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Kallikrein–Kinin and Renin–Angiotensin Systems in Renovascular Hypertension in Rats (40794)

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Since 1971, Brunner *et al.* (1) and other authors have proposed two mechanisms for the genesis of experimental renovascular hypertension. To establish these mechanisms, the levels of plasma renin activity (PRA) were considered as well as the response to the blocking of angiotensin II (AII) in reversing hypertension.

The two-kidney Goldblatt model (2KG) seems to be renin–angiotensin dependent since these rats show an increase in PRA and AII block provokes a fall in blood pressure. In contrast the one-kidney Goldblatt model (1KG) has normal or subnormal values of PRA and fails to respond to AII block.

The above findings suggest that another renal factor would be involved in the genesis of hypertension. Among the possible factors, renal kallikrein appeared as the most appealing, owing to its increasing importance in the maintenance of blood pressure and of salt and water excretion.

The important drop in the excretion of urinary kallikrein observed in experimental hypertensive rats (2, 3), spontaneously hypertensive rats (4), and essential hypertensive patients (5, 6), suggests that this deficiency could play some role in the genesis of hypertension.

The synthesis of renal kallikrein has been postulated to occur in the distal tubule (7). This enzyme also plays a role in renal blood flow (8) and in salt and water excretion (9). Roblero *et al.* (10) demonstrated the presence of renal kallikrein in the perfusate of rat kidney which suggests that it may have a systemic function. It has been recently shown that the release of renal kallikrein into the circulation is stimulated by high perfusion pressure and by high sodium concentration in the perfusion fluid (unpublished data).

The purpose of this work was to further study the participation of the renin–angiotensin system (RAS) and the kallikrein–kinin system (KKS) in the pathogenesis of renovascular hypertension by measuring simultaneously, in the same animal, the activity of both systems during the evolution of the Goldblatt hypertensive models.

Material and methods. Male Sprague–Dawley rats weighing between 80 and 100 g were employed. They were operated according to the Goldblatt technique and separated into three series of three groups each, which were sacrificed during the 5th, 10th, and 15th week postsurgery, respectively.

Each series consisted of three groups of 10 rats.

First group: 2KG rats. They were anesthetized under tribromosthanol anesthesia (Avertin–Winthrop 20 mg/100 g body wt ip). After laparotomy, a U-shaped silver clip of 0.2 mm inner diameter was placed on the left renal artery; the right kidney was left intact.

Second group: 1KG rats. In this group, the same operation was performed but a week later the right kidney was removed.

Third group: Sham-operated rats. This control group was only sumbitted to laparotomy. To study the effect of the renal mass reduction on urinary kallikrein excretion, another series was run (eight rats). The animals were submitted to unilateral nephrectomy under the same conditions of the previous experimental groups, once their normal levels of urinary kallikrein had been verified.

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After surgery the rats of all groups were allowed to have food and water *ad libitum*. The weight of the animals was controlled weekly.

Blood pressure measurements. The development of hypertension was monitored by measuring blood pressure weekly by the tail-cuff method using a 1010 crystal microphone as pulse detector. Hypertension was considered to be established when the blood pressure was 150 mm Hg or more in three consecutive measurements.

Urine sampling. Before sacrificing the rats, they were placed in metabolic cages (A.M.C.) for 24 hr without food; water was allowed *ad libitum*. The urine collected was stored at −20°C until the moment of analysis.

Blood sampling. Rats were laparotomized under anesthesia (Nembutal Abbot, 30 mg/kg body wt ip). After verifying the right position of the silver clip, the renal vessels were quickly ligated and the rats were bled through a polyethylene catheter inserted into the carotid artery. Two aliquots were taken. The first was collected in iced silicone tubes containing 1 mg/ml EDTA and centrifuged in a refrigerated Sorvall at 1200*g* for 15 min. The plasma was separated and stored at −20°C for subsequent AI determination(s). The second aliquot, with no anticoagulant added, was used for sodium and creatinine measurements.

Urinary kallikrein activity (*UKA*). Urine dialized overnight against NaCl 0.1% was employed for measuring kallikrein activity by the oxytocic method (3) and was expressed as micrograms bradykinin (BK) equivalent excreted in 24 hr per 100 g body wt. Bradykinin Sandoz was used as standard.

Plasma renin activity (*PRA*). PRA was measured by radioimmunoassay (RIA) of AI according to the method of Haber (11). Reagents were purchased from New England Nuclear. Values are expressed as nanograms of AI generated per milliliter plasma per hour (ng/AI/ml/hr).

Sodium measurements. Sodium was measured using an Eppendorf flame Photometer in nondialized urine and serum.

Creatinine measurements. The method of Folin and Wu (12) was used to obtain creatinine clearance values in urine and serum.

All values are expressed as the mean ± SEM. Data were analyzed according to Student's test.

Results. No differences were observed between hypertensive and control groups in urinary and plasma sodium or in diuresis.

