Correlation between Granulation of Juxtaglomerular Cells and Extractable Renin in Rats with Experimental Hypertension.* (24535)

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Twenty years ago, Goormaghtigh theorized without much evidence that granules in the juxtaglomerular cells contained renin(1). This concept was strengthened somewhat when Peart, Gordon, Cook and Pickering reported that renin in rabbit kidney was present only in areas containing glomeruli and not in zones which contained only tubules(2). Recently, the Hartrofts have developed a superb stain for juxtaglomerular granules which permits a fairly accurate quantitation of them (3). It seemed of interest to determine both juxtaglomerular granulation and renin content in the same kidney of certain experimental rats. If the 2 parameters varied together, it would strengthen Goormaghtigh's old theory. If they did not, it would militate against its validity.

Methods. The Hartroft stain for juxtaglomerular granules was utilized as previously described(3,4). Four separate sections were examined for each kidney, and the average of these 4 sections provided the "juxtaglomerular index"(3,4). For renin assay, the rat kidney was homogenized in cold isotonic saline (5 cc of saline/g of kidney) in a Potter-Elvehjem homogenizer for 8-10 minutes. Then the homogenate was shaken at room temperature for 2 hours to allow time for extraction of renin. The homogenate was then centrifuged at 3000 rpm while being maintained at 20°C for one hour. The supernatant extract was obtained for subsequent injection into "bioassay" animals weighing approximately 250 g. Rats for the bioassay were anesthetized with nembutal and nitrous oxide and the femoral artery was cannulated. The cannula and its tubing were connected by saline to a mercury manometer. 0.3 cc of the supernatant fluid from kidney homogenate was injected intraarterially in the bioassay procedure. If renin was present in the extract, a sharp rise in blood pressure occurred, reaching a maximum in 3 minutes. Blood pressure would then gradually fall to the baseline over the next 30-50 minutes. After one injection, the rats would become variably tachyphylactic to subsequent doses of extract. For this reason, all rats used to bioassay renin were not given more than one injection of the extract. The difference in blood pressure readings just before and 3 minutes after injection of the extract gives an estimate of the amount of renin contained in the extract. In *Exp. I*, a silver clip was applied to one renal artery to partially narrow its lumen. The rats were usually quite hypertensive 8 weeks after application of the clip. Renin assays on both "clipped" and the "untouched" kidneys were carried out 6 months after the original clipping procedure. In Floyer's useful terminology, the "clipped" kidney has a clip on its renal artery(5). The "untouched" kidney, which has never been surgically manipulated, is contralateral to the "clipped" kidney. Holtzman rats were used for clipping as well as for bioassay of kidney extracts. Normal blood pressure of the Holtzman strain averages 121 mm Hg (S.D. $= \pm$ 13) using the Friedman microphonic method(6) with light ether anesthesia. In *Exp. II*, desoxycorticosterone hypertension was produced by injecting 125 mg of desoxycorticosterone trimethylacetate subcutaneously and repeating this dose after a 4 week interval. Rats were allowed to drink only a 1% NaCl solution while the steroid was being absorbed. The rats were usually hypertensive by the sixth week of steroid administration, at which time renin assays on their kidneys were performed. The extract was bioassayed simultaneously on 2 test rats

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TABLE I. Granulation of Juxtaglomerular Cells and Renin Content of "Clipped" and "Untouched" Kidneys in 9 Rats with Unilateral Renal Hypertension.

	Final B.P. before obtaining kidney (mm Hg)	"Clipped" kidney JG index	B.P. response to extract from "clipped" kidney (mm Hg)	"Untouched" kidney JG index	B.P. response to extract from "untouched" kidney (mm Hg)
	146	18	+50	0	+1
	162	57	+44	2.5	−2
	156	31	+38	4	−6
	162	54	+57	2	0
	154	37	+40	1.5	−4
	164	50	+42	3.5	+1
	138	26	+40	11	+4
	174	39	+54	10	+7
	138	58	+38	7	+7
Mean	155	41	+45	4.6	+1

instead of one, and the results in the 2 rats were averaged. Kidneys from 6 normal rats served as controls. Only rats of the Wistar strain were used in this experiment. This includes rats receiving desoxycorticosterone, normal control rats, and rats receiving kidney extract in the bioassay procedure. The blood pressure of normal Wistar rats averages 102 mm Hg (S.D. = ±8). The rats in the 2 experiments were of 2 different strains with differing juxtaglomerular indices. Therefore the normal rats in *Exp. II* cannot be validly compared with the rats in *Exp. I*.

