GENETIC INFLUENCE ON THE RENIN-ANGIOTENSIN SYSTEM

Evidence for a Renin Inhibitor in Hypertension-Prone Rats*

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In experimental hypertension due either to high salt intake or to renal manipulations, we reported previously that rats from a strain with a genetic predisposition to hypertension produced a humoral factor which was transmittable in parabiosis and induced hypertension in rats genetically resistant to this condition. We surmised that the factor is identical in these two "kinds" of experimental hypertension and have evidence that it is produced by the kidneys of the hypertension-prone rats (1–4).

The renin-angiotensin system is known to be coupled to sodium metabolism and is thought to be responsible for the development of renal hypertension. The mechanisms involved are by no means clear. We have therefore explored this system in both strains of rats, and have studied its possible relation to the hypertensinogenic factor encountered in hypertension-prone rats. The most striking single observation was that the kidney of the hypertension-prone rats produced a renin inhibitor that could not be demonstrated in animals from the hypertension-resistant strain. The implications of these findings are discussed.

Materials and Methods

The rats employed in this experiment were developed in this laboratory and are called "R" and "S" because of their resistance or sensitivity, respectively, to developing hypertension induced by salt (NaCl). Detailed reports on breeding, feeding, and care have been published (5, 6) and only details pertinent to the current studies will be included here. Special low salt chow (0.4% NaCl), high salt chow (8% NaCl), and tap water were used. The animals were weaned at 21–23 days and then were maintained on tap water and the low salt chow until they were ready for the experiment. Purified hog renin was purchased from Nutritional Biochemicals Corp., Cleveland, Ohio, and angiotensin-II (Hypertensin-Ciba, Ciba Pharmaceutical Co., Summit, N. J.) was donated to us.[1] Statistical analysis was done by standard techniques; some of the computations were performed on a computer.[2]

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[1] We are grateful to Dr. Robert Gaunt of Ciba for this gift.

[2] We thank Keith Thompson for help with this program.

Experiment I

Pressor Responses to Angiotensin in R and S Rats.—A solution of 0.5 μg/ml of angiotensin[3] in 0.9% NaCl and bacitracin (0.05%) was prepared and the pH was adjusted to 7.5 with Na_2CO_3. This standard solution was kept at 4°C and prepared every 2 wk. 320 rats of either sex (160 from each strain), maintained on low NaCl since weaning, were used as they became available when they weighed 100–110 g (30–40 days of age). At that age, groups of 16 rats from the same strain were subjected to the procedures and diets described below. Of the 16 rats in each group, however, only 10 were ultimately used for the pressor response tests, the criterion for selection being that their body weight should be 180–195 g at the time of the test. In each of the following studies (A–E), four groups of 10 rats were tested: two groups, each consisting of 10 R and 10 S rats, had been maintained on high NaCl chow for 3 wk, and two similar groups had been on low NaCl chow for the same period. The tests were performed under sodium pentobarbital anesthesia (35 mg/kg body weight, intraperitoneally). A polyethylene tube (PE 10) was inserted into the jugular vein and connected to a microsyringe (The L. S. Starrett Co., Athol, Mass.) for the administration of angiotensin. The carotid artery on the opposite side was similarly cannulated and the carotid blood pressure recorded by means of a Grass polygraph and a Statham pressure transducer. After the blood pressure had become stabilized a standard dose of 5.6 ng angiotensin/kg body weight was administered and the blood pressure response recorded. Each rat was subsequently tested on higher doses to ascertain that the log dose/response curve was linear.

Study A

Intact Rats.—These four groups (R high salt, R low salt, S high salt, S low salt) were kept for 3 wk with no manipulations until the day of the sensitivity test.

Study B

Unilateral Renal Artery Constriction.—In these animals a silver clip was applied to the left renal artery by the technique of Wilson and Byrom (7); they were then placed on their respective diets and, 3 wk later, the test was performed as described above.

Study C

Unilateral Nephrectomy.—Uninephrectomy was performed through a skin incision over the left lumbar region. The study was otherwise identical with study B.

Study D

Unilateral Renal Artery Constriction with Contralateral Nephrectomy (Goldblatt Procedure).—The right kidney was removed and, 2 days later, a silver clip was applied to the left renal artery. Thereafter the animals were handled as in study B.

Study E

Bilateral Nephrectomy.—The four groups were maintained on their respective diets as in study A, but after 3 wk bilateral nephrectomy was carried out in one stage. 24 hr after nephrectomy, the sensitivity test was done.

Experiment II

Pressor Responses to Renin in R and S Rats.—A solution of 20 units/ml of hog renin in distilled water was prepared and kept at 4°C. Using different rats, experiment II was identical

[3] The term angiotensin will be used hereafter to refer to the active vasopressor, angiotensin II.

with experiment I, except that hog renin (0.1 unit/kg body weight) instead of angiotensin was used. Each rat was tested by only one injection to avoid tachyphylaxis.

