Renin Activity Content in Various Tissues of Dogs Under Different Physiopathological States[1] (34770)

K. HAYDUK,[2] R. BOUCHER,[3] AND J. GENEST
Clinical Research Institute of Montreal, Montreal, Canada

The presence of renin or a renin-like enzyme in tissue other than plasma and kidneys has been reported in arterial tissues and in several organs of various species (1–11). In the present study, we report the systematic measurement of the renin activity content (RAC) in arteries and in many organs of dogs under normal conditions and the changes observed under different physiopathological states such as severe sodium restriction, bilateral nephrectomy, experimental congestive heart failure, constriction of one renal artery with or without contralateral kidney.

Materials and Methods. Mongrel dogs were divided into the following groups: (a) Group 1: 11 male dogs weighing 17 to 27 kg and kept in a constant temperature and humidity room and receiving Purina chow, Ballard's meat and tap water *ad libitum* for at least 2 weeks. On the day of sacrifice, 10 ml of peripheral blood were drawn for measurement of plasma renin activity according to Granger's modification (12) of Boucher's procedure (13). The dogs were then anesthetized with pentothal (30 mg/kg) and aliquots of arterial tissues (mesenteric artery and saphenous artery branches, and aorta), right and left ventricles, lung, liver, spleen, skeletal muscle (rectus femoris), adrenal glands, and kidneys were taken. (b) Group 2: 5 dogs which received daily 15 g of a "sodium-free" diet (Nutritional Biochemicals Corp., Cleveland), 40 ml of demineralized water and 3 mg of NaCl/kg of body weight/day for a 4-week period. (c) Group 3: 6 male dogs bilaterally nephrectomized under pentothal anesthesia (30 mg/kg). An infusion of valine-5-angiotensin II-aspartic β-amide (Ciba) was administered at a rate of 200 ng/kg/min, 30 min before anesthesia and was continued until removal of the second kidney in order to suppress renal renin secretion. Five dogs were kept alive for 24 hr and one for 48 hr. Four of the 6 dogs were bled to death and then aliquots of the various organs were removed. Two of the 6 dogs were handled as those of group 1. (d) Group 4: 5 dogs with congestive heart failure and ascites following total removal of the tricuspid valve (e) Group 5: 12 dogs with constriction of one renal artery. Under pentothal anesthesia and by a lumbar approach, the left renal artery was dissected free from surrounding tissues. A Goldblatt clamp was placed on the artery and completely closed. It was then opened counterclockwise, 1.5 to 2 turns according to the size of the artery. The contralateral kidney remained untouched in 10 dogs and was removed in 2 others. Three months following clamping, this group was divided into (i) subgroup A: 6 dogs which presented an increase in mean arterial pressure of 50 mm Hg or more, (ii) subgroup B: 4 dogs which showed either no change in blood pressure or a mean increase of less than 25 mm Hg, and (iii) subgroup C: 2 dogs with severe hypertension secondary to unilateral clipping and removal of the contralateral kidney.

The dogs of groups 3 to 5 were fed Purina chow, Ballard's meat and tap water *ad libitum*. Procedures for anesthesia and organ removal in dogs of groups 2, 4, and 5 were

[1] Work supported through grants MT-2915 and MT-1853 from the Medical Research Council of Canada.

[2] Research Fellow of the Deutsche Forschungsgemeinschaft (1968) and the Medical Research Council of Canada (1969).

[3] Research Associate of the Medical Research Council and professor of Medicine, University of Montreal.

identical to those of group 1.

Procedure for tissue renin activity content determination. The determination of renin activity content (RAC) was mostly based on the micromethod for renin activity determination in rat, as described by Boucher *et al.* (13). Aliquots of the organs studied were deep-frozen immediately after removal and about 500 mg were kept for determination of (RAC). The tissues were frozen and thawed three times, homogenized first with mortar and pestle, and then transferred with 0.9% NaCl in a tissue crusher (Fisher No. 7727) to obtain complete homogenization. The total volume of the homogenate was brought to 10 ml by adding 0.9% NaCl and centrifuged for 10 min at 10,000 rpm. This homogenization procedure was performed at 2 to 5°. One ml of supernatant from homogenates of adrenal glands, liver, and spleen extracts, 2 ml of heart and lung extracts and 3 ml of skeletal muscle extracts were incubated with 2 ml of trisphosphate buffer, excess homologous renin substrate and 1 ml of Dowex (50W-X2 (NH_4^+) at pH 5.5 for 12 hr. Weighed aliquots of arterial tissue of about 100 mg were homogenized in the tissue crusher under addition of about 2.5 ml of 0.9% NaCl. The total of the arterial homogenate was incubated under the same conditions as described for the organ extracts; plasma (1 ml) was incubated under similar conditions.

