Renin Activity in Urine, Plasma, and Renal Cortex of Dogs During Changes in Sodium Balance[1] (35273)

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Few investigations have been made on urinary excretion of renin since Houssay *et al.* (1) in 1942 described urinary renin activity (URA) in dogs infused with high doses of hog renin. Orti *et al.* (2) suggested in 1957 that an aldosterone-stimulating substance was present in the urine of sodium-depleted and of adrenalectomized rats. It is possible that this substance, which was not present in the urine of rats maintained on salt load, could be renin. Brown *et al.* (3), Oelkers (4), and Lumbers and Skinner (5) investigated URA in normal humans; the latter workers (6) also studied URA during natriuresis. Anichini and Gross (7) found increased URA after clipping one renal artery in rats. Rapelli and Peart (8) infused renin in rats and observed increased URA excretion during treatment with mercuric chloride and maleic acid. In the present study, we have used a recently developed method (9, 10) to determine plasma renin activity (PRA) and URA simultaneously in healthy, sodium-restricted, sodium-deprived, and sodium-loaded dogs. In addition, renin activity in renal cortex (RRA) was measured in one group of animals.

Experimental protocols. Nineteen dogs were divided into two groups:

1. Group A consisted of 6 female mongrel dogs weighing 16 to 23 kg. The animals were kept in metabolic cages and at constant temperature and humidity. They were fed for 4 days 15 g of a virtually sodium-free diet (Nutritional Biochemicals Corp., Cleveland) plus 150 mg NaCl (= 2.57 mEq of Na), and 40 ml of demineralized water/kg of body weight/day.

After this control period, the dogs were kept on the same food and water intake for 12 days, but without sodium supplement. From days 16 to 22, the dogs were given a sodium load of 300 mg of NaCl (= 5.13 mEq of Na)/kg/day in addition to the usual sodium-free diet for 6 days. The following parameters were measured after 4 days of control diet, 6 and 12 days of total sodium deprivation and 6 days of sodium load: PRA in the conscious dog; URA; hematocrit; plasma and urinary sodium; plasma potassium and blood pressure. The dogs were anesthetized with thiopental (Pentothal, Abbott; 20 mg/kg) for catheterization and blood pressure measurement.

2. Group B consisted of 13 male mongrel dogs weighing 17–25 kg. Nine of these dogs were fed 15 g of the same "sodium-free" diet with a small supplement of 3 mg of NaCl and 40 ml of demineralized water/kg/day for 4 weeks. The other 4 dogs served as controls and received the same diet but supplemented with 100 mg of NaCl/kg/day. At the end of the 4-week period, the dogs were anesthetized with pentobarbital (Nembutal, Abbott; 30 mg/kg), catheterized, and both kidneys were removed. PRA in the conscious dog, URA, and RRA were measured. In both groups of animals, the urine was collected during the entire experiment.

Methods. PRA and URA were measured according to Granger's modifidation (9) of the micromethod of Boucher *et al.* (10). Ten ml of blood were drawn from a peripheral vein into a syringe containing 0.2 ml of a 15% EDTA solution. One ml each of plasma and urine were incubated simultaneously at 37° and pH 5.5 for 12 hr with 1 ml of Dowex 50W-X2 $(NH_4)^+$ and excess homologous renin substrate dissolved in 2 ml of trisphosphate buffer. For each incubation of urine, 0.05 ml of a 15% EDTA solution was added. Sodium and potassium were analyzed in a

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Technicon flame photometer model IIIA. Hematocrit was determined in heparinized microhematocrit tubes centrifuged for 8 min in a microcapillary centrifuge (Int. Equipment Co.). The blood pressure was recorded on a Grass Polygraph model No. 7 after puncture of the femoral artery of the anesthetized dogs.

The results are expressed as mean value ± standard error (SE). Whenever possible, statistical analysis was made using Student's *t* test; when the differences were not normally distributed, the ranking test of Wilcoxon was applied.

Results. Identification of renin in urine: Urine samples of 5 to 12 dogs were examined in the following experiments. The direct injection of untreated urine into the nephrectomized rat produced in some experiments a short-lasting increase of blood pressure. This pressor agent disappeared after dialysis of the urine against distilled water for 12 hr at 4°. However, the dialysis did not affect the formation of pressor material during the incubation of urine with renin substrate. When incubated in excess, purified hog renin and urine enzyme formed the same amount of pressor material with dog plasma. The formation of pressor material by urine was proportional to incubation time (6–12 hr) and amount of enzyme added (0.5–2.0 ml of urine). Boiling for 15 min prior to the incubation led to a destruction of the urine enzyme, but not of the pressor substance. The addition of trypsin destroyed the pressor material completely. The recovery of synthetic angiotensin (Hypertensin, Ciba) was 75–80% (range 40–200 ng). Incubation of urine without renin substrate did not produce any pressor material, nor did incubation of urine with substrate at 4°. The pressor curve of the substance formed by the urine enzyme was identical to that of synthetic angiotensin.