Glomerular filtration rate (G.F.R.) was obtained according to the values of endogenous creatinine clearance on the 15th week. Control: 0.885 ± 0.1 ml/min ($n = 6$); 1KG: 0.80 ± 0.07 ml/min ($n = 8$); 2KG: 0.888 ± 0.1 ml/min ($n = 6$).

Table 1 summarizes data of body weight, blood pressure, UKA, and PRA. No significant difference in body weight was observed during the experimental period. Blood pressure was significantly increased in all experimental groups ($p < 0.0005$) throughout the observation period; no difference was found between 1KG and 2KG groups.

Plasma renin activity (*PRA*). No statistical difference was found between control and 1KG rats in all series (Fig. 1, Table 1). 2KG rats show a significant increase on the 5th week ($P < 0.0005$), the 10th week ($P < 0.0005$), and the 15th week ($P < 0.002$) after clamping.

Urinary kallikrein activity (*U.K.A.*). As shown in Fig. 2 and Table 1, the 1KG model shows a significant decrease on the 5th ($P < 0.001$), 10th ($P < 0.001$), and 15th week ($P < 0.005$) after clamping. In the 2KG model there is no significant difference in UKA as compared to control on the 5th and 10th weeks. Only on the 15th week is there a significant decrease ($P < 0.001$) compared to the 15th week control.

In control uninephrectomized animals, kallikrein levels decreased significantly 8 days after surgery ($P < 0.001$) but returned to normal values at Day 30 (Fig. 3).

Discussion. When the KKS and RAS activities are analyzed simultaneously in the 1KG and 2KG models, two different pathophysiological patterns are observed.

In the 1KG model, the PRA levels remain normal whereas UKA values markedly decrease throughout the observation period.

In the 2KG model, UKA levels stay nor-

TABLE I. SUMMARIZES BODY WEIGHT, BLOOD PRESSURE, URINARY KALLIKREIN ACTIVITY (UKA) PLASMA RENIN ACTIVITY (PRA)[a]

Weeks after clamping	Group	n	Body weight (g)	Blood pressure (mmHg)	UKA (μg BK/24 hr /100 g body wt)	PRA (ng Ang I/ml/hr)
5	Control	10	279 ± 3.5	125.3 ± 1.9	1.76 ± 0.23	4.21 ± 0.98
	1 kg	9	276 ± 11	207 ± 2.7	± 0.1	5.7 ± 0.63
			N.S.	$P < 0.0005$	$P < 0.001$	N.S.
	2 kg	8	248 ± 17	195 ± 6.2	1.79 ± 0.26	16.7 ± 1.98
			N.S.	$P < 0.0005$	N.S.	$P < 0.0005$
10	Control	10	324.4 ± 7.1	123 ± 3.5	1.93 ± 0.2	4.45 ± 0.67
	1 kg	10	330 ± 11	216 ± 5.8	0.75 ± 0.14	5.2 ± 0.50
			N.S.	$P < 0.0005$	$P < 0.001$	N.S.
	2 kg	10	344 ± 10	178.3 ± 4.9	1.87 ± 0.3	21.9 ± 2.41
			N.S.	$P < 0.0005$	N.S.	$P < 0.0005$
15	Control	10	431 ± 13	123.5 ± 2.3	1.56 ± 0.17	4.86 ± 0.64
	1 kg	9	401.8 ± 9.4	208.3 ± 6.3	0.78 ± 0.15	4.2 ± 0.46
			N.S.	$P < 0.0005$	$P < 0.005$	N.S.
	2kg	8	407 ± 7.2	190.6 ± 8.3	0.87 ± 0.15	17.8 ± 3.1
			N.S.	$P < 0.0005$	$P < 0.001$	$P < 0.002$

[a] 1KG = One-kidney Goldblatt rats; 2KG = two-kidney Goldblatt rats; Control = Sham-operated rats. All values are mean ± SEM. The P values were obtained from 1KG versus Control and 2KG versus Control.

mal up to the 10th week while the PRA is markedly increased until the end of the observation period (15th week).

Keeping in mind that RAS has a pressor effect while the KKS has a depressor effect, we can assume from our results that hypertension of renal origin would be the result of an unbalanced state between both systems. Thus hypertension could be due either to an increase in activity of the pressor system (2KG model) or to a decrease in the depressor system (1KG model).