Experiment III

Determination of Angiotensin Formation by Renin in the Presence of Plasma from Intact and Nephrectomized Rats.—128 rats (64 from each strain) of either sex were used. All were kept on low salt chow and tap water until they reached a weight of 200–240 g. At this stage, half (32 from each strain) were nephrectomized bilaterally 24 hr before bleeding, the other half just before bleeding. The latter half was considered "intact"; the purpose of the nephrectomy just prior to bleeding was to avoid the stimulating effect of such bleeding on renin secretion. Blood was obtained from the abdominal aorta under anesthesia using a disposable syringe moistened with a 15% solution of the ammonium salt of ethylenediaminetetraacetic acid (EDTA) as an anticoagulant. It was kept on ice during the collection period; after centrifugation at 0–4°C for 10 min at 10,000 rpm, the plasma was removed and stored frozen at −20°C

TABLE I

Classification of Tubes Used in Measurements of Renin Activity In Vitro

Tube No.	Contents	Substrate*	Additional incubation time
		mg	*min*
1	3 ml plasma + 0.1 ml H_2O	0	0
2	3 ml plasma + 0.1 ml H_2O	0	15
3	3 ml plasma + 0.1 ml H_2O	150	15
4	3 ml plasma + 0.1 ml H_2O	150	30
5	3 ml plasma + 0.01 unit (0.1 ml) hog renin	0	0
6	3 ml plasma + 0.01 unit (0.1 ml) hog renin	0	15
7	3 ml plasma + 0.01 unit (0.1 ml) hog renin	150	15
8	3 ml plasma + 0.01 unit (0.1 ml) hog renin	150	30

* Rat renin substrate was used. For other details, see text.

until it could be assayed. Renin activity level was measured by the micromethod of Boucher et al. (8).[4] Renin substrate from rat plasma also had been prepared for the assay according to this method. Angiotensinase was destroyed before testing both in the renin substrate preparation and in the plasma samples by treatment with EDTA (8) and the addition of one drop of diisopropylfluorophosphate (DFP) in isopropyl alcohol (1:20 dilution) to the incubation mixture (9). To determine whether the treatment was effective in inhibiting angiotensinase activity, known quantities of angiotensin were added to the plasma and incubated at 37°C for 30 min; no destruction of angiotensin was detected in 30 min of incubation with treated plasma. S rats, only, were used as bioassay preparations. Rats of either sex, weighing 120–160 g were nephrectomized bilaterally under ether anesthesia 18 hr prior to the assay. At the time of assay, under sodium pentobarbital anesthesia (35 mg/kg), the rats were intubated with a tube (PE 240) via tracheotomy; two polyethylene tubes (PE 10) were inserted into the right jugular vein, one for the administration of standard and one for sample; and a polyethylene tube (PE 50) was inserted into the left carotid artery for direct blood pressure recordings as described earlier. The response of the unknown was bracketed between known doses of standard angiotensin at two different levels. All results of this bioassay were expressed in terms of

[4] We thank Dr. R. Boucher and Dr. J. Genest for teaching us this method.

TABLE II

Pressor Responses to Angiotensin in Intact Rats and Those with Unilateral Renal Artery Constriction, Uninephrectomy, Unilateral Renal Artery Constriction with Contralateral Nephrectomy, and Bilateral Nephrectomy

Operation	A. Intact				B. Unilateral clip				C. Uninephrectomy				D. Unilateral clip with contralateral nephrectomy				E. Bilateral nephrectomy			
Diet	Low salt		High salt		Low salt		High salt		Low salt		High salt		Low salt		High salt		Low salt		High salt	
Strain	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R
Pressor response *mm* Hg	7	5	13	9	12	10	16	10	7	10	18	5	11	13	22	8	5	5	9	8
	12	7	15	10	8	7	13	12	14	6	12	11	12	7	12	12	6	6	8	8
	11	4	17	6	12	7	13	10	12	8	11	5	16	9	14	10	5	5	6	7
	9	5	13	6	11	10	10	6	14	6	15	8	12	8	17	7	7	8	6	8
	12	6	14	7	9	8	17	6	7	5	15	10	14	10	22	9	4	7	9	6
	8	8	14	6	15	6	16	10	7	9	15	10	16	9	15	16	6	8	9	5
	8	6	15	4	8	8	18	8	11	8	18	10	15	7	19	10	8	7	6	5
	11	6	15	7	14	8	13	6	10	10	13	6	15	6	15	14	6	4	7	5
	9	7	11	4	12	12	20	3	11	10	8	11	14	11	10	12	5	8	8	4
	8	6	14	7	16	10	14	9	10	4	16	6	12	8	11	9	4	5	5	7
Number of Rats	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
Mean pressor response *mm Hg*	9.5	6.0	14.1	6.6	11.7	8.6	15.0	8.0	10.3	7.6	14.1	8.2	13.7	8.8	15.7	10.7	5.6	6.3	7.3	6.3
SE	0.6	0.4	0.5	0.6	0.9	0.6	0.9	0.9	0.8	0.7	1.0	0.8	0.6	0.7	1.4	0.9	0.4	0.5	0.5	0.5

Each number is the response of one rat to the injection of angiotensin (5.6 ng/kg body weight). Each rat was subsequently tested on higher doses to ascertain that the log dose/response curve was linear.

