Effects of Sodium Restriction on Renin, Norepinephrine, and Cation Content of Cardiovascular Tissues of Dogs[1] (35035)

K. HAYDUK,[2] H. M. BRECHT,[3] A. VLADUTIU,[3] J. M. ROJO-ORTEGA, R. BOUCHER, AND J. GENEST

Clinical Research Institute of Montreal, Montreal, Canada

Sodium restriction causes an increase in plasma renin activity (PRA) (1–3) and in renal renin activity (RRA) (4) coinciding with an increased juxtaglomerular cell granularity (5). Plasma sodium level is generally stable and decreases only in severe and prolonged sodium restriction (6). The present study was performed to investigate the effect of severe sodium restriction on (a) renin activity of various cardiovascular tissues: branches of saphenous and mesenteric arteries, thoracic aorta, and left ventricle; (b) concentrations of cations in plasma and their content in vascular tissues (branches of saphenous and mesenteric arteries, carotid and renal arteries, thoracic aorta and left ventricle); and (c) norepinephrine content in the same tissues. Juxtaglomerular granulation index (JGI) and activity of glucose-6-phosphate dehydrogenase (G6PD) in the cells of the adrenal zona glomerulose were also measured.

Material and Methods. Eleven male mongrel dogs, weighing 17 to 25 kg, were divided into: (a) group A, 7 dogs received during a 4-week period a virtually sodium-free diet (Nutritional Biochemicals Corp., Cleveland, Ohio; 15 g/kg of body wt given daily by gavage) with 3 mg of NaCl and 40 ml of demineralized water/kg/day; (b) group B, 4 dogs used as controls during the same period on the same dietary regimen, but receiving in addition a "normal" supplement of 100 mg of NaCl/kg/day.

Plasma renin activity was measured according to Granger's modification (7) of the micromethod of Boucher *et al.* (8). Determination of tissue renin activity was performed as recently described (9). Measurement of the norepinephrine content in tissues was done according to the method of Anton and Sayer (10) with modifications of Genest *et al.* (11).

The procedure for determination of water and cation contents in vascular tissues has been described (9). Measurements of the juxtaglomerular cell granulations was done by the juxtaglomerular index (JGI) of Hartroft and Hartroft (5).

The enzymatic activity of the glucose-6-phosphate dehydrogenase was measured according to an arbitrary scale from 0 to 3 (0: no activity; 1: slight activity; 2:moderate activity; 3:high activity) (12).

Results. PRA and RRA are significantly increased in the sodium-restricted dogs whereas the mean renin activity of vascular tissues is slightly but not significantly increased (Table I). A marked increase in left ventricle renin activity is observed, but its statistical significance could not be appraised because of the small number of control observations.

The mean norepinephrine content in all vascular tissues studied (Table II) is lower in the sodium-restricted dog than in those receiving the sodium supplement, but these differences are not significant.

There are no consistent nor significant differences in plasma electrolyte concentration between the dogs of group A when compared to those of group B. Mean plasma sodium

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[3] Medical Research Fellow of the Medical Research Council of Canada.

TABLE I. Effects of Severe Sodium Restriction on Plasma (PRA), Renal (RRA), and Vascular Tissue Renin Activity[a] of Dogs.

	Group A sodium-restr. dogs ($n = 7$)[b]	Group B control dogs ($n = 4$)[c]	p Group A vs B
PRA, control (before experiment)	0.2 ± 0.04	0.25 ± 0.1	NS[d]
After 2 weeks	3.2 ± 0.8	0.32 ± 0.08	<0.05
After 4 weeks	3.8 ± 0.8	0.5 ± 0.24	<0.02
Tissue renin activity after 4 weeks:			
Renal cortex	90,000 ± 14,300	18,800 ± 3800	<0.01
Saphenous artery	3.9 ± 0.5	2.9 ± 0.14	NS
Mesenteric artery (2nd to 5th branches)	7.7 ± 0.6	4.25 ± 2.1	NS
Thoracic aorta	5.4 ± 0.9	3.8 ± 2.2	NS
Left ventricle	98.2 ± 23.0	29.6 ± 9.1 ($n = 3$)	—

