Release of Renin by Rat Kidney Slices; Relationship to Plasma Renin after Desoxycorticosterone and Renal Hypertension (33493)

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Rats adrenals incubated *in vitro* produce corticosteroids (1) and the rate at which glucocorticoids and mineralocorticoids are released into the medium is a reliable index of *in vivo* adrenocortical activity (2). This index also reflects pituitary–adrenal activity and can be used for the indirect assessment of corticotropin (ACTH) in the blood (3). At present no direct chemical measurement of ACTH is available and thus, indirect procedures must be used.

Similarly, since direct chemical measurement of renin in plasma is not possible, indirect methods are employed. Therefore, it was deemed of interest to determine whether rat kidney slices release renin *in vitro*, and if so, whether such release reflects *in vivo* activity. It was found that rat kidney slices do indeed release renin *in vitro*. The release was studied under standardized conditions and compared to plasma renin levels and kidney renin content. The present report concerns the effect of deoxycorticosterone acetate (DOCA) administration with 0.9% saline, a treatment which inhibits the release of renin *in vivo* (4), and the effect of renal hypertension, a condition which presumably increases *in vivo* release of renin.

Materials and Methods. Male rats of a Wistar strain bred in this laboratory and weighing 120–160 g were used. Operations were performed under ether anesthesia. Renal hypertension was induced by putting a silver clip on the left renal artery in intact rats (5). Systolic blood pressure of unanesthetized trained rats was determined 3–5 times a week with a tail sphygmographic method (5).

Plasma renin activity was measured according to Pickens *et al.* (6) with the following modifications. All steps before and after the incubation were done at 0–5°. Following decapitation, blood was collected in cooled polypropylene tubes containing 25 mg of disodium ethylenediaminetetraacetic acid in 0.2 ml of 0.9% saline. Following dialysis, to 2 ml of plasma, 0.1 ml of a 1:20 solution of di-isopropylfluorophosphate (DFP, at least 99.9% pure) in isopropyl alcohol was added and adjusted to pH 5.5. This solution was incubated for 4 hr at 37°, 1.5 ml demineralized water and 0.5 ml of 0.9% saline were added, the pH was brought to 6.3, and the tubes were placed in boiling water for 10 min. The protein precipitate was removed by centrifugation and pH adjusted to 7.0. This solution was evaporated to dryness under reduced pressure at 45–50°. The residue was dissolved in 0.5 ml of demineralized water. Angiotensin was determined by a 2 + 1 point assay in nephrectomized rats treated with autonomic blocking agents as described previously (5). Plasma renin activity was expressed as mμg of angiotensin II (Hypertensin, Ciba) equivalents produced per 2 ml of plasma.

The renin content of the kidney was determined according to the method of Gross and Sulser (7). Material of the same kidney was used for the *in vitro* study and for the assay of the renin content. The kidney was homogenized in 0.9% saline (5 ml/g tissue), and following centrifugation (30 min, 16,000*g*), the supernatant was injected via a lateral tail vein (0.1 ml/rat) into rats that had been nephrectomized 16–20 hr previously. Urethane anesthesia (1.0–1.5 g/kg, i.p.) was employed. Pressor responses were determined by monitoring carotid blood pressure with a Statham transducer (P23AC) and a Grass polygraph (model 5D). Each rat received only one dose of the kidney extract.

For the measurement of renin release *in vitro*, the incubation procedure for the forma-

TABLE I. Effect of DOCA Administration on *in Vitro* Release of Renin, Plasma Renin Activity, and Kidney Renin Content.[a]

Duration of treatment (days)	Group	Pressor response (mm Hg)		Plasma renin activity (angiotensin II equivalents mμg/2 ml)
		Medium	Kidney extract	
2	Control	20 ± 1.9 (9)	52 ± 2.5 (9)	—
	DOCA	11 ± 1.5 (9)[c]	52 ± 3.8 (9)	—
4	Control	25 ± 2.7 (7)	50 ± 4.1 (7)	4.4 ± 0.7 (7)
	DOCA	12 ± 2.9 (7)[c]	55 ± 4.8 (7)	2.2 ± 0.4 (6)[b]

[a] Results are given as mean ± SE; number of animals is given in parentheses.
[b] $p < 0.05$.
[c] $p < 0.01$.

tion of adrenal corticosteroids *in vitro* as described by Van Goch *et al.* (3) was used. Immediately after decapitation of each rat both kidneys were removed, cleaned, and a piece of approximately 110 mg (90–130 mg) was removed from the upper pole of the kidney. The tissue was weighed, cut into 8–10 slices, and preincubated in 2 ml of Krebs–Ringer bicarbonate buffer medium containing 200 mg of glucose/100 ml of buffer in a Dubnoff metabolic incubator at 37° under oxygen containing 5% carbon dioxide. The medium was changed after 15 min and the tissue was then incubated for 1 hr. After this time the tissue was discarded, and the amount of renin released into the medium was determined by its pressor response (0.2 ml/rat) in nephrectomized rats as described above for the assay of renin content of the kidney. Results are expressed as mean ± standard error of the mean (SE). Statistical analysis of the data was performed using Wilcoxon's two sample test (8).

Results. (I) *Preliminary experiments.* The release of renin by rat kidney slices *in vitro* at 37° occurred within 15 min and continued for at least 4 hr. Assay of the renin content of the kidney slices revealed no significant decrease following incubation up to 4 hr. However, the total amount of renin released *in vitro* amounted to 2–3% of the renin content of the kidney slices. This difference is within the variation of the assay and therefore quantitative interpretation of any change was not possible. During incubation at 4° the release of renin started after 2–5 hr. When purified rat renin was added to the incubation medium and this mixture was subsequently incubated for 1 hr a recovery of 80–100% was found. The renin used was prepared according to the method of Haas *et al.* (9) and had a specific activity of 3.0 Goldblatt units /mg of dry weight. In subsequent experiments the kidney slices were incubated for 1 hr at 37°, and in all cases a preincubation of 15 min was used.