Statistical significance of difference in mean pressor response to angiotensin. Statistics used in text: Intact rats: S > R, ($P < 0.01$); S high > S low, ($P < 0.01$); R high = R low, ($P > 0.05$). Bilateral nephrectomy: S = R, ($P > 0.05$); high = low, ($P > 0.05$). Among S rats: intact > bilateral nephrectomy, ($P < 0.01$). Among R rats: intact = bilateral nephrectomy, ($P > 0.05$).

Other statistics. Unilateral clip: S > R, ($P < 0.01$); S high > S low, ($P < 0.01$). Uninephrectomy: S > R, ($P < 0.05$); S high > S low, ($P < 0.01$). Unilateral clip + contralateral nephrectomy (Goldblatt): S > R, ($P < 0.01$); S high = S low, ($P > 0.05$); R high = R low, ($P > 0.05$). Among S rats: on low salt, Goldblatt = unilateral clip, ($P > 0.05$); Goldblatt > intact, ($P < 0.01$); unilateral clip > intact, ($P < 0.01$); uninephrectomy > intact, ($P < 0.05$); Goldblatt > uninephrectomy, ($P < 0.05$); all groups > bilateral nephrectomy, ($P < 0.01$). On high salt, intact = Goldblatt = uniclip = uninephrectomy, ($P > 0.05$); all groups > bilateral nephrectomy, ($P < 0.01$). Among rats R: on low salt, Goldblatt > intact, ($P < 0.01$); uniclip > intact, ($P < 0.05$); Goldblatt = uniclip, ($P > 0.05$); Goldblatt > bilateral nephrectomy, ($P < 0.05$); unilateral clip > bilateral nephrectomy, ($P < 0.05$). On high salt, Goldblatt > intact, ($P < 0.01$); Goldblatt > bilateral nephrectomy, ($P < 0.01$); intact = bilateral nephrectomy, ($P > 0.05$); Goldblatt > unilateral clip, ($P < 0.05$); unilateral clip = uninephrectomy, ($P > 0.05$).

nanograms of angiotensin per sample. The standard deviation of 13 measurements of angiotensin formation in portions of the same plasma from a pooled sample was ± 5%.

For each plasma sample (pooled from eight rats of the same strain), eight tubes were prepared. To each tube, 3 ml of angiotensinase-free plasma was added. Hog renin (0.01 unit) in 0.1 ml of distilled water was added to four tubes; to the other four tubes, 0.1 ml of water, only, was added. All tubes were preincubated for 3 hr at 37°C in a water bath with vigorous agitation using an Evapo-Mix (Buchler Instruments, Inc., Fort Lee, N. J.). After the preincubation, rat renin substrate or water was added and incubation continued as shown in Table I. The concentration of added substrate was sufficient to measure maximum velocity of renin activity during 30 min incubation; twice as much substrate added to the mixture did not increase the production of angiotensin and larger doses of renin produced proportionally greater amounts of angiotensin.

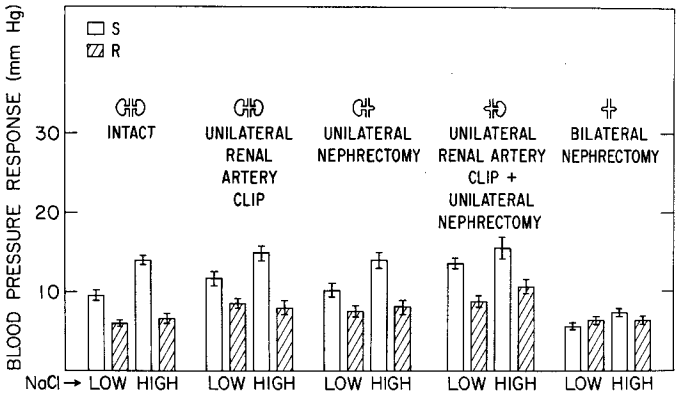

FIG. 1. Mean pressor responses to angiotensin. Columns represent the mean values from Table II of: A. intact; B. unilateral renal artery clip; C. uninephrectomy; D. unilateral renal artery clip with contralateral nephrectomy; E. bilateral nephrectomy groups. Vertical bar represents mean ± 1 SE.

