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Blood Kininogen in Renal Hypertensive Rats (38269)

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The striking decrease in urinary kallikrein in renal hypertensive rats and the correlation found between blood pressure levels and kininogenase activity in the urine led to the assumption that kallikrein is involved in the renal impairment which promotes the blood pressure rise (1, 2). Moreover, nephrectomy produces a considerable increase in kininogen which is significantly higher than that observed in rats submitted to sham operation or to different surgical traumas (3, 4). Furthermore, preliminary experiments disclosed a rise in plasma kininogen in a small group of rats having a moderate and long-lasting renal hypertension, thus providing further support to the theory that a kallikrein-like enzyme could be released by the kidney as a regulatory mechanism (5).

In order to shed some light upon kidney involvement in the kallikrein–kinins system, an experiment was designed to investigate whether a change in blood kininogen can be observed in an earlier period of renal hypertension. Besides kininogen, blood nitrogen urea was measured, in order to assess the magnitude of the renal impairment provoked by the renal ligature.

Material and Methods. Twenty Sprague–Dawley male rats (80–90 g body wt) were operated according to Grollman's technique (6). A figure-in-eight ligature was placed in one kidney, and the contralateral was removed a week later. From this group, 15 rats were used for kininogen determinations; they were sacrificed at intervals 47–123 days after the kidney removal. A group of 14 sham-operated rats of the same age was used as control. Blood-pressure determinations were carried out routinely once or twice a week. The photometric method recommended by Gilson (7) was adopted, and the blood-pressure tracings were recorded using a Grass polygraph.

Kininogen and urea measurements. To estimate plasma kininogens, trypsin was used, according to the method of Fasciolo *et al.* (8); instead of the hind leg perfusion bioassay, two different biological preparations were used to test kinins: isolated rat uterus and cat jejunum. Rat uterus is a very sensitive and reliable organ to measure pure kinins. The specificity of this test has been challenged when crude samples of kinins are used since the tissue is also sensitive to other substances (angiotensin, neurohypophysial hormones, etc.). Besides, peptides from plasma substrates other than kininogen, which potentiate the uterus to the kinins action, are released during trypsin hydrolysis (9, 10). Cat jejunum has been selected as one of the most specific biological preparations to detect kinins. It relaxes under catecholamines, does not respond to angiotensins, and is not directly affected by kallikrein. The day before the experiment blood pressure was measured in one hypertensive and in one control rat. Twenty-four hours later, under bromethone anesthesia (Avertin, Winthrop) given intraperitoneally (0.1 ml diluted 1 × 40 per 100 g body wt), the animals were bled. A DE-90 polyethylene catheter, filled with heparin solution (19 mg/ml), was introduced into one carotid artery. The blood collected in a polyethylene tube was immediately centrifuged. Samples of 0.2 ml in duplicate were taken from each plasma for kininogen determinations. Urea nitrogen was measured in the plasma, using the micromethod of Skeggs (11) adapted to a Technicon Autoanalyzer, and was expressed in mg of urea per 100 ml of blood.

Isolated organs. Nonpregnant female rats and small cats (500–700 g) were used. A uterus horn from a rat was immersed in Tyrode modified solution (12). Longitudinal strips about 3–4 mm wide and 5–6 cm long were cut from the cat jejunum and bathed in Krebs solution, according to Ferreira and Vane (13). The preparations,

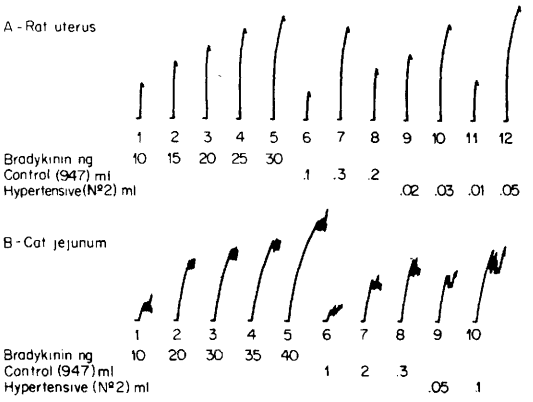

FIG. 1. Effect on rat uterus and cat jejunum of trypsin hydrolysate of plasma from one normal (No. 947) and one hypertensive rat (No. 2). (A) In 2–5, effect of 10, 15, 20, 25, and 30 ng bradykinin; in 6–8, effects of 0.1, 0.3, and 0.2 ml of normal rat plasma hydrolysate; in 9–12, effects of 0.02, 0.03, 0.01, and 0.05 ml of hypertensive rat plasma hydrolysate. (B) Similar assay upon cat jejunum.

kept in Krebs solution in a refrigerator for 18–72 hr, were more sensitive than the freshly prepared. Bradykinin (Br) (ampoules from Sandoz, Basel) diluted in Tyrode solution (100 ng/ml) immediately before the experiment was used as standard. Kininogen was expressed in μg of bradykinin per ml of plasma.