For the measurement of renal renin activity content (RRAC), a weighed aliquot (about 1 g) of renal cortex was homogenized, diluted 1:200 in bidistilled water, centrifuged for 10 min at 10,000 rpm, and 0.1 ml of the supernatant was incubated for 1 hr, as described above.

Following incubation, the mixture was processed as described in Boucher's micromethod. The amount of angiotensin formed during incubation was determined by a bioassay using nephrectomized rats, in comparison to standard valine-5-angiotensin II-aspartic β-amide. Results are expressed as nanograms of angiotensin formed per 1 ml of plasma or 1 g of tissue/1 hr of incubation.

Results. Recovery of added angiotensin. The recovery of added valine-5-angiotensin II-aspartic β-amide was performed in all organs studied. In each 3 different experiments with 50 and 250 ng of added angiotensin II the recovery was between 70 and 80%.

Recovery of added renin. The recovery of renin was determined by comparing the liberation of angiotensin after adding the same amount of renin at different steps of the procedure. For all tissues studied, the recovery of renin (hog renin, Nutritional Biochemicals Corp., Cleveland)—before and after homogenization—was between 90 and 100% in 2 different experiments. The added renin, when incubated without tissue homogenate and with excess renin substrate, formed 80 ng of angiotensin in 12-hr incubation.

Effect of incubation time and of enzyme concentration on the liberation of angiotensin. A linear relation between time of incubation and formation of angiotensin was found up to 12 hr. Longer incubation periods were not studied.

A similar linear relation between amounts of enzyme and liberation of angiotensin was found.

Renin activity content of dog tissues. The results obtained in the five groups of dogs are described in Table I. The highest RAC, after the kidney, is found in adrenal glands, followed by liver and spleen. In the same dog, the adrenal RAC is higher than in the liver and in turn the liver RAC is higher than in the spleen, despite the fact that values overlap when comparing one dog to another. In only one dog with one renal artery clipped and with contralateral nephrectomy, was the RAC higher in the spleen than in the liver. Skeletal muscle and arterial tissues always show the lowest RAC. A careful attempt was made to separate the cortex from the medulla in adrenal glands. Findings in three experiments indicate that the renin content is located mostly in the medulla, being about 2-fold greater than in the cortex. Dogs maintained on severe sodium restriction for 1 month show a significant increase in PRA, RRAC, and RAC in all tissues, with the exception of adrenal glands and aorta. Dogs nephrectomized 24 or 48 hr previously did not show any significant change in tissue RAC when compared to control animals. The

TABLE I

PLASMA RENIN ACTIVITY[a] AND RENIN ACTIVITY CONTENT[a] IN TISSUES OF DOGS UNDER VARIOUS PHYSIOPATHOLOGICAL CONDITIONS.