OBSERVATIONS

Experiment I

Pressor Response to Angiotensin.—The pressor responses to angiotensin (5.6 ng/kg body weight) in the five studies (A–E) are summarized in Table II and Fig. 1. Since there was no difference in response between sexes, they have been combined. Analysis of variance indicated significant influence by the renal operations, the two diets, and the two strains ($P < 0.01$); also significant interaction between strain and operation, and between strain and diet ($P < 0.05$). S rats had a higher response than R rats in all but one study (bilateral nephrectomy), confirming our previous findings (10). High salt diet increased the response of S rats, but not of R rats, in the studies involving intact rats, uninephrectomized rats and those with renal artery clip. Hence, in general, S rats were more sensitive to angiotensin than R rats, and more influenced by diet

TABLE III

Pressor Responses to Renin in Intact Rats and Those with Unilateral Renal Artery Constriction, Uninephrectomy, Unilateral Renal Artery Constriction with Contralateral Nephrectomy, and Bilateral Nephrectomy

Operation	A. Intact				B. Unilateral clip				C. Uninephrectomy				D. Unilateral clip with contralateral nephrectomy				E. Bilateral nephrectomy			
Diet	Low Salt		High salt		Low salt		High salt		Low salt		High salt		Low salt		High salt		Low salt		High salt	
Strain	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R
Pressor response *mm Hg*	10	15	24	14	12	17	11	10	12	8	17	25	25	14	14	14	22	21	20	15
	7	19	19	24	13	16	24	12	12	18	15	8	32	11	16	16	19	15	16	17
	11	26	18	15	12	12	22	19	20	11	16	10	14	16	26	11	23	17	27	18
	14	18	14	16	15	11	20	13	9	16	22	24	11	18	24	15	25	18	17	18
	10	14	15	17	15	11	20	16	14	12	9	12	14	20	18	10	26	22	17	23
	15	17	14	15	17	14	22	8	13	6	9	17	13	23	24	10	18	26	26	24
	9	20	26	16	18	16	22	20	20	15	10	7	28	16	28	18	21	23	23	20
	10	14	23	14	18	12	25	20	18	20	20	8	24	14	18	9	16	16	26	17
	12	19	18	12	18	18	26	12	10	10	14	14	28	22	19	21	32	23	35	11
	14	18	14	18	22	20	17	19	16	13	11	9	17	20	13	16	21	17	27	16
Number of rats	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
Mean pressor response *mm Hg*	11.2	18.0	18.5	16.1	16.0	14.7	20.9	14.9	14.4	12.9	14.3	13.4	20.6	17.4	20.0	14.0	23.3	19.8	23.4	17.9
SE	0.8	1.1	1.4	1.0	1.0	1.0	1.4	1.4	1.2	1.4	1.4	2.1	2.4	1.2	1.6	1.2	1.7	1.2	1.9	1.2

Each individual figure is the response in one rat to the injection of hog renin (0.1 unit/kg body weight). Each rat was tested by only one injection to avoid tachyphylaxis.

Statistical significance of difference in mean pressor response to renin. Statistics used in text: Intact rats: S low < R low, ($P < 0.01$); R low = S high, ($P > 0.05$); R low = R high, ($P > 0.05$). Bilateral nephrectomy: S low = S high = R low, ($P > 0.05$). Among S rats: on low salt, bilateral nephrectomy > intact, ($P < 0.01$). On high salt, bilateral nephrectomy > intact, ($P < 0.05$). Among R rats: bilateral nephrectomy = intact, ($P > 0.05$). Other statistics. Intact rats: S low < R high, ($P < 0.05$). Among unilateral clip: S high > R low, R high, ($P < 0.01$); S high > S low, R high, ($P < 0.05$). Among Goldblatt operation: R high < S low, S high, ($P < 0.01$); S low = S high, ($P > 0.05$). Bilateral nephrectomy: R high < S low, S high, ($P < 0.01$); S low = S high, ($P > 0.05$). Among S rats: on low salt, Goldblatt > intact, uninephrectomy, ($P < 0.01$); intact = uninephrectomy, ($P > 0.05$); unilateral clip > intact, ($P < 0.05$); Goldblatt > unilateral clip, ($P < 0.05$); bilateral nephrectomy > unilateral clip, uninephrectomy, ($P < 0.01$); unilateral clip = uninephrectomy, ($P > 0.05$). On high salt, intact = Goldblatt, ($P > 0.05$); intact > uninephrectomy, ($P < 0.05$); uninephrectomy < Goldblatt, unilateral clip, bilateral nephrectomy, ($P < 0.01$); Goldblatt = unilateral clip = bilateral nephrectomy, ($P > 0.05$); Among R rats: on low salt, Goldblatt = intact, ($P > 0.05$); Goldblatt, intact > uninephrectomy, ($P < 0.05$); Goldblatt = bilateral nephrectomy, ($P > 0.05$); bilateral nephrectomy > unclip, uninephrectomy, ($P < 0.05$); unilateral clip = uninephrectomy, ($P > 0.05$). On high salt, bilateral nephrectomy > uninephrectomy, ($P < 0.05$).

and operation. After bilateral nephrectomy, however, the two strains had similar responses ($P > 0.05$).