The assumption that blood pressure levels reflect the balance between opposite

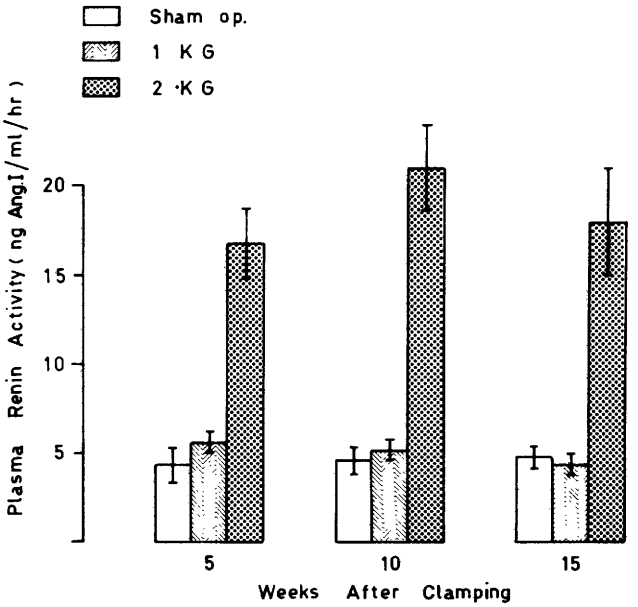

FIG. 1. Plasma renin activity (PRA) at the 5th, 10th, and 15th weeks after clamping in sham-operated, 1KG, and 2KG rats. The bars represent mean values of PRA. The SEM is expressed on the top of each bar.

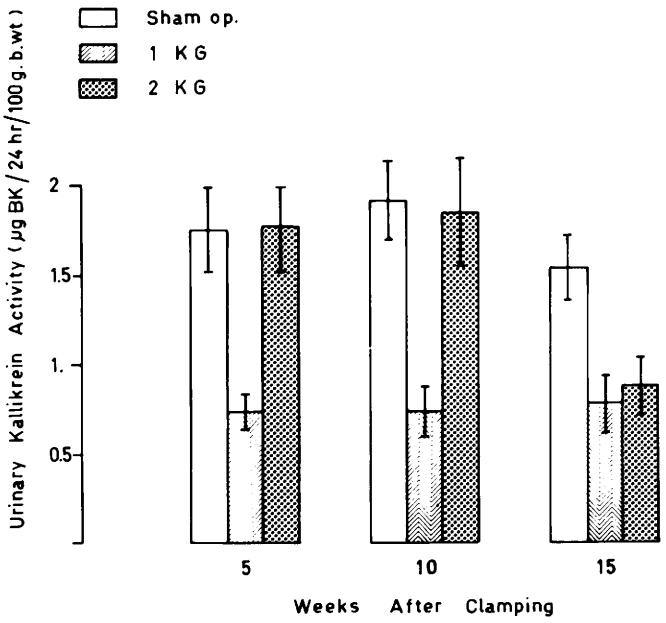

FIG. 2. Urinary kallikrein activity (UKA) at the 5th, 10th, and 15th weeks after clamping in sham-operated, 1KG, and 2KG rats. The bars represent mean values of UKA. The SEM is expressed on the top of each bar.

systems is also supported by Bartter's syndrome, where both systems significantly increase without producing any change in blood pressure (13).

Furthermore, the administration of a converting enzyme inhibitor which blocks both the conversion of AI to AII and the degradation of bradykinin to inactive peptides causes the prevalence of the depressor system which, in turn, provokes a drop in blood pressure (14).

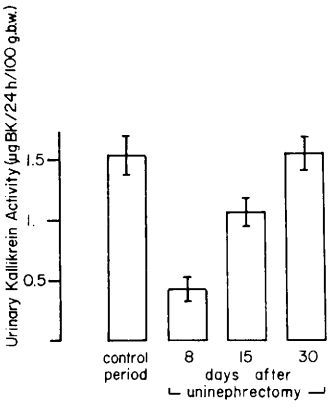

FIG. 3. Urinary kallikrein activity (UKA) in uninephrectomized rats. The bars represent mean values of UKA. The SEM is expressed on the top of each bar.

On the other hand, the UKA began to decrease on the 15th week in the 2KG model. These results show that the renin-dependent model has become a kallikrein-deficient model. Gavras (15) failed to lower blood pressure by blocking AII in the same timing (15th week): he could achieve it only after sodium depletion or furosemide administration. It must be stressed that both procedures constitute potent stimuli for the synthesis and secretion of renal kallikrein (16, 18) and the model would be no longer kallikrein-deficient but only renin-dependent, therefore it would be able to respond to AII blockade.

Results found in uninephrectomized controls show that the lower urinary-kallikrein excretion in one-kidney Goldblatt hypertensive rats is not a consequence of the reduction of the renal mass, which is in agreement with a previous work (3).

Summary. Plasma renin activity (PRA) and the levels of urinary kallikrein (UK) were studied simultaneously in Goldblatt

one- and two-kidney hypertensive rats (1KG and 2KG) 5, 10, and 15 weeks after clamping the left renal artery.

Increase in PRA was statistically significant in 2KG rats ($P < 0.0005$ on the 5th and 10th week, and $P < 0.002$ on the 15th week). PRA was not significantly different to that of controls in 1KG rats. Contrariwise, the urinary kallikrein levels were significantly lower in 1KG rats ($P < 0.002$) in relation to values found in normotensive rats.

In 2KG rats, urinary kallikrein levels became significantly lower than those in controls only 15 weeks after surgery.

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