PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 148, 562–567 (1975)

The Renin–Angiotensin System in Rats Made Hypertensive by Ligation of the Kidney Poles (38584)

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Ligation of both poles of one kidney followed by the removal of the contralateral one elicits some degree of arterial hypertension in up to 80% of the rats submitted to the operation. In these animals, the weights of the medial zone of the kidney and of the adrenal glands are directly correlated with the arterial pressure (1), and most of the hypertensive rats have heavily granulated juxtaglomerular cells in some areas of the kidney poles; in contrast, in the medial zone there is hypogranularity. Loyke (2), who induced hypertension by the same procedure, showed that the synthetic peptide SQ 20858 significantly reduces the arterial pressure. SQ 20858 inhibits angiotensin I converting enzyme as well as the inactivation of kinins (3, 4). Engel (5), using the figure-of-eight ligature procedure to produce renal hyperrenal hypertension in rats, showed that SQ 20858 is hypotensive. These findings suggest the participation of the renin–angiotensin system in this model of renal hypertension.

We have studied some variables of the renin–angiotensin system in plasma and in the kidney and other organs when hypertension is induced by ligation of the kidney poles. This experimental model of renal hypertension allowed a separate study of the compressed part of the kidney (the poles) and the unconstricted part, the medial zone.

Methods. Male Sprague–Dawley rats, 90–120 g, were used. Hypertension was produced by ligating both poles of the left kidney and removing the right kidney 1 wk later (1). Sham-operated rats without ligatures were used as controls. All animals were fed on Purina rat chow and tap water *ad lib.*

Arterial blood pressure and body weight were measured weekly. Pressure was measured indirectly by means of a tail cuff. The animals were under light ether anesthesia. The pressure on the tail cuff and the tail pulse were recorded simultaneously on a Grass model 7 polygraph fitted with a 7P8 preamplifier and a model 1010 Grass crystal microphone as a pulse detector. Usually three or four measurements were taken until the rats began to wake up. The average of all values was used. When the pulse was not detectable, the tail was slightly warmed. Pole-ligated rats were classified as hypertensive when their indirect arterial pressures were 150 mmHg or higher for more than 3 consecutive wk. The rats were classified as normotensive when indirect arterial pressure was 140 mmHg or lower during the last 3 wk and never higher than 150 mmHg.

Twenty-three control, 27 ligated kidney hypertensive, and 21 ligated kidney normotensive rats were used. Six to 10 wk after ligation of the renal poles, the animals were anesthetized with sodium pentobarbital (Nembutal), 40 mg/kg intraperitoneally. A polyethylene tubing was placed on the trachea. Mean arterial pressure was measured directly via the femoral artery, which was cannulated with a polyethylene tube (PE-50). The pressure was recorded on a Grass polygraph by means of Statham pressure transducers. Blood samples were then taken from the femoral artery catheter into cold syringes containing EDTA (for Renin and Renin Substrate) or heparine (for converting enzyme), the blood being kept at 4° until the plasma was separated. Plasma was kept frozen until used for the biochemical measurements. The following measurements were made in the plasma:

Plasma renin concentration: Measured in 0.1 ml of plasma by the micro-method of Boucher *et al.* (6), and values were expressed

[1] This work was supported in large part by the Medical Research Council of Canada Group Grant to the Hypertension Multidisciplinary Group of the Clinical Research Institute of Montreal.

in nanograms of angiotensin formed per milliliter of plasma per hour of incubation.

Plasma concentration of renin substrate: Plasma (0.2 ml) was incubated for 1 hr with an excess of homologous renin (0.2 ml of the homogenate of 1 g of rat renal cortex in 50 ml of distilled water). Incubation, elution and bioassay were conducted under the same conditions as for the plasma renin, and values are expressed in nanograms of angiotensin formed per milliliter of plasma.

Plasma "converting enzyme" activity: Method of assay has been described previously (7). Values are expressed in nanomoles of histidyl-leucine liberated from angiotensin I per milliliter of plasma per minute of incubation.

After the blood samples had been collected, the kidney was removed and divided longitudinally into two equal parts. One part was fixed with Zenker-formol, stained with Bowie's dye (8), and used for the study of granularity of juxtaglomerular cells, measured semi-quantitatively as the juxtaglomerular index (JGI) devised by Hartroft and Hartroft (9). The JGI was studied for the whole kidney and separately for the medial zone and for each pole of the kidney, including the areas surrounding the ligatures on the poles. The second half of the kidney was used for the measurement of renal renin concentration (6).

In 10 ligated hypertensive, nine control, and nine ligated normotensive rats, kidney slides were made for histochemical estimation (8) of the glucose-6-phosphate dehydrogenase (G6PD) content of the macula densa. Glomeruli were counted separately in the medial zone and poles of the kidney; those whose macula densa were stained were assigned to two groups, according to whether they were lightly or heavily coloured.