Experiment II

Pressor Response to Renin.—The results are summarized in Table III and Fig. 2. Analysis of variance indicated that the two strains differed in their reaction to renin and that the responses were influenced by the renal operations. In contrast to the results with angiotensin, intact S rats on low salt showed significantly lower response to renin than R rats; in all other groups, however, S rats proved to be more sensitive to renin than R rats, and bilateral nephrectomy particularly increased the renin response of S rats. This contrasts sharply

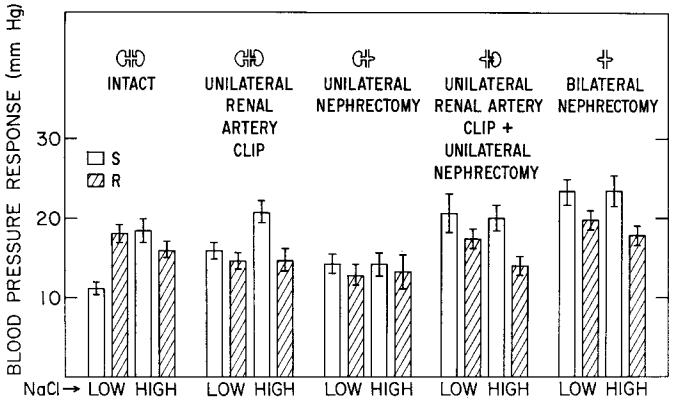

FIG. 2. Mean pressor responses to renin. Columns represent the mean values from Table III of: A. intact; B. unilateral renal artery clip; C. uninephrectomy; D. unilateral renal artery clips with contralateral nephrectomy; E. bilateral nephrectomy groups. Vertical bar represents mean ± 1 SE.

with their reduced response to angiotensin after nephrectomy. High salt diet had no uniform influence on the responses; intact S rats had an increased response, while R rats showed no change.

Experiment III

Determination of Angiotensin Formation by Renin in Presence of Plasma from Intact and Nephrectomized R and S Rats on Low Salt Diet.—

A. Determination of endogenous renin in R and S rats: Angiotensinase-free plasma was incubated at 37°C for 3 hr without addition of substrate or hog renin. At 3 hr (tube No. 1) plasma from intact R and S rats contained 123 ± 8.6 (SE) and 92 ± 7.7 ng of angiotensin, respectively ($R > S$, $P < 0.05$). Addition of excess substrate after 3 hr (tube Nos. 3 and 4) did not increase the reaction rate, which remained approximately 0.8 ng/min for R plasma and 0.5 ng/min

for S plasma. Addition of hog renin (0.01 units) to plasma during preincubation caused a marked increase in angiotensin production: 530 ± 28.1 and 405 ± 16.5 ng for intact R and S rats, respectively, after 3 hr (tube No. 5). These results indicate that endogenous renin substrate was in excess for the endogenous renin concentration during preincubation. Nephrectomy had the expected results of eliminating endogenous renin activity, and increasing the angiotensin produced by exogenous renin (Table IV).

TABLE IV

Endogenous Renin Activity Level, and Activity of Exogenous Renin in the Presence of Plasma from Intact and Nephrectomized Rats

Tube No.	Preincubation content	Addition of substrate	Additional incubation	Intact		Nephrectomy	
				R	S	R	S
				ng	*ng*	*ng*	*ng*
1	Plasma	−	0	123 ± 8.6	92 ± 7.7	0	0
2	Plasma	−	15 min	123 ± 10.4	95 ± 8.4	0	0
3	Plasma	+	15 "	136 ± 11.0	99 ± 7.8	0	0
4	Plasma	+	30 "	148 ± 10.1	107 ± 8.0	0	0
5	Plasma + hog renin	−	0	530 ± 28.1	405 ± 16.5	1077 ± 40.9	1014 ± 43.8
6	Plasma + hog renin	−	15 min	537 ± 31.9	413 ± 17.5	1173 ± 36.9	1106 ± 54.1
7	Plasma + hog renin	+	15 "	647 ± 34.0	440 ± 14.3	1207 ± 52.7	1138 ± 39.4
8	Plasma + hog renin	+	30 "	761 ± 39.7	474 ± 10.0	1318 ± 46.4	1246 ± 35.9

Each value is the average of four analyses on different samples. The figures represent ng angiotensin found in the mixture after each incubation was stopped by cooling to 0–4°C.