Results. Figure 1 shows typical responses of both biological preparations under the action of two plasma extracts, one obtained from an hypertensive rat and another from the control. In this particular experiment, the contractile effect produced by the hypertensive sample upon uterus and jejunum was approximately 3 times higher than that of the control, but the absolute values of bradykinin equivalents of both plasmas were greater in the test performed on the uterus than on the jejunum. These quantitative differences between uterus and jejunum responses to the same extract were reproduced in most of the experiments as shown in Tables I and II. These tables summarize kininogen values found in 14 control rats and 15 kidney-ligated rats, of which all but one had abnormal blood pressure at the end of the experiment. A significant increase ($P < .001$) in kininogen levels of all the hypertensive rats, as compared to controls, was found both in uterus and jejunum bioassays.

Kininogen measured by using rat uterus. The average values were : 7.75 ± 6.2 μg Br equivalent per ml plasma (Br/ml) for the hypertensive group, and 2.15 ± .64 μg Br/ml for the control one. The lowest kininogen level among the rats

TABLE I. [a]

Control Rats		Hypertensive Rats				
	Kininogen		Kininogen	Blood Pressure		Days
Rat No.	(μg/ml)	Rat No.	(μg/ml)	Last control	Average	elapsed
35	3	3	5	205	161	46
36	1.8	18	3.4	175	170	47
46	1.3	10	6.9	200	190	80
47	1.5	2	17.6	200	187	81
48	3.7	5	4	190	186	82
50	2.3	7	3.7	170	146	83
54	1.6	13	5.4	200	174	86
57	2	16	24.6	160	165	88
64	1.8	9	6.1	150	154	107
68	2.1	19	3.7	150	151	113
69	2.5	14	3	190	166	114
72	2.5	15	13.6	180	181	120
73	1.3	4	2.7	120	134	122
74	2.1	6	3.4	150	154	123
75	2.1	11	4.1	150	168	123
Mean values and SD	2.15 ± .64		7.7 ± 6.2	172 ± 25		

[a] Kininogen in μg/ml of plasma measured by uterus bioassay in control and hypertensive rats. Blood pressure in mm Hg: average during the experimental period (between kidney operation and bleeding days) and blood pressure found in the last control; days elapsed from the day of uninefrectomy up to the day of bleeding.

TABLE II. Kininogen in μg/ml of Plasma in Control and Hypertensive Rats Measured by Using Cat Jejunum Bioassay; and Blood Urea, in mg/100 ml.

Control Rats			Hypertensive Rats		
Rat No.	Kinin (μg/ml)	Urea (mg%)	Rat No.	Kininogen (μg/ml)	Urea (mg%)
35	1.2	—	3	4.8	—
36	1.9	—	18	5.9	—
46	.9	49	10	6.4	199
47	1.6	24	2	7.2	94
48	1.4	54	5	4.8	62
50	1.9	58	7	3.8	101
54	1.5	34	13	3.1	51
57	1	34	16	4.3	60
64	1.1	58	9	1.7	56
68	1	39	19	3	73
69	—	34	14	2.7	81
72	1.7	21	15	5.8	36
73	1.1	15	4	2.2	24
74	1.3	17	6	2.6	30
75	1.2	—	11	3.8	26
Mean values and SD	1.34±.33	36.4±19		4.1±1.5	68±26

bearing the kidney ligature was 2.7 μg Br/ml, found in one animal whose last blood pressure recording was 120 mm Hg, while the average during the experimental period was of 134 mm Hg (maximum : 145 mm). The highest values of plasma kininogen, 24.6, 17.6, and 13.6 μg Br/ml, correspond to 3 rats whose final blood pressure recordings were 160, 200, and 180 mm Hg, respectively. However, some rats with identical blood pressure differed in their amount of blood kininogen. Although all the hypertensive rats exhibited levels of kininogen above the normotensive average, the correlation coefficient (r) between blood pressure and substrate level was 0.228 and statistically nonsignificant ($P = .3$).

In Fig. 2, it is possible to compare the average kininogen levels obtained in the plasma of control and hypertensive groups by using uterine muscle assay. The hypertensive group was divided into two subgroups: (1) 10 rats with a final blood pressure over 150 mm Hg; (2) 5 rats with 150 mm Hg or less. In the most hypertensive subgroup, kininogen reached its highest average level: 9.9 ± 6.8 μg Br/ml; the average in the less hypertensive subgroup was 3.91 ± 1.2 μg Br/ml. The latter figure is significantly different from the average 2.15 ± 0.6 μg Br/ml found in normal rats ($P < 0.02$).

