Studies on the Role of the Adrenal in Renin Kinetics (35953)

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In a previous study of experimental renal hypertension (1) in the rabbit, a transient increase in renin substrate concentration was observed after each surgical procedure. At the time, it was postulated to be a nonspecific stress reaction, possibly involving the pituitary adrenal axis. A similar rise is seen during the survival period following bilateral nephrectomy in the rat (2).

In later studies of experimental hypertension, we have developed a procedure for measuring the initial velocity of the renin-substrate reaction (RV_0) (3). This measurement, which defines the initial rate of release of angiotensin by renin, has been shown to be independent of substrate concentration. Renin reaction velocity increases after bilateral nephrectomy (2, 4). We have also seen increases after nonspecific stress. In some forms of experimental hypertension this measurement also increases with rising blood pressure (3). Since the adrenal and the kidney have numerous interrelations in theories concerning the pathogenesis of hypertension, we believed it to be important to study carefully the role of the adrenal in affecting these two measurements involved in the renin reaction, namely renin substrate and renin reaction velocity (RV_0).

Materials and Methods. I. Animal techniques and protocols. Male Sprague-Dawley rats, weighing 230–260 g, were divided into groups of eight on which the procedures specified below were performed, followed by blood sampling at sacrifice. Each sample was run separately, but only the mean and standard errors are given in Tables I–III.

1a. Normal rats on tap water.

1b. Normal rats receiving 0.9% saline solution in place of drinking water (normal + sal).

2a. Rats bilaterally adrenalectomized 5 days previously and given tap water (Adx).[2]

2b. Rats bilaterally adrenalectomized 5 days previously and given in the interval only saline solution to drink (Adx + sal).

3. Rats sham nephrectomized via a left flank incision 24 hr previously (sham).

4. Rats bilaterally adrenalectomized 5 days previously, given saline solution in the interval, and sham operated 24 hr previously (Adx + sal + sham).

5. Rats given ACTH (Parke Davis) 2.5 units ip every 4 hr for 1 day and bled 10 hr after the last injection.

6a. Rats bilaterally nephrectomized 24 hr previously (Nx).

6b. Rats given saline solution for the previous 5 days and bilaterally nephrectomized 24 hr previously (Nx + sal).

7a. Rats bilaterally adrenalectomized 5 days previously and bilaterally nephrectomized 24 hr previously (Adx + Nx).

7b. Rats bilaterally adrenalectomized 5 days previously, given saline solution in the interval and bilaterally nephrectomized 24 hr previously (Adx + sal + Nx).

II. Preparation of plasma samples. Blood samples were taken under light ether anesthesia from the carotid artery into cooled plastic centrifuge tubes containing the disodium salt of EDTA to give a final concentration in plasma of 0.38%. After centrifugation at 4°, the plasma was separated and frozen.

III. Biochemical procedures. A. Determination of plasma renin substrate concentration. Plasma renin substrate concentra-

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[2] All animals subjected to the adrenalectomy procedure were given 10 mg of tetracycline hydrochloride (Roerig) im after the procedure.

TABLE I. *RS* and RV_0 in Intact and Adrenalectomized Rats Undergoing Sham Operation or ACTH Administration.[a]

Expt. condition	*N*	*RS* ± SEM (ng/ml)	*p*	RV_0 ± SEM (ng/ml/min)	*p*
Normal	8	609 ± 20	—	5.92 ± 0.31	—
Adx + sal	8	591 ± 10	NS[b]	5.92 ± 0.07	NS[b]
Sham	8	960 ± 18	<.001[b]	8.23 ± 0.27	<.001[b]
Adx + sal + sham	8	648 ± 31	NS[c]	5.54 ± 0.42	NS[c]
Adx	8	518 ± 21	<.02[b]	5.83 ± 0.17	NS[b]
ACTH	8	788 ± 17	<.001[b]	7.61 ± 0.13	<.001[b]

[a] *RS* = renin substrate; RV_0 = renin reaction velocity; SEM = standard error of the mean; *p* values determined by Student's *t* test.

NS = not significant: [b] compared with normal; [c] compared with Adx + sal.

tion (*RS*) was determined on the basis of the amount of angiotensin II liberated by the addition to 0.1 ml of plasma of an excess of hog renin (2.5 DU, as reported by Nutritional Biochemical Corporation, Cleveland). Incubation was carried out at pH 5.1 and at 37° for 12 min. The validity of this measurement has been confirmed previously (1).