Statistical significance of difference: intact groups, tube Nos. 1, 3, 4, 6, R > S, ($P < 0.05$); tube Nos. 5, 7, 8, R > S, ($P < 0.01$); tube No. 2, R > S, ($P > 0.05$). Nephrectomy groups, tube Nos. 5, 6, 7, 8, R = S, ($P > 0.05$).

B. Determination of velocity of reaction between renin and substrate in presence of plasma from R and S rats: Hog renin (0.01 units) and rat renin substrate (150 mg) mixed and incubated for 15 and 30 min produced 134 ± 9.5 and 249 ± 14.0 ng of angiotensin, respectively (Table V). If hog renin was preincubated with R plasma for 3 hr before addition of substrate, no change in activity was observed. The values entered in Table V (116.5 ± 5.9 and 230.5 ± 16.7) are the increments observed after addition of excess substrate calculated for each sample by subtracting the value of tube No. 5 from the values in tube Nos. 7 and 8, respectively; they do not differ significantly from the control values ($P > 0.05$). Preincubation with S plasma resulted in significant inhibition as shown by Fig. 3 and the values in Table V (35.3 ± 2.6 and 68.8 ± 8.3),

TABLE V

Activity of Hog Renin in the Presence of Plasma from Intact and Nephrectomized R and S Rats on Low Salt

Operation	Intact		Nephrectomy*	
Incubation period	15 min	30 min	15 min	30 min
	ng	*ng*	*ng*	*ng*
A. Inhibition tests:				
R-plasma + hog renin + substrate	100	216	138	258
	125	258	104	236
	125	258	158	247
	116	190	118	223
Mean ± SE	116.5±5.9	230.5±16.7	129.5±11.8	241.0±7.5
S-plasma + hog renin + substrate	40	88	136	243
	29	49	116	231
	33	75	107	205
	39	63	139	252
Mean ± SE	35.3±2.6	68.8±8.3	124.5±7.8	232.8±10.2
B. Controls:				
R-plasma + substrate	21	31	0	0
	8	21	0	0
	9	26	0	0
	13	22	0	0
Mean ± SE	12.8±3.0	25.0±2.3	0	0
S-plasma + substrate	8	17	0	0
	6	14	0	0
	8	16	0	0
	8	17	0	0
Mean ± SE	7.5±0.5	16.0±0.7	0	0

	15 min	30 min
	ng	*ng*
C. Hog renin + substrate + water	125	220
	150	285
	150	255
	112	235
Mean ± SE	134.3±9.5	248.8±14.0

The values have been obtained by subtracting the control values (amount of angiotensin formed during 3 hr preincubation) as follows: hog renin activity in the presence of plasma: 15 min, tube No. 7 − tube No. 5; 30 min, tube No. 8 − tube No. 5. Endogenous renin activity: 15 min, tube No. 3 − tube No. 1; 30 min, tube No. 4 − tube No. 1.

Statistical significance of difference: hog renin activity: intact, 15 min and 30 min, R > S ($P < 0.01$); nephrectomy, 15 min and 30 min, R = S ($P > 0.05$). Endogenous renin activity: intact, 15 min R = S ($P > 0.05$); 30 min R > S ($P < 0.01$). Each value represents one sample.

* 24 hr after bilateral nephrectomy.

($P < 0.001$). 24 hr after bilateral nephrectomy, neither R nor S plasma showed any evidence of inhibition of exogenous renin.

The observations may be summarized as follows:

(*a*) The pressor response of intact hypertension-prone (S) rats on low NaCl diet was significantly higher to angiotensin and lower to renin than that of similarly fed intact hypertension-resistant (R) rats.

(*b*) High NaCl intake was without effect on the pressor response of R animals; in some of the S groups, however, added dietary NaCl clearly increased the pressor response. In all S groups there was a tendency for high NaCl to

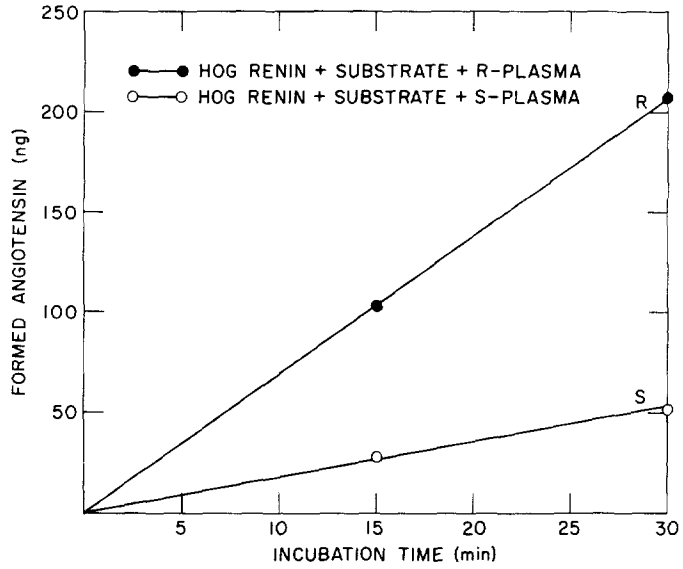

FIG. 3. The velocity of reaction between hog renin and rat renin substrate in the presence of plasma from intact R and S rats. The values from Table V have been corrected for endogenous renin activity. One dot represents the mean value of four analyses on different samples.