Kininogen measured by using cat jejunum. In 14 control rats, a mean value of 1.34 ± .33 μg Br/ml was found, whereas the corresponding value in the hypertensive group was 4.1 ± 1.5 (Table II). Similar to the results of the uterus bioassay, the difference between both groups was significant ($P < 0.001$) and the average in kininogen levels obtained in hypertensive rats with the cat jejunum test was approximately thrice as much as compared to the control group (Table II and Fig. 3). An important difference between the two bioassays can be drawn by examining the individual and average figures. In the hypertensive group, the highest figures (7.2, 6.4, 5.9, and 5.8 μg Br/ml) were recorded from rats Nos. 2, 10, 18, and 15, which exhibited in the last control group blood pressures of 200, 200, 180, and 175 mm Hg, respectively. The lowest figure (2.2 μg Br/ml) was found in rat No. 4, whose plasma had also displayed the smallest oxytocic activity. The individual kininogen figures show much less scattering when the plasma extracts are tested in cat jejunum instead of rat uterus. Furthermore, kininogen mean values of normal and hypertensive rat plasma determined by the jejunum method were respectively 1.6 and 1.7 times lower than the averages obtained with the uterus bioassay.

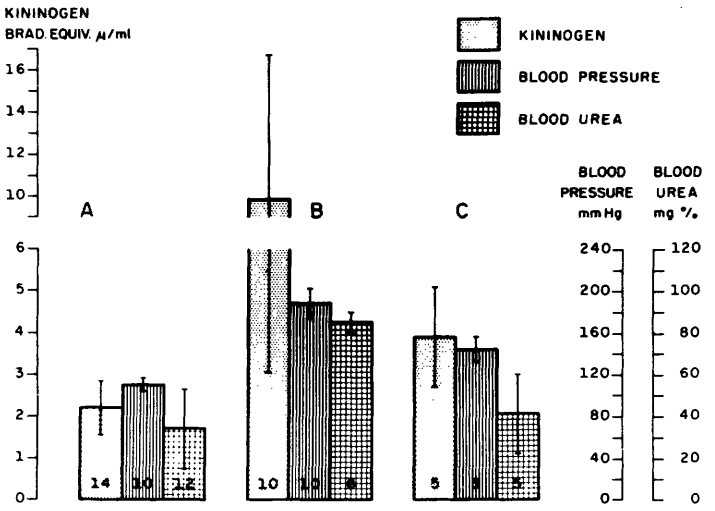

FIG. 2. Average values and SD of plasma kininogen determined by isolated rat uterus bioassay; blood pressure and blood urea in 3 groups of rats: (A) control rats; (B) hypertensive rats with blood pressure over 150 mm Hg; (C) hypertensive rats with blood pressure of 150 mm Hg or less. Figures in the columns indicate the number of rats.

Urea determinations. Table II and Figs. 2 and 3 indicate the uremia individual and average values found in both groups of animals. Nine out of thirteen rats presented higher uremia than the control group whose average was 36.4 ± .19 mg/100 ml (Fig. 2). The mean value for the hypertensive group was 68 ± .26. It is worth noting that one rat (No. 15), whose blood pressure in the last record was 185 mm Hg and exhibited one of the highest levels of plasma kininogen measured with both bioassays, had normal uremia: 36 mg/100 ml. This result indicates that abnormal uremia is not necessarily required to find elevated kininogen in the plasma. However, in the whole, the magnitude of uremia does not correlate with kininogen levels, since the analysis of both parameters yielded a correlation coefficient of 0.06 with a $P > 0.7$. No significant difference was found in blood urea between the control group and the less hypertensive subgroup of rats.

Discussion. The conspicuous increase in

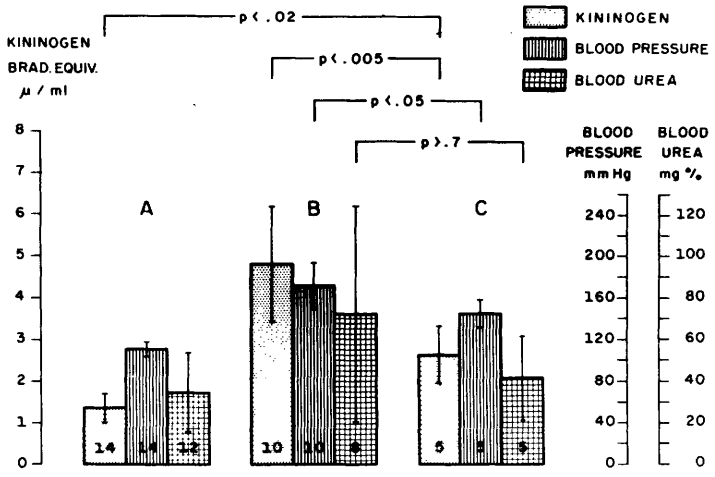

FIG. 3. Average values and SD of plasma kininogens determined by isolated cat jejunum; blood pressure and blood urea in 3 groups of rats: (A) control rats; (B) hypertensive rats with blood pressure over 150 mm Hg; (C) hypertensive rats with blood pressure of 150 mm Hg or less. Figures on the columns indicate the number of rats.

kininogen observed in hypertensive rat plasma confirms a previous report (3) based on a small group of animals and shows that the biochemical alterations in the kallikrein system can also appear in the first period of hypertension and can probably be registered soon after the triggering operation. Surgical trauma or a typical inflammatory process cannot account for the change in kininogen levels, since the immediate effect of the surgical stress or infection was not present when the blood samples were taken.