	GROUP 1	GROUP 2	GROUP 3[b]	GROUP 4[c]	GROUP 5		
	CONTROL DOGS (N = 11)	DOGS UNDER SEVERE SODIUM RESTRICT. (N = 5)	DOGS 24-48 HOURS AFTER NEPHRECT. (N = 6)	DOGS WITH CONGESTIVE HEART FAILURE	UNILATERAL "CLIPPED" KIDNEY, CONTRALATERAL K. UNTOUCHED		
					SUB-GROUP A WITH HYPERTENSION (N = 6)	SUB-GROUP B WITHOUT HYPERTENSION (N= 4)	SUB-GROUP C UNILATERAL GOLDBLATT CLAMP, CONTRALATERAL KIDNEY REMOVED, WITH HYPERTENSION (N = 2)
	"p" value						
PLASMA	0.23 ± 0.09	4.67 ± 0.73	0.04 ± 0.04	1.38 ± 0.41[b]	0.38 ± 0.38	0.10 ± 0.10[b]	0
	"p"< 0.001						
RENAL CORTEX NORMAL KIDNEY	23500 ± 4900	104000 ± 16000	-	19000 ± 1000[b]	-	-	30000[d]
	"p"< 0.01						
CLAMPED KIDNEY	-	-	-	-	30400 ± 12400	33750 ± 13600[b]	27500
UNTOUCHED CONTRALATERAL K.	-	-	-	-	7100 ± 6200	15000 ± 5400[b]	-
RIGHT VENTR.	31.2 ± 5.3	-	-	27.3	36.8 ± 5.8	-	50.9
LEFT VENTR.	33.0 ± 6.5	105.3 ± 30.1	39.5 ± 13.0	30.4 ± 4.9[b]	46.5 ± 5.5	35.8 ± 6.2[b]	64.5
	"p"< 0.01						
LUNG	17.8 ± 2.2	35.3 ± 9.3	27.3 ± 8.4	11.2	14.3 ± 4.0	27.4 ± 6.6[b]	29.8
	"p"< 0.05						
LIVER	140.2 ± 14.8	469.1 ± 94.3	108.6 ± 37.2	137.5	151.3 ± 16.0	121.4 ± 34.6[b]	128.4
	"p"< 0.001						
SPLEEN	77.2 ± 8.6	234.0 ± 51.7	81.5 ± 21.8	57.4	91.3 ± 9.6	75.9 ± 22.3[b]	162.3
	"p"< 0.001						
SKELETAL MUSC.	4.3 ± 0.7	17.5 ± 7.2	7.1 ± 2.7	4.8	3.4 ± 0.6	5.0 ± 3.2[b]	6.3
	"p"< 0.02						
ADRENALS	446.1 ± 94.8	541.3 ± 190.4	258.2 ± 54.3	246.5	872.9 ± 255.7	551.9 ± 175.5[b]	1637
	b						
SAPHENOUS ARTERY BRANCHES	2.38 ± 0.33	3.93 ± 0.50	-	2.78 ± 0.67[b]	3.28 ± 0.89	-	1.95
	"p"< 0.02						
MESENTERIC ARTERY BRANCHES	2.49 ± 0.30	7.72 ± 0.64		3.42 ± 0.38[e]	2.61 ± 0.37	8.00 ± 2.39[f]	7.02
	"p"< 0.001						
THORACIC AORTA	3.46 ± 0.48	5.37 ± 0.89	-	3.0 ± 0.5[b]	3.10 ± 0.35	9.42 ± 1.55[g]	5.45
	b						

[a] Expressed in ng angiotensin/l ml plasma or 1 g tissue/1 hour incubation. Mean ± S.E.

[b] No significant difference when compared to control dogs of Group 1.

[c] Findings a) in plasma, arterial tissue, left ventricle and kidneys from 5 dogs; b) in the other organs from 2 dogs.

[d] Refers to the contralateral kidney which was removed at the time of the Goldblatt clamp.

[e] "p"< 0.05 when compared to control dogs (Group 1)

[f] "p"< 0.01 " " " " " "

[g] "p"< 0.001 " " " " " "

RAC in mesenteric artery branches and aorta was significantly greater in dogs with unilateral renal clipping, which did not develop significant hypertension, than in normal or hypertensive animals. In dogs with unilateral renal clipping and contralateral nephrectomy which developed significant hypertension, the tissue RAC also increased.

Discussion. These findings show that renin or a renin-like enzyme is present in the organs studied. When no renin substrate is added, no pressure response in the rat bioassay at the exception of a minimal one from some incubations with adrenal glands and heart could be observed. The pressor response curve of the eluate from the incubation mixture was identical to the standard angiotensin in the rat. Boiling the organ extracts before incubation destroyed their angiotensin-forming capacity. The loss of pressor activity by the addition of trypsin suggests that the vasoactive substance is a peptide.

The findings obtained show a distinct distribution pattern of RAC in the organs studied. The RAC of tissue homogenates exceeds the PRA by far when plasma and tissues are compared (ml for g). Thus the small

amount of extracellular fluid or plasma contained in the tissue studied is only a minimal part of the RAC found.