increase the pressor response to angiotensin, although this was statistically significant ($P < 0.01$) only among the intact controls, those with a unilateral renal artery clip, and the uninephrectomized rats. The response to renin was increased by high NaCl in the control and uninephrectomized S groups only.

(*c*) In animals from the S strain, only, bilateral nephrectomy influenced the pressor responses to renin and angiotensin in opposite directions; responses to renin increased, while those to angiotensin decreased.

(*d*) In vitro, plasma from hypertension-prone rats had less endogenous renin activity than plasma from hypertension-resistant rats. As expected, all plasma renin activity disappeared within 24 hr after bilateral nephrectomy.

(*e*) Plasma from intact S rats inhibited hog renin activity in vitro, whereas

plasma from intact R rats showed no inhibition. 24 hr after bilateral nephrectomy, no inhibition could be demonstrated in plasma from either strain.

DISCUSSION

The systems studied in these experiments involve the known reactions shown in Fig. 4. Renin, a proteolytic enzyme secreted by the kidney, reacts with its substrate (a plasma protein, angiotensinogen) to release a physiologically inactive decapeptide, angiotensin I. This decapeptide is split by a "converting enzyme" to an octapeptide, angiotensin II, which is both a pressor agent and a major stimulus to the secretion of aldosterone. In turn, angiotensin II is

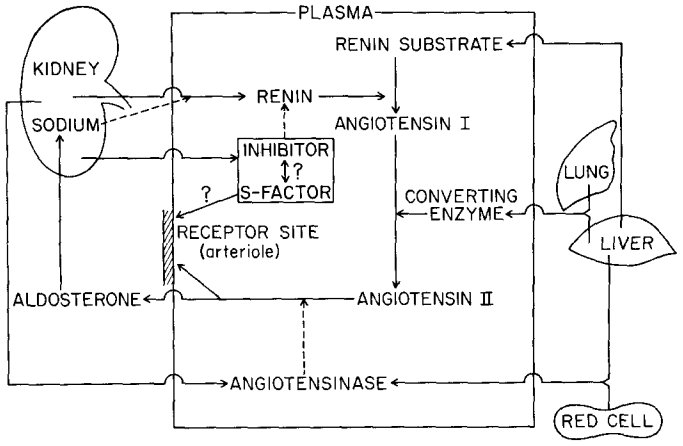

FIG. 4. Schematic diagram of the renin-angiotensin system. Solid arrow indicates stimulating action. Broken arrow indicates inhibiting action. Question mark indicates speculation.

readily inactivated by proteolytic enzymes grouped together under the classification "angiotensinase".

The pressor response to renin of intact S rats on low salt was mild, despite their high sensitivity to angiotensin. This suggests the existence of a block in the series of reactions from renin to the production of angiotensin. Our in vitro experiments suggest that this block might be controlled by a factor located in the plasma of S rats, disappearing after bilateral nephrectomy.

The generally greater pressor response of S rats to angiotensin might be explained by assuming that these animals had less angiotensinase than R rats, but the decreased pressor response of S rats after nephrectomy makes this assumption unlikely. The kidney is one of the major sources of angiotensinase, and nephrectomy should decrease angiotensinase and increase the pressor response. Neither can the difference be explained by termination of renal excretion, since nephrectomy had no effect on the response of R rats. We do not have the ex-

planation for this strain difference in the response to angiotensin, but we use its existence in our evaluation of the response to renin.

Intact S rats on low salt showed a lower pressor response to renin than comparable R rats and this was not due to a reduced sensitivity to angiotensin. It must have been caused, therefore, either by inhibition of the production of angiotensin in the S rats or by enhanced production in the R rats. This is supported by the in vitro results, which showed conclusively that there was, indeed, inhibition of renin activity by plasma from S rats, whereas plasma from R rats caused no stimulation. It may appear logical to suggest that S rats might have a lower activity of the converting enzyme which generates angiotensin II. We have not yet ruled out this possibility conclusively. A difference in converting enzyme, however, cannot explain the results of the in vitro experiments in which only the inactive angiotensin I was generated (11), and only S rats were used for its bioassay. Since the bioassay measures response to the conversion product, namely angiotensin II, converting enzyme must have been present in every assay, presumably in comparable concentrations.