[a] Renin activity (ng of angiotensin/ml of plasma or g of tissue/hr incubation; mean ± SE).
[b] 15 g "sodium-free" diet/kg of body weight, plus 3 mg of NaCl/kg/day supplement for 4 weeks.
[c] Same dietary regimen plus 100 mg of NaCl/kg/day for 4 weeks.
[d] NS = not significant.

concentration of the dogs of group A is 139.3 ± SE 3.3 meq/liter whereas those receiving a sodium supplement is 146.8 ± SE 2.9. This difference is not significant.

Sodium content is significantly decreased in all vascular tissue studied, with the exception of the renal artery which shows no change (Table III). Potassium content is slightly, although not significantly, increased in all tissues, except for the carotid artery; there were no significant changes in calcium, magnesium, or water content.

The JGI is significantly ($p < 0.01$) increased in the group of sodium-restricted dogs (46.6 ± SE 6.6) when compared to the control group (12.3 ± SE 5.8), as previously reported by many workers. The zona glomerulosa of the adrenal gland of the sodium-restricted animals shows a marked hyperplasia with an increase in G6PD activity (grade 3) when compared to that of control dogs (grade 2).

Discussion. During severe sodium restrictions, PRA and RRA are increased, as previ-

TABLE II. Effects of Severe Sodium Restriction on Norepinephrine Content[a] in Vascular Tissues of Dogs.

	Group A sodium-restricted dogs ($n = 7$)[b]	Group B control dogs ($n = 4$)[c]
Saphenous artery	1.27 ± 0.27	1.37 ± 0.30
Saphenous vein	0.58 ± 0.15	0.72 ± 0.20
Superior mesenteric artery (2nd to 5th branches)	5.48 ± 0.54	6.39 ± 0.72
Superior mesenteric vein (2nd to 5th branches)	3.70 ± 0.59	4.33 ± 0.36
Left ventricle	0.44 ± 0.10	0.57 ± 0.08
Thoracic aorta	1.42 ± 0.20	1.92 ± 0.35

[a] Expressed in μg/g of wet weight, mean ± SE. There is no significant difference between the values obtained in both groups.
[b] 15 g of "sodium-free" diet/kg of body weight, plus 3 mg of NaCl/kg/day supplement for 4 weeks.
[c] Same dietary regimen plus 100 mg of NaCl/kg/day for 4 weeks.

TABLE III. Effects of Severe Sodium Restriction on Sodium Content[a] in Vascular Tissues of Dogs.

	Sodium		
	Group A sodium-restr. dogs ($n = 7$)[b]	p A vs B	Group B control dogs ($n = 4$)[c]
Saphenous artery	226 ± 9.2	<0.001	301 ± 6.9
Mesenteric artery (2nd to 5th branches)	246 ±10.2	<0.01	317 ± 8.0
Carotid artery	233 ±12.7	<0.01	311 ±11.7
Renal artery	252 ± 5.2	NS	251 ±10.4
Left ventricle	175 ± 5.1	<0.01	209 ± 5.2
Thoracic aorta	235 ± 9.5	<0.001	329 ± 7.1

[a] Expressed in μEq/g of dry weight, mean ± SE.

[b] 15 g of "sodium-free" diet/kg of body weight, plus 3 mg of NaCl/kg/day supplement for 4 weeks.

[c] Same dietary regimen plus 100 mg of NaCl/kg/day for 4 weeks.

[d] NS = not significant.

ously reported by many workers and this is accompanied by a decreased vascular reactivity of exogenous renin and angiotensin. The slight, although not significant, mean decrease of tissue norepinephrine content in the sodium-restricted dogs is in contrast with findings in rats of De Champlain *et al.* (13), who showed that rats have under sodium restriction an increased myocardial NE storage and a lower blood pressure. In our study, the dogs under severe sodium restriction presented no significant change in blood pressure.