Animal experiments. Group 1. During prolonged sodium deprivation plasma sodium fell from 156.0 ± 2.3 to 151.2 ± 1.3 mEq ($p < 0.01$). Plasma potassium increased from 4.79 ± 0.08 to 5.6 ± 0.22 mEq ($p < 0.01$), and hematocrit from 42.0 ± 0.5 to 53.4 ± 3.0% cells. The urinary sodium excretion fell markedly ($p < 0.001$). The daily urine volume was slightly increased after 6 days and decreased again after 12 days of sodium deprivation. PRA and URA increased during sodium deprivation and decreased when sodium was readministered (Table I). We have no explanation why dog No. 2 showed higher PRA and URA after 6 days of sodium deprivation than after 12 days.

Group 2. As shown in Table II, sodium restriction increased URA as well as PRA and RRA. During the experiments, proteinuria as measured by the Uristix method (Ames Co.) was not detected.

Discussion. The micromethod of Boucher *et al.* (10) which has been shown to measure renin activity in plasma and kidney was used for determination of renin activity in urine. Our studies provide strong evidence that the enzyme activity found in urine is identical to "renin activity" measured in plasma and kidneys. Furthermore, it seems very unlikely that sodium restriction increases an enzyme other than renin in urine, plasma, and renal cortex simultaneously.

The results indicate that URA, PRA, and RRA increase in parallel in response to sodium restriction (Tables I, II). The mechanism by which renin is excreted in the urine is not known. The ratio of renin activity to protein in the urine when compared to the ratio in plasma is so high that renin could not be excreted solely by glomerular filtration of renin from the peripheral plasma. If all URA originates from the peripheral blood, one would have to assume that all the other proteins with the exception of renin are reabsorbed. The fact that URA did not change with increased proteinuria (4), also suggests that at least a part of the renin enters the urine in the kidney without originating from the peripheral blood. The renin could enter the urine from structures which are rich in renin activity and topographically closely related to the glomerulus. In preliminary experiments in 3 dogs with one kidney constricted with a Goldblatt clamp, the contralateral kidney being untouched, on the fourth day after clamping, we found an increase of renin activity in the urine and in the renal venous blood on the clamped side. The URA was enhanced, whether expressed per milliliter or by total excretion per hour.

TABLE I. Renin Activity in Urine and Plasma of Dogs.

Dog no.	Diet:[a] Day:	Daily urinary excretion of renin activity (ng of angiotensin/hr)				Plasma renin activity (ng of angiotensin/ml/hr)			
		Control	"Sodium-free"		Sodium replacement	Control	"Sodium-free"		Sodium replacement
		4	10	16	22	4	10	16	22
1		94	1950	9333	70	1.04	10.83	25.00	0.42
2		647	2467	1667	672	1.04	6.67	4.17	0.52
3		450	10917	24583	—[b]	0.52	6.67	18.33	—[b]
4		68	4900	38000	113	0.83	10.00	16.67	0
5		94	—[c]	14000	125	0.83	8.33	16.67	0.16
6		73	656	1050	0	0.31	1.67	2.92	0.16
Mean ± SE		238 ±104	4178 ±1849	14772 ±5969	196 ±123	0.76 ±0.12	7.36 ±1.33	13.96 ±3.53	0.25 ±0.09
p value			<0.01	NS	<0.01		<0.01	NS	<0.01
Days 4 and 16			p <0.01				p <0.01		

[a] Diet: The dogs received daily 15 g of a virtually sodium-free diet and 40 ml of demineralized water/kg of body weight. From day 0 to day 4: 150 mg NaCl (2.57 mEq of Na); and from day 16 to 22: 300 mg NaCl (5.13 mEq of Na)/kg of body weight/day were added.

[b] Dog died from pulmonary infection.

[c] Renin activity was not measured because of severe urinary infection subsequently cured by antibiotics.

TABLE II. Renin Activity[a] in Sodium-Restricted and Control Dogs.[b]

	Control dogs (n=4)	p value	Sodium-restricted dogs (n=9)
Plasma	0.51 ± 0.24	<0.01	5.68 ± 1.07
Urinary excretion/day	133 ± 68	<0.01	4701 ± 1473
Renal cortex	18750 ± 3750	<0.01	108750 ± 13810

[a] Renin activity (ng of angiotensin/ml) or (g/hr of incubation).

[b] All dogs were kept on 40 ml of demineralized water and 15 g of a virtually sodium-free diet/kg of body weight/day for 4 weeks. The control dogs received a NaCl-supplement of 100 mg/kg/day; the sodium-restricted animals were supplemented with 3 mg/kg/day.

It is of interest that despite an otherwise decreased function (Na excretion, urine volume) more renin is excreted by the clamped kidney than by the untouched one. These findings support the hypothesis that the high amounts of renin excreted in the urine do not originate from filtration of peripheral blood alone.

Summary. Plasma and urine renin activity were measured in dogs during control diet and sodium deprivation. Renin activity increased in both plasma ($p < 0.01$) and urine ($p < 0.01$) in parallel fashion during sodium deprivation and decreased with sodium replacement. In another group of dogs, sodium restriction had the same stimulatory effect on plasma ($p < 0.01$), urine ($p < 0.01$), and renal renin activity ($p < 0.01$). The results indicate that enhanced urinary excretion of renin is directly correlated to the increase in plasma and renal renin activity.

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