Increase in response to renin, as well as increased reaction velocity between renin and substrate after nephrectomy, were reported as due to increase in substrate concentration (12–14). Here, however, we found that the substrate was in excess for the amount of renin tested both in vivo and in vitro; larger doses of renin than those presented have produced greater pressor response in vivo and proportionately greater angiotensin formation in vitro. After nephrectomy, the amount of substrate presumably increased markedly in both R and S rats as demonstrated by the increased production of angiotensin in vitro. Nonetheless, the R rats showed no increased sensitivity to renin after nephrectomy, indicating that the increased substrate had not affected the pressor response. We conclude, therefore, that there was sufficient endogenous substrate also in the S rats.

The reaction velocity between renin and substrate was suppressed by plasma from intact S rats. This inhibition was not caused by limited concentration of substrate, since this was present in excess. It was furthermore not due to changes in renin concentrations, since these were kept constant. On the other hand, plasma from R rats and from nephrectomized S rats did not influence this reaction rate. The disappearance of the suppression after nephrectomy, therefore, suggested that the suppressing agent found in S rat plasma was produced by the kidney.

Tigerstedt and Bergman first showed that the pressor response to renin was increased after nephrectomy (15). This finding was confirmed by many other investigators. But in our experiments, only S rats showed an increased response to renin after nephrectomy. Some investigators have also observed a potentiation in the pressor response to angiotensin in nephrectomized dogs (16, 17). In rats, it has been claimed that the pressor response to angiotensin is unaltered by nephrectomy (18, 19), but

Sokabe et al. (14) and Gross et al. (20) reported that pressor responses to both renin and angiotensin were increased after nephrectomy. These results differ from ours in that nephrectomized R rats showed no change in response to either substance, while S rats had an increased renin response and a decrease in angiotensin response. Bing and Magill (19) speculated that potentiation of the response to renin induced in normal animals by cross-circulation with nephrectomized ones was due to an activator or some humoral principle. Their findings are difficult to reconcile with ours, since we observed no evidence of an activator.

Page and Helmer found that transfusion with blood from normal dogs reduced the sensitivity of nephrectomized dogs to renin (16). Gross et al. similarly found a decrease in sensitivity to renin in nephrectomized rats after cross-circulation with normal rats (21). These observations are compatible with ours and could be explained by the presence of an inhibitor in normal plasma.

Blaquier reported that there was no difference in the Michaelis constant of hog renin whether plasma from normal or from 24-hr nephrectomized rats was added. He concluded that there was no evidence for inhibition of the reaction (13). However, Montague reported that the Michaelis constant did change, indicating either an inhibitor of renin in normal plasma or an activator after nephrectomy (22). Assuming that the genetic substratum varied among the animals used by these investigators, their discrepant results might support our findings, since we found evidence for an inhibitor in intact S rats but not in R rats.

Bumpus and his associates (23–25) recently isolated a renin inhibitor of phospholipid nature from dog kidney. They reported that the same substance was present in hog kidney and in canine and human blood. These workers found the concentration of their inhibitor in plasma to be unchanged 48 hr after bilateral nephrectomy, whereas we found no evidence for inhibitor in plasma of S rats 24 hr after nephrectomy. The two inhibitors, therefore, are not necessarily identical.

We noted in the introduction that a hypertensinogenic factor which is specific for S strain rats is transmittable in parabiosis in both NaCl-induced and in renal hypertension. The current study suggests that an inhibitor controlling the reaction between renin and its substrate exists in the kidney and plasma of S rats, but not in R rats. The relation between this inhibitor and the hypertensinogenic factor is still speculative, but the fact that both appear to originate in the kidney of S rats suggests a possible connection or even identity.

We propose that as shown in Fig. 4, renin inhibitor is operating as a part of the blood pressure-raising system, rather than the opposite and that it also controls the renin activity level.

SUMMARY

Two strains of rats with opposite genetic propensity for hypertension were tested for: (*a*) the sensitivity to injections of angiotensin and renin, and (*b*) the influence of their plasma on the reaction velocity of renin and its substrate in vitro.

Intact hypertension-prone (S) rats on low salt had higher sensitivity to angiotensin and a lower sensitivity to renin than hypertension-resistant (R) rats. High NaCl diet did not change the response of the R rats to these injections, but increased the response to renin and angiotensin in intact S rats. Bilateral nephrectomy caused increased response to renin and a decreased response to angiotensin in the S rats, so that both strains were equivalent after bilateral nephrectomy. In vitro, plasma from intact S rats inhibited the activity of hog renin. Plasma from R rats showed no inhibition. The inhibitor disappeared after bilateral nephrectomy. It was speculated that renin inhibitor may be involved in the development of hypertension by increasing sensitivity to angiotensin and other hypertensinogenic stimuli.

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