The high RAC found in adrenal glands is of interest because of the close relationship between angiotensin, aldosterone, and catecholamines. The significant increase in PRA, RRAC, and RAC in most tissues of dogs under severe sodium restriction supports the assumption that the factor responsible for the liberation of angiotensin in tissues is renin. The findings of an increase in RAC in mesenteric artery branches and aorta of dogs with constriction of one renal artery and without hypertension, when compared to the values obtained in normal dogs and in hypertensive dogs following unilateral renal clipping is surprising and remains to be explained.

After completion of the present study, the findings of Skeggs and co-workers (14) on "pseudo-renin," a new angiotensin-forming enzyme, were published. These workers found a "renin-like" enzyme which resembles renin in its ability to form angiotensin I from the synthetic tetradecapeptide renin substrate and from purified hog renin substrate A, but which differs from renin in having a maximal activity at a much lower pH value and in being found in many tissues other than kidney (the highest content being found in the salivary gland, in spleen, thymus, aorta, and others) and also in plasma. This new enzyme called "pseudo-renin" can be separated from renin by chromatography on DEAE-cellulose. The relationship between the renin activity we are measuring and the "pseudo-renin" of Skeggs and co-workers needs further investigation. It is of interest in our studies that severe sodium restriction increases the renin activity content in the tissues studied and that there is no significant decrease 24 to 48 hr following nephrectomy. Our findings are consistent with those recently reported by Nasjletti *et al.* (15) who postulated an extrarenal renin-like enzyme in plasma and in tissues of bilaterally nephrectomized rats.

Summary. Plasma renin activity and renin activity content in several tissues of dogs under conditions of severe sodium restriction, congestive heart failure, clipping of one renal artery, and nephrectomy were studied and compared to the values obtained in control dogs. The highest extrarenal RAC was found in adrenal glands followed by liver, spleen, heart, lung, skeletal muscle, and arterial tissue. Severe chronic sodium restriction increased RAC in all tissues, with the exception of the adrenal glands and aorta; whereas, the RAC following nephrectomy did not decrease significantly. In renovascular hypertension secondary to unilateral renal clipping, there is a slight, but not significant, increase in RAC in adrenal glands.

1. Werle, E., Vogel, R., and Goldel, L., Naunyn-Schmiedebergs Arch. Exp. Pathol. Pharmakol. **230**, 236 (1957).

2. Hunter, C. A., and Howard, W. F., Amer. J. Obstet. Gynecol. **79**, 838 (1960).

3. Stakemann, G., Acta Pathol. Microbiol. Scand. **50**, 350 (1960).

4. Gould, A. B., Skeggs, L. T., and Kahn, J. R., J. Exp. Med. **119**, 389 (1964).

5. Gross, F., Schaechtelin, G., Ziegler, M., and Berger, M., Lancet **1**, 914 (1964).

6. Ryan, J. W., Science **158**, 1589 (1967).

7. Skeggs, L. T., Lentz, K. E., Gould, A. B., Hochstrasser, H., and Kohn, J. R., Fed. Proc., Fed. Amer. Soc. Exp. Biol. **26**, 42 (1967).

8. Capelli, J. P., Wesson, L. G., Jr., Aponte, G. E., Feraldo, C., and Jaffe, E., J. Clin. Endocrinol. Metab. **28**, 221 (1968).

9. Genest, J., Simard, S., Rosenthal, J., and Boucher, R., Can. J. Physiol. Pharmacol. **47**, 87 (1969).

10. Rosenthal, J., Boucher, R., Rojo-Ortega, J. M., and Genest, J., Can. J. Physiol. Pharmacol. **47**, 53 (1969).

11. Takeda, T., DeBusk, J., and Grollman, A., Amer. J. Physiol. **216**, 1194 (1969).

12. Granger, P., thesis for the degree of Ph.D., McGill University, Montreal, February, 1969.

13. Boucher, R., Ménard, J., and Genest, J., Can. J. Physiol. Pharmacol. **45**, 881 (1967).

14. Skeggs, L. T., Lentz, K. E., Kahn, J. E., Dorer, F. E., and Levine, M., Circ. Res. **25**, 451 (1969).

15. Nasjletti, A., Matsunaga, M., and Masson, G. M. C., Endocrinology **85**, 967 (1969).

Received Dec. 3, 1969. P.S.E.B.M., 1970, Vol. 134.