Finally, iso-renin content was measured (10) in the left ventricle, aorta, adrenal glands, and brain tissue. For these studies the brain was dissected to yield the hypothalamus, frontal cortex, and medulla oblongata.

Results. Arterial pressure in the animals and their body weights, at the time of the final experiment, are shown in Table I. Five of the 27 hypertensive rats, despite high indirect arterial pressures, had a normal direct mean arterial pressure under pentobarbital anesthesia. It was assumed that they became normotensive because of the anesthesia, since each had a pronounced cardiac hypertrophy with ventricular weight over 2.75 g/kg, as opposed to 2.46 ± 0.17 (SD) g/kg in the control group of 23 rats.

Plasma renin–angiotensin system. No differences were found in plasma renin concentration between the three groups of animals (Table II). Renin substrate was significantly lower in the hypertensive rats. The mean of the converting enzyme values of the hypertensive animals was higher than in the control and in the ligated normotensive rats; however, this difference was statistically significant only in comparison with the ligated normotensive rats. Among the pole-ligated rats a positive correlation was found between the indirect arterial pressures and the plasma concentration of converting enzyme ($r = 0.32$, $P < 0.05$).

Juxtaglomerular index, renal renin content, and glucose-6-phosphate dehydrogenase of the macula densa. Granules in the juxtaglomerular cells of the control rats were uniformly scattered in the cortex of the whole kidney. In the medial zone of the ligated kidney there were fewer granules, but they were still uniformly scattered. In the poles of the ligated kidney, the degree of juxtaglomerular cell

TABLE I. ARTERIAL PRESSURE AND BODY WEIGHT AFTER KIDNEY POLE LIGATION.

		Arterial pressure (mmHg)		
	n	Indirect[a]	Mean direct	Body weight (g)
Control	23	117 ± 14	113 ± 14	351 ± 39
Ligated hypertensive	27	182 ± 21	159 ± 33	339 ± 47
Ligated normotensive	21	120 ± 12	125 ± 14	327 ± 41

Values are means ± SD.

[a] Tail arterial pressure. Mean of the last 3 wk.

TABLE II. EFFECTS OF KIDNEYS POLE LIGATION ON RENIN, RENIN SUBSTRATE, AND ANGIOTENSIN I—CONVERTING ENZYME CONCENTRATION IN PLASMA.

	Renin			Substrate			Converting enzyme		
	n	ngAII/ml/hr	*P* value	*n*	ngAII/ml	*P* value	*n*	nmol His-Leu/ml/min	*P* value
Control	18	9.0 ± 6.2	—	7	116 ± 30	—	21	306 ± 67	—
Ligated hypertensive	21	9.0 ± 8.0	NS NS[a]	9	76 ± 39	<0.05 <0.10[a]	25	342 ± 76	NS <0.01[a]
Ligated normotensive	17	7.3 ± 3.5	NS	7	107 ± 31	NS	20	298 ± 85	NS

Values are means ± SD.

[a] *P* value versus ligated normotensive. All other *P* values are versus controls.

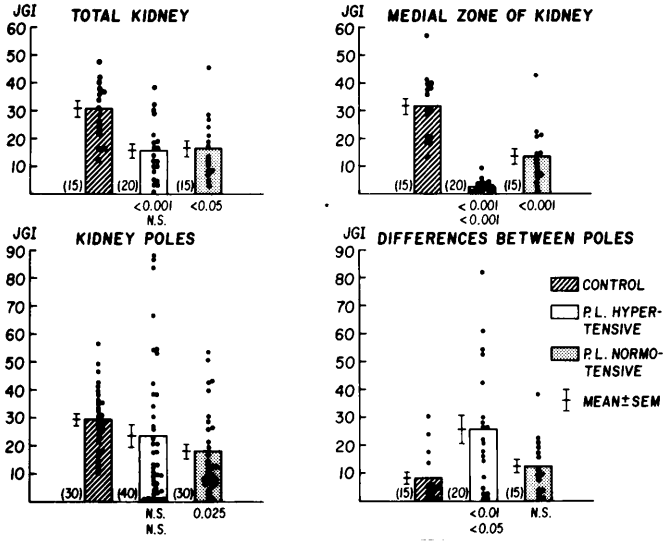

FIG. 1. Effect of kidney pole ligation on juxtaglomerular granularity index (JGI). Number of samples in parentheses. Most *P* values are versus controls; the second *P* value under the P.L. Hypertensive histogram is versus the P.L. Normotensive group.

granularity was not uniform: most of the juxtaglomerular cells with granules were in the ischemic zones or around the ligature, while in the rest of the poles the juxtaglomerular cells were depleted of granules. Results are shown in Fig. 1. The most constant findings in the hypertensive rats were the lower JGI in the medial zone and the presence of areas with heavily granulated juxtaglomerular cells in the poles. Because of the topographical variability of juxtaglomerular cells with granules on the kidney poles of the hypertensive rats, JGI values were highly dependent on the areas used for the study.