B. Determination of the initial velocity of the hog renin rat substrate reaction (RV_0). The conditions for the measurement of RV_0 have been detailed elsewhere (3). After preliminary studies to support conformity with these requirements in the rat, we adopted the following procedure: to 0.9 ml of thawed rat plasma, at pH 5.1, was added 0.02 DU of hog renin in 0.1 ml of saline to make a total volume of 1.0 ml. Two such tubes were prepared from each plasma, to be incubated at 4 and 8 min at 37°, following which the reaction was stopped by placing the tubes in boiling water for 5 min. Following centrifugation at 1750*g* for 30 min, the supernatant was separated and frozen until assayed.

Control tubes incubated without added renin under the conditions described did not result in a measurable yield of angiotensin.

C. Bioassay for angiotensin. As previously reported (3), angiotensin was assayed in the pentobarbital anesthetized–pentolinium blocked rat using val-5-angiotensin II amide (Hypertensin, Ciba) as a standard.

Renin substrate is expressed as nanograms of angiotensin II generated per milliliter of plasma (ng/ml). Initial velocity is expressed as nanograms of angiotensin II generated per milliliter per minute (ng/ml/min) and represents the average of the 4 and 8 min incubations.[3]

Results. Table I summarizes the effects of sham operation before and after removal of the adrenals on plasma renin substrate concentration (*RS*) and on the initial velocity of the hog renin–rat substrate reaction (RV_0). As shown, sham operation significantly increased both *RS* and RV_0 only in those animals with the adrenal glands intact. Similarly, administration of ACTH produced a significant increase in *RS* and RV_0.

Observations on the effects of adrenalectomy suggested that salt administration of itself might alter *RS*. Hence, this phenomenon was studied in more detail. Table II shows that salt supplementation increases *RS* in all three types of animals: normals; adrenalectomized; nephrectomized. RV_0 is unaffected by these procedures except after nephrectomy, when it is increased by prior salt administration.

The role of the adrenal in the changes which follow nephrectomy was next considered. Table III summarizes the effects of bilateral nephrectomy in normal, adrenalectomized, and adrenalectomized-salt supplemented rats. Nephrectomy alone caused the expected increases in *RS* and RV_0. When the adrenals were removed 5 days before nephrectomy and no salt was given, the animals were in poor condition and the observation showed a wide scatter. Nevertheless a

[3] For the sake of comparison with other laboratories, the velocity (*K*) can be expressed as amount of angiotensin generated per unit of renin, by dividing RV_0 by the amount of renin used (0.02).

TABLE II. Effect of Salt Administration in Normal, Adrenalectomized, and Nephrectomized Rats on *RS* and RV_0.[a]

Expt. condition	*N*	*RS* ± SEM (ng/ml)	*p*	RV_0 ± SEM (ng/ml/min)	*p*
Normal	8	609 ± 20	—	5.92 ± 0.31	—
Normal + sal	8	682 ± 24	<.002[b]	6.52 ± 0.26	NS[b]
Adx	8	518 ± 21	<.001[b]	5.83 ± 0.17	NS[b]
Adx + sal	8	591 ± 10	<.005[c]	5.92 ± 0.07	NS[c]
Nx	8	2437 ± 10	<.001[b]	9.81 ± 0.10	<.001[b]
Nx + sal	8	2656 ± 42	<.001[d]	10.90 ± 0.20	<.001[c]

[a] Abbreviations as in Table I.
NS = not significant: [b] compared with normal; [c] compared with Adx; [d] compared with Nx.

significantly lessened increase in *RS* was observed without an alteration in RV_0. In order to clarify this point, animals adrenalectomized and salt-fed were subjected to nephrectomy. Results were similar but the variability of *RS* determination was reduced.

Discussion. The observations reported herein concern two measurements—plasma renin substrate concentration (*RS*) and the initial velocity of the (hog) renin–(rat) substrate reaction (renin reaction velocity [RV_0]). These measurements have previously been shown to be independent of each other although they frequently vary concurrently (3).

Renin substrate. Renin substrate rises transiently after various operative procedures (1). The present findings support our previous assumption that this elevation is related to activation of the pituitary–adrenal axis. As shown in Table I, the increase in *RS* is prevented by prior adrenalectomy and can be duplicated by ACTH administration.

The changes in *RS* following salt administration (Table II) are more difficult to explain. When the drinking water given to normal and bilaterally nephrectomized rats contained 0.9% saline solution, a uniform increase in *RS* to approximately 110% of pretreatment values was observed. Furthermore, saline administration to adrenalectomized rats prevents the fall in *RS* usually seen in such animals not receiving saline, as also reported by Nasjletti and Masson (5). It might be postulated that the increase in *RS* in those animals receiving salt is related to changes in plasma renin levels; however, the observation that saline solution raises *RS* in the bilaterally nephrectomized rats clearly fails to support this theory. Furthermore, Carretero and Gross (6) have shown that the infusion of large amounts of (hog) renin into bilaterally nephrectomized rats does not result in a decrease of *RS* to normal.