The released material resembles renin by its typical pressor response in nephrectomized rats, its denaturation by heat (10 min at 80°), and by the fact that it reacts with rat renin substrate to produce an angiotensin-like product.

(II) *Effect of DOCA administration.* The effect of DOCA (solution in oil, N.V. Organon, Oss, Holland) administration (once daily, 10 mg/kg, s.c.) is shown in Table I. The DOCA-treated animals were given 0.9% saline as drinking fluid. The rats were decapitated 7 hr after the last injection. *In vitro* renin release and renin content of the left kidneys were determined. DOCA administration for 2 and 4 days decreased the release of renin *in vitro* without having an effect on the renin content of the kidney. Plasma renin activity was also found to be depressed.

(III) *Renal hypertension.* The results of the experiments on renal hypertension of 14-and 45-days duration are summarized in Table II. The depicted blood pressures of the rats which were used for the measurement of plasma renin activity were measured 1 day prior to decapitation. Mean blood pressures of the comparative groups were in the same range. The clipped (i.e., left) kidneys showed

TABLE II. Effect of Renal Hypertension on *in Vitro* Release of Renin, Plasma Renin Activity, and Kidney Renin Content.[a]

Group	Pressor response (mm Hg)				Plasma renin activity (angiotensin II equivalents mμg/2 ml)	Blood pressure (mm Hg)
	Medium		Kidney extract			
	Left kidney (clipped)	Right kidney	Left kidney (clipped)	Right kidney		
Control	15 ± 1.3 (9)	14 ± 1.2 (12)	51 ± 4.5 (13)	49 ± 6.0 (8)	2.7 ± 0.2 (13)	123 ± 2.6 (13)
Hypertensive (14 days)	25 ± 3.5 (12)[c]	8 ± 1.6 (9)[b]	48 ± 3.8 (14)	22 ± 5.8 (8)[b]	4.2 ± 0.5 (8)[c]	179 ± 8.4 (8)[c]
Control	15 ± 0.7 (18)	16 ± 1.5 (10)	60 ± 2.3 (20)	60 ± 4.5 (10)	3.1 ± 0.4 (18)	128 ± 1.9 (18)
Hypertensive (45 days)	25 ± 1.7 (14)[c]	3 ± 1.1 (8)[c]	54 ± 3.4 (18)	24 ± 5.5 (8)[c]	10.6 ± 3.0 (13)[c]	223 ±10.5 (13)[c]

[a] Results are given as mean ± SE; number of animals is given in parentheses.
[b] $p < 0.05$.
[c] $p < 0.01$.

an increased release of renin *in vitro*, but no significant changes were detected in the renin content of the clipped kidneys. The right kidneys (not clipped) of the hypertensive rats showed a decreased release of renin *in vitro*. Renin content of the right kidneys of the hypertensive rats was also found to be decreased. Plasma renin activities of both groups of hypertensive rats were elevated.

Discussion. The present experiments show that rat kidneys can release renin *in vitro* and that such release by kidneys of DOCA-treated rats and by the clipped kidneys of renal hypertensive rats parallels plasma renin activity. The *in vitro* release of renin apparently is not merely a reflection of the total renin content of the kidney, since increased as well as decreased release of renin were observed under conditions which had no detectable effect on the kidney renin content.

Evidence for the inhibition of *in vivo* release of renin and for the depletion of renin in the kidney by administration of DOCA and 0.9% saline for several weeks has been reviewed by Gross *et al.* (4). Our findings show that treatment with DOCA and 0.9% saline for as little as 2–4 days inhibits the *in vivo* and *in vitro* release of renin without affecting the renin content of the kidney.

During the acute stage of renal hypertension increased levels of plasma renin activity have been detected (10–12), while in chronic stages of renal hypertension normal plasma renin activity was found (10, 12). The present study shows that in the renal hypertensive rat, plasma renin activity is increased acutely, maintained for at least 6 weeks, and is parallelled by an increased release of renin from the clipped kidney *in vitro*.

Release of rat renin by renal tissue *in vitro* has not been previously reported as far as we are aware. However, the possibility that renal tissue might release renin *in vitro* was suggested by the work of Bing (13). This author showed that renin was released on perfusion of completely ischemic kidneys. This renin release increased with the time of perfusion and was higher at 37° than at 2°. The release of renin was found to increase after pretreatment of the kidneys with phenol, toluol, ether, or acetone. A further

indication that the kidney might release renin *in vitro* was provided by the experiments of Robertson *et al.* (14), showing the long-term release of renin by organ cultures of renal cortex tissue. Similar results were reported by Szalay and Gyévai (15). During the preparation of this manuscript the occurrence of release of renin from dog kidney slices *in vitro* was reported by Yamamoto *et al.* (16).

Summary. The release of renin by rat kidney slices *in vitro* using a simple short-term incubation procedure is reported. The *in vivo–in vitro* relationships were studied in DOCA-treated rats and in renal hypertensive rats. DOCA decreased the *in vitro* release of renin and also decreased plasma renin activity. *In vitro* release of renin at a supranormal level was detected during renal hypertension, while plasma renin activity was found to be enhanced. The reported changes in renin release were not necessarily correlated with changes of the renin content of the kidney.

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