The renin content of the different portions of the kidney paralleled those of the JGI, but the differences in renin content in the medial zone of the kidney in the three experimental groups were not as great as for JGI (Fig. 2). Mean values in medial zone of the kidney of ligated hypertensive and normotensive rats were lower than in the control group ($P < 0.001$ and $P < 0.025$, respectively).

The histochemical G6PD activity in the macula densa of the ligated hypertensive rats was significantly ($P < 0.001$) less than in the control and ligated normotensive animals (Fig. 2). It was noted that the G6PD activity was not uniformly distributed in the poles of the kidney of the ligated hypertensive rats.

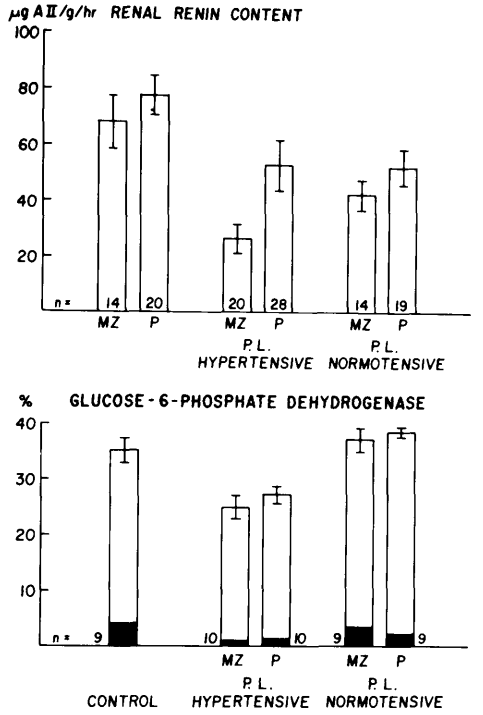

FIG. 2. Renal renin contents, and glucose-6-phosphate dehydrogenase activity determined histochemically on the macula densa. MZ = medial zone of the kidney, *P* = poles of the kidney. Renin is expressed as micrograms of angiotensin II released per hour per gram of tissue. G6PD activity is expressed as the percentage of glomeruli whose macula was stained with the blue formazan deposit. Solid shading represents the percentage of maculae densae which were heavily coloured. Control values are given for the whole kidney.

The macula densa G6PD activity and the JGI followed a parallel decrease in the medial zone of the kidney of the ligated hypertensive rats; however, the decrease of the JGI was more pronounced.

No correlation was found between JGI and renin content in the poles of the ligated kidneys, or between arterial pressures and either JGI or renal renin content. In the medial zone, JGI and renal renin content were positively correlated (Fig. 3); JGI in the medial zone was inversely correlated with arterial pressure arterial, but there was no significant correlation between renin content and pressure (Fig. 3).

Tissue iso-renins. Pole ligation, whether it led to hypertension or not, tended to produce lower iso-renin activity in adrenals, myocardium, and especially aorta (Table III) but had no effect on the three brain regions studied.

Discussion. Ligature of the kidney poles causes large changes in renin content and JGI, and this contributes new information on the morphodynamic aspects of the renin–angiotensin system in rats with this type of renal hypertension. Loyke and Markrell (11) have reported a decrease in the JGI of the whole kidney and Ebihara (12), in figure-of-eight hypertension, has reported, without quantitative analysis, that hypergranularity occurs in the constricted areas. Our analysis shows that the low total JGI in the kidney pole-ligated rats is largely due to a much decreased JGI in the medial zone, while areas of heavily granulated juxtaglomerular cells can still be present in the poles.

Renin content in the different portions of the kidney follows the pattern described for JGI values. The lack of correlation between the two sets of values can be explained by their topographical variation. In rats made hypertensive by figure-of-eight ligature, Ebihara (12) reports a raised renin content in the constricted part of the kidney and a low renin content in the unconstricted part. This difference disappears by the 20th postoperative day, at which time all parts of the kidney show significantly less renin than the kidneys of control animals. Hence, with respect to JGI and renal renin content, the pattern is the same for kidney pole-ligated and figure-of-eight ligature hypertensive rats.

The changes found in the JGI and renin content in the medial zone of the pole ligated kidney, recall the ones observed in the contralateral kidney of Goldblatt rats (13–15).

The decrease in the JGI and renin content of the medial zone of the kidney can be explained by the same mechanisms proposed for the "untouched" kidney in the two-kidney Goldblatt hypertension. Tobian *et al.* (16) have reported that the increase in perfusion pressure of the "untouched" kidney responds with a reduced renin production as a result of an increased aldosterone secretion induced by the renin liberated in the ischemic kidney.