We have compared the findings of renin activity and of NE content of tissues from sodium-restricted dogs to those of another control group which was maintained on Purina Chow, Ballard's meat and water *ad libitum*. This comparison shows a significant ($p < 0.05$) decrease of NE in some tissues (saphenous artery, aorta, and branches of mesenteric vein) and a significant ($p < 0.02$ to 0.001) increase of renin activity in all vascular tissues studied with the exception of the thoracic aorta.[4]

A decrease in sodium content in aortic walls of rats receiving a low sodium diet was previously reported by Tobian (14), without any change in plasma sodium concentration. The present results extend these findings in branches of saphenous and mesenteric arteries, carotid artery, thoracic aorta, and left ventricle of dogs. It is probably of physiological significance that the renal artery of dogs under severe sodium restriction shows no decrease in sodium content. The sodium content of the renal artery in control dogs receiving a "normal" NaCl supplement is the lowest of all arterial tissues studied and it is of interest that, in the dogs of group A under severe sodium restriction, the value for this artery becomes greater than that of all other vascular tissues. Friedman *et al.* (15) have stressed the importance of sodium for maintenance of a normal vasoconstriction. The

[4] The difference in the significance of the results in the group of sodium restricted dogs when compared to the control group of dogs which received the same diet plus an average NaCl supplement of 2 g for a 20-kg dog versus the control group maintained on Purina Chow and Ballard's meat is most probably due to the much higher salt content of this latter diet. In this experiment, we have used as "normal" NaCl intake of dogs, the values for daily NaCl requirement given for dogs by Berger (13a) and which range from 584 to 4380 mg/day. By contrast, the mixture of Purina Chow and Ballard's meat given in our Institute to a 20-kg dog contains an average of 5 g of NaCl/day. This higher sodium intake of the control group of dogs kept on Purina Chow and Ballard's meat is probably responsible for the lower tissue renin activity and higher norepinephrine content and as a result, for the greater significance of the changes observed in the dogs maintained on sodium-free diet.

present finding shows that the renal artery maintains, during periods of severe sodium restriction, a "normal" sodium content and suggests a normal response and receptor sensitivity to various stimuli.

Previous correlative studies from our laboratory in rats under a sodium-restricted diet have shown an increase in PRA as well as in JGI. The present dog experiments, as well as those in rats (16), show that in such experimental conditions the storage (high JGI and RRA) and the renin release (high PRA) from the juxtaglomerular cells are increased. The role of the increased G6PD activity in the cells of the adrenal zona glomerulosa associated with the marked hyperplasia of this zone confirms the recently reported work of Pohanska and Pike (17) and is consistent with the hypothesis that this enzyme activity can be regarded as an index of aldosterone synthesis.

Summary. Seven dogs were maintained for 1 month on a severely sodium-restricted diet. Another group of 4 dogs was maintained on the same dietary regimen with the addition of a "normal" NaCl supplement and served as controls. We studied: (a) renin activity in plasma (PRA), renal cortex (RRA), saphenous and mesenteric arteries (2nd to 5th branches), aorta, and left ventricle; (b) norepinephrine (NE) content of branches of mesenteric and saphenous arteries and veins, aorta, and left ventricle; (c) cation and water contents in saphenous, mesenteric (2nd to 5th branches), carotid and renal arteries, aorta, and left ventricle; (d) juxtaglomerular granulation index (JGI); (e) glucose-6-phosphate dehydrogenase (G6PD) activity in the cells of the adrenal zona glomerulosa. The most important findings were: (i) a significant increase of PRA and RRA and a slight, although not significant, increase in renin activity in the vascular tissues studied; (ii) a constant, but not significant, decrease in norepinephrine content in the same tissues; (iii) an unaltered plasma sodium concentration; (iv) a significant decrease of sodium content in all vascular tissues studied, with the exception of the renal artery; and (v) a significant increase in JGI associated with a greater G6PD activity in the adrenal zona glomerulosa.

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