Results. Exp. I. The "clipped" kidney was compared with the "untouched" kidney in 9 rats (Table I). This was believed to be an interesting comparison since "clipped" kidneys tend to increase their juxtaglomerular granulation, while granulation in "untouched" kidneys becomes drastically reduced(4). "Clipped" kidneys had a mean juxtaglomerular (JG) index of 41 and a mean renin response of +45 mm Hg. The "untouched" kidneys had a mean JG index of 4.6 and a mean renin response of +1 mm Hg. Among the "untouched" kidneys, 3 had relatively high juxtaglomerular indices of 11, 10, and 7. Two of these 3 rats had a very mild hypertension. Renin responses of these 3 kidneys were +4 mm Hg, +7 mm Hg, and +7 mm Hg, respectively. The JG indices of the other 6 "untouched" kidneys were 4 or less, and the renin responses varied from −6 mm Hg to +1 mm Hg, indicating an almost complete absence of renin. The renin contents paralleled quite closely the juxtaglomerular indices. The JG index of normal kidneys from this strain averages 30 (S.E. of mean = ±1.9), indicating that the JG index of the "clipped" kidneys in this experiment is significantly elevated.

Exp. II. Kidneys of Wistar rats with desoxycorticosterone hypertension (Table II) were compared with kidneys of normal Wistar rats (Table III). It has been shown previously that juxtaglomerular granules virtually

TABLE II. Granulation of Juxtaglomerular Cells and Renin Content of the Kidney in 8 Rats with Desoxycorticosterone Hypertension.

	Final B.P. before obtaining kidneys (mm Hg)	JG index	B.P. response to kidney extract (mm Hg)
	154	2	−14
	150	1	− 4
	168	1	− 6
	160	2	− 6
	162	2	− 7
	154	1	−10
	162	1	− 5
	162	1	−14
Mean	159	1.4	− 8

TABLE III. Granulation of Juxtaglomerular Cells and Renin Content of 6 Normal Rat Kidneys.

	JG index	B.P. response to kidney extract (mm Hg)
	55	+52
	67	+52
	48	+22
	29	+18
	44	+20
	42	+21
Mean	48	+31

disappear in rats given desoxycorticosterone and a high sodium intake(3,4). In Table II, the JG indices and renin responses of 8 such hypertensive rats are presented. Average JG index in the group receiving desoxycorticosterone was 1.4 and no index was higher than 2 in any individual rat. Renin response averaged –8 mm Hg for the group. In this group, the extracts uniformly produced a drop in blood pressure of bioassay test animals, indicating an almost complete absence of renin in these kidneys. Virtual absence of juxtaglomerular granules corresponded quite well with the extremely low renin content.

In Table III, the results with the 6 normal control rats are given. The average JG index for the group was 48 and the average renin response was +31 mm Hg. There is an abundance of both renin and juxtaglomerular granulation in kidneys of these rats.

Discussion. Renin assays obtained on our rats receiving desoxycorticosterone confirm the observations of Gross and Sulser(7). However, renin assays on our rats with renal hypertension are not in agreement with those of Williams, *et al.*(8). In their studies the "ischemic" kidney of the rat usually did not have more extractable renin than the contralateral "untouched" kidney. Our experimental method was slightly different from theirs, which probably accounts for the difference in results. The fact that renin closely parallels granulation of juxtaglomerular cells, does not prove that these granules contain renin. The observation, however, is most compatible with such a hypothesis. It may be that both renin content and granules are separately and independently influenced by certain physiological states, such as a rise in the pressure of blood perfusing a kidney. Further experiments are necessary before the question can be finally resolved.

In kidneys with a very low juxtaglomerular index, the kidney extract usually produced a depressor response in the test animal. In these kidneys, the renin content is probably minimal, allowing depressor substances in the extract to become readily apparent. In these kidneys there is no doubt that depressor substances are present. It is possible that they are related to the depressor effects exerted by normal kidneys when introduced into the circulation of rats with renal hypertension(9). However, the presence of these depressor substances did not prevent hypertension in these rats.

Summary. Normal rat kidneys and kidneys with a narrowed renal artery have abundant granules in the juxtaglomerular cells and large quantities of extractable renin. On the other hand, kidneys of rats with desoxycorticosterone hypertension and the "untouched" kidney of rats with unilateral renal hypertension (resulting from narrowing of the contralateral renal artery) both have a virtual absence of juxtaglomerular granules as well as absence of extractable renin. There is so little renin in these kidneys that the renal extracts usually produce a depressor response. Thus, there exists a striking correlation between the amount of juxtaglomerular granulation and the amount of extractable renin in a kidney. This does not prove that these granules are composed of renin but the observations are compatible with such a hypothesis.

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