We found no differences between experimental groups in plasma renin concentra-

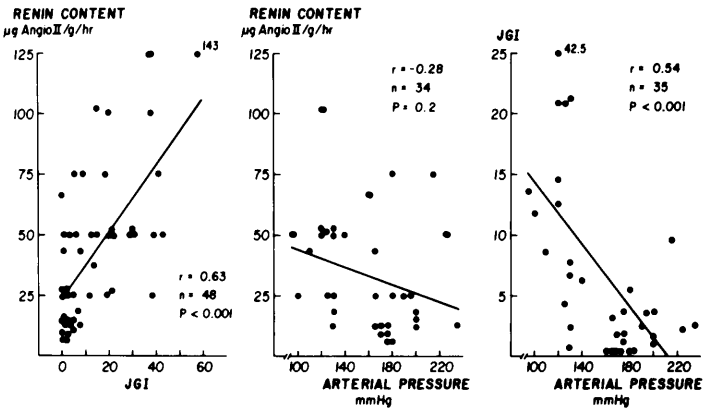

FIG. 3. Correlations between JGI, renin content of the medial zone of the kidney, and arterial pressure for rats with ligated kidney poles. Arterial pressure was measured indirectly on tail under light ether anesthesia, values are means of those obtained during the last 2 wk.

TABLE III. TISSUE ISO-RENINS.

	Control		Pole-ligated hypertensive			Pole-ligated normotensive		
	n	Mean ± SD	*n*	Mean ± SD	*P*	*n*	Mean ± SD	*P*
Heart (ng/g/hr)	8	16.3 ± 4.6	11	12.2 ± 3.8	<0.05 NS[a]	6	12.9 ± 3.0	NS
Adrenals (ng/mg Prot./hr)	11	0.95 ± 0.67	14	0.75 ± 0.42	NS NS[a]	8	0.41 ± 0.13	<0.05
Aorta (ng/g/hr)	7	68.5 ± 25.9	7	35.0 ± 17.8	<0.001 NS[a]	6	32.8 ± 8.1	<0.001
Hypothalamus (ng/g/hr)	8	14.1 ± 6.2	11	15.2 ± 3.5	NS NS[a]	6	15.8 ± 4.2	NS
Frontal cortex (ng/g/hr)	8	10.4 ± 2.1	11	10.1 ± 3.0	NS NS[a]	6	9.5 ± 1.2	NS
Medulla oblongata (ng/g/hr)	8	17.4 ± 3.2	11	15.8 ± 5.9	NS NS[a]	6	15.3 ± 4.7	NS

[a] *P* value versus ligated normotensive. All other *P* values are versus controls.

tion, but this does not exclude the possibility of an increase in the velocity of the renin—substrate interaction or of the interaction between angiotensin I and its converting enzyme. Such a possibility is favored by the lower plasma concentration of renin substrate and the slightly higher plasma concentration of converting enzyme in the hypertensive rats.

Whether the animals were hypertensive or not after ligature of the kidney poles, the iso-renins activity was lower in the aorta, adrenal glands, and left ventricular myocardium. We do not know what the significance of these observations is.

The question of whether or not the variation observed in the renin–angiotensin system is related to the systemic increase in the arterial pressure remains to be answered; however, the large variation of renin in the kidney and iso-renin content in some tissues suggest a possible participation of this mechanism in those areas.

Summary and Conclusions. The renin–angiotensin system was studied in experimental renal hypertension produced by ligation of the poles of the left kidney followed by contralateral nephrectomy. Plasma renin concentration was the same in hypertensive and controls animals. The plasma concentration of renin substrate was lower and that of angiotensin I converting enzyme was higher in hypertensive animals.

The juxtaglomerular index decreased in

the medial zone of the kidney, while heavily granulated areas appeared in the poles. Ligated kidneys of rats that remained normotensive showed juxtaglomerular indices intermediate between the control and the hypertensive rats. Differences in renal renin content between the groups correspond to those for the juxtaglomerular index, but were smaller.

No differences between the experimental groups were observed in iso-renin content in the brain; however, in all animals with ligated kidney poles, hypertensive or normotensive, there was a tendency for iso-renin in the adrenals, left ventricular myocardium, and especially aorta to be lower than in controls.

The role played by the changes of the renin-angiotensin system, in this kind of renal hypertension, remains to be elucidated.

These results suggest similarities between hypertension produced by compression of the kidney and hypertension produced by partial occlusion of the renal artery. Furthermore, when the kidney is compressed in this way, the medial zone behaves like the contralateral kidney in two-kidney Goldblatt hypertension.

The authors thank Miss L. Laporte and Mrs. I. Grimal for the technical assistance, and Mrs. M. Myette for secretarial assistance.

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Received July 1, 1974. P.S.E.B.M. 1975, Vol. 148.