The changes seen in *RS* after bilateral nephrectomy have been reported previously (2, 7) and are confirmed here. The rise in *RS* after bilateral nephrectomy is strikingly reduced when the adrenals have been previously removed (Table III). However, a substantial increase still occurs. Thus, the adrenal contributes in some way to the rise in *RS*

TABLE III. Effects of Nephrectomy in Intact and Adrenalectomized Rats on *RS* and RV_0.[a]

Expt. condition	*N*	RS ± SEM (ng/ml)	*p*	RV_0 ± SEM (ng/ml/min)	*p*
Normal	8	609 ± 20	—	5.92 ± 0.31	—
Nx	8	2437 ± 10	<.001[b]	9.81 ± 0.10	<.001[b]
Adx + Nx	8	1378 ± 216	<.001[c]	9.59 ± 0.29	NS[c]
Adx + sal + Nx	8	1121 ± 41	NS[d]	9.97 ± 0.10	NS[d]

[a] Abbreviations as in Table I.
[b] NS = not significant: compared with normal; [c] compared with Nx; [d] compared with Adx + Nx.

in the anephric animal, but it is clearly not the only factor involved.

Renin reaction velocity. The changes in RV_0 are in some respects parallel to those for *RS*. Thus, RV_0 rises with nonspecific stress (sham operation) and the change can be prevented by prior adrenalectomy or mimicked by ACTH administration.

RV_0 does not increase after salt administration in either the normal or adrenalectomized animal. Some increase is observed when salt is administered to the rat before nephrectomy. In this series of experiments *RS* and RV_0 do not change concurrently.

Another difference appears when nephrectomy is studied. In our study *RS* and RV_0 are both increased equally at 24 hr. However, Smeby *et al.* (4), who have studied this change more closely, report that there is a disparity between the time of first appearance of changes in *RS* and change in rate of "angiotensin generation," which is a measurement similar to RV_0. We herewith report a further difference, namely that in the adrenalectomized animal, nephrectomy causes the same increase in RV_0 but only one-half the expected change in *RS*.

These studies again demonstrate a dissociation between *RS* and RV_0. They support the view that *RS* is not ordinarily an influence controlling the velocity of the renin reaction since it is in excess in the physiologic situations dealt with herein. Similarly, we believe fluctuations in *RS* to be relatively unimportant in the control of blood pressure (1).

On the other hand, we believe the changes in RV_0 to be of more physiologic importance than alterations in *RS*. The rate at which the pressor peptide is produced could be more important than the concentration of plasma renin in controlling blood pressure. We have demonstrated this to be the case in the rabbit with Goldblatt hypertension: when the untouched kidney is removed from an animal with a previously constricted renal artery, an increase in blood pressure was seen only in those animals with an increase in RV_0 (renin levels and *RS* remained constant throughout). When the pressure did not rise after nephrectomy, RV_0 was likewise unchanged (3). Furthermore, the augmented pressor response to renin exhibited by the renoprival rat (2) and the Goldblatt hypertensive rabbit (8) could be a consequence of an increased reaction velocity with an augmented production of angiotensin.

As discussed elsewhere (3) changes in initial velocity (RV_0) reflect the action of inhibitors or activators of the reaction. The role of the adrenal in mediating these changes in RV_0, possibly by secreting an activator of the reaction, has been herewith demonstrated. The reaction velocity of the renin and substrate reaction is clearly augmented by the stress response. However, the changes which appear after nephrectomy are not a consequence of adrenal stimulation—it must be assumed that they represent the elimination by nephrectomy of an inhibitor possibly secreted by the intact renal medulla.

If bilateral nephrectomy acts solely by removing a renal inhibitor of the reaction, then the further hypertension which follows unilateral nephrectomy in an animal with one renal artery clamped probably also reflects diminution in the secretion of a renin inhibitor, rather than any adrenal mediation. While this study shows that adrenal stimulation can indeed alter renin reaction velocity, we believe the preponderant effect is in the kidney and we are working toward further characterization of the renal inhibitory factor.

This study further supports the existence of activators and inhibitors of the renin–angiotensinogen reaction and suggests that such substances may originate in the kidney and adrenal. Whether they play a primary role in maintaining normal or elevated blood pressure is a question for further study.

Summary. Renin substrate and renin reaction velocity rise transiently with stress reactions such as sham operation and these changes are shown to be mediated via pituitary–adrenal stimulation. Salt administration raises slightly the renin substrate level but does not change renin reaction velocity. The augmentation of renin substrate which follows nephrectomy is diminished by prior adrenalectomy. The concomitant rise in renin reaction velocity is not modified by adrenalectomy. Reasons are given for assigning a primary role to loss of a renal inhibitor in the

increased renin reaction velocity which accompanies the hypertension which is seen in the partially or completely renoprival rat.

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