The difference in the absolute amount of kininogen yielded by each one of the smooth muscle preparations can be explained by the lesser specificity of the uterus. This difference supports the fact of the potentiating effect of some nonactive peptides set free from plasma albumins by trypsin (10). Apparently, cat jejunum, which due to its high specificity has been routinely used to detect bradykinin-type peptides (13), is less affected by interfering factors, and provides more precise data about kinins occurrence in trypsin hydrolysates. The kininogen average quantitated in bioassays on rat uterus —either in control or hypertensive rats—is overestimated by a factor of 1.6–1.7 when compared with averages obtained with cat jejunum. The difference is very close to the factor 1.9 found in the rat uterus bioassay by Louhija and Sipilä (14) in crude plasma trypsin hydrolysates before and after removing the potentiating peptides. It is difficult to ascribe the difference in kininogen levels between control and hypertensive rats only to interfering effects of peptides derived from substrates other than kininogen, because the control plasma can also generate the same interfering factors, particularly if albumin is the protein involved.

The average values of kininogen found in the blood of control rats: 1.34 ± 0.33, and 2.15 ± 0.64 μg Br/ml in jejunum and uterus bioassays respectively, are below and slightly above the figures $2 \pm .9$ μg Br/ml found by the method of Briseid *et al.* (15). According to this procedure, the conversion of kininogens into kinins is achieved through the activation of plasma kallikrein by acetone, and no trypsin is employed. Probably the definitive estimate of absolute kininogen levels must wait for the development of more specific micromethods for kininogen determination. However, the results obtained with the cat jejunum bioassay disclose consistent data supporting that kinins precursors are deeply affected in rats bearing a ligature in the kidney.

The rise in uremia found in most of the rats rendered hypertensive through the Grollman technique is a common observation. The average uremia in the hypertensive group is twice as much as the control level, and, in general, the greatest increase of blood urea was observed in rats presenting the highest blood pressure and kininogen levels.The three parameters are intermingled and probably recognize a common causal factor which might be the functional impairment of the remaining renal tissue. That uremia is not a necessary factor for the rise of kininogen levels is sustained by the coexistence of very high kininogen levels with normal blood urea in some of the hypertensive rats. On the other hand, uremia per se does not affect kininogen contents in the blood. Normal rats given furosemide exhibit a high urea concentration in the blood, but have rather normal or below normal levels of kininogen (16).

In spite of its relevance, we have no consistent data about kininogen level in the earliest postoperation phase, because unilateral nephrectomy by itself produces significant increase in blood kininogen which last 14–20 days (3) and furthermore most of the rats with ligated kidney show irregular blood pressure changes in the first weeks of renal hypertension.

The significant increase in blood kininogen observed in all hypertensive rats by using two different bioassays points in favor of the theory that the kallikrein–kinin system is affected by the kidney deterioration and that the rise in kininogen can be caused by an enzymatic deficiency of the renal tissue. If a decrease in kallikrein activity in the urine reflects a renal impairment to produce this enzyme, then such a deficiency might account for the substrate rise, in a parallel fashion as angiotensinogen rises when renin is lacking. In this connection, the experiments reported by Roblero *et al.* (17), showing that kidneys perfused with artificial fluids are able to release a kallikrein type enzyme in the perfusate and urine, make more feasible the theory that a kallikrein of renal origin is involved in some systemic regulation.

Summary. In renal hypertensive rats whose hypertension was induced by a figure-in-eight ligature of one kidney and the removal of the contralateral one, a significant increase in blood kininogen was found ($P < 0.001$). Kininogen determination was carried out using trypsin as

kininogenase, and the kinins formed were tested by using two bioassays: isolated rat uterus and cat jejunum. Kininogen average values in 15 hypertensive rats were equivalent to 7.75 ± 6.2 μg Br/ml when using the uterus, and 4.1 ± 1.5 with the cat jejunum bioassay. In a control group of 14 rats, both biological tests gave the average values of 2.15 ± .64 and 1.4 ± .33 μg Br/ml, respectively. In the hypertensive rats, a significant increase in uremia was also observed. Results indicate that the renal impairment produced by the kidney ligature alters the kallikrein substrate in the blood and lend support to the theory that renal kallikrein might be involved in some systemic regulation.

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