses averaging 260 times greater than nonimmune hypertensives to elicit equipressor responses, and were refractory to renin, but not to norepinephrine.

Thus, while previous studies have not excluded direct participation of endogenous AI in renal clip hypertension in rats, evidence from our experiments makes it extremely difficult to sustain any pressor function therein for circulating AI or AII. Our results also preclude involvement of AII produced from circulating AI by conversion within arteriolar walls, close to receptor sites, since AI immunity would block this mechanism of action.

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