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Urinary Kallikrein in Dogs with Constriction of One Renal Artery (39141)

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(Introduced by F. C. Bartter)

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Urinary kallikrein is a renal enzyme that cleaves kininogen, an α-globulin substrate, to produce kallidin (lysyl-bradykinin), a potent vasodilator kinin (1). In the 40 years since kallikrein was first detected in human urine, a number of workers have shown that kallikrein excretion is altered in patients (2–5) and animals (6–8) with various forms of hypertension. We have shown that urinary kallikrein is subnormal in patients with essential hypertension and supranormal in patients with primary aldosteronism (4, 9, 10). We have also shown that urinary kallikrein is regulated by the activity of sodium-retaining steroids, and thus, by the level of sodium intake (11). The purpose of this study was to determine the effects of chronic renal arterial stenosis on kallikrein excretion in the dog.

Methods. Nine female mongrel dogs weighing 20 to 25 kg were anesthetized with sodium thiamylol and halothane and then the left renal artery was narrowed by suture ligatures according to the method of Lupu and his co-workers (12). The artery was narrowed progressively until a Carolina electromagnetic flow meter showed a greater than 75% reduction in blood flow to the kidney. During the same period of anesthesia bilateral ureterostomies were performed after excision of the bladder, by division of the trigone and suturing of each ureterovesical junction to the lower anterior abdominal wall. At least 10 days later (18.2 ± 5.6, mean ± SEM), when the dogs had fully recovered, they were made to lie on an animal operating table and passively restrained. An infusion of 5% dextrose in water, 15 to 20 ml/min was given into a jugular vein. Urine was collected via small polyethylene catheters placed in each ureter. After urine flow had stabilized, approximately 45 to 90 min after the start of the infusion, urine was collected for 20- or 30-min periods. An aliquot was stored with toluene at 4° until assayed. Venous blood was drawn through a catheter in a jugular vein at midperiod. All infusates contained inulin and *p*-aminohippurate (PAH), and the clearances of inulin (C_{IN}) and of PAH (C_{PAH}) were determined by standard techniques (13). Urinary kallikrein was measured by a modification (11) of the radiochemical esterolytic method of Beaven, Pierce, and Pisano (14). Duplicate aliquots from selected urines bioassayed on guinea pig ileum showed that TAME esterase activity under the conditions of our assay is analogous to kinin generating activity. Sodium and potassium were determined by internal standard flame photometry. The data presented are the means of three periods. Statistical analysis consisted of either a paired *t* test or a simple linear regression for correlation. A P value of 0.05 or less was considered to be significant.

Results. Urinary volume was significantly ($P < .005$) reduced from the left kidney (i.e., constricted renal artery), 3.2 ± 0.5 ml/min (mean ± SEM), compared to the right (control) kidney, 6.4 ± 0.4 (Table I). The ratio of urinary output for the right to that of the left kidney was 2.41 ± 0.36 (range 1.01 to 4.04). The renal clearance of PAH was significantly ($P < .02$) reduced by the left kidney, 94 ± 10 ml/min, compared to the right kidney, 199 ± 37 (Table I). The ratio of PAH clearance for the right to that of the left kidney was 2.20 ± 0.33 (range,

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TABLE I. RESULTS OF CHRONIC CONSTRICTION OF ONE RENAL ARTERY IN NINE DOGS.[a]

	Kidney	Mean ± SE	P^b		Kidney	Mean ± SE	P^b
V	R	6.4 ± 0.4	<.005	$U_{Ka}V$	R	61.9 ± 8.1	<.005
	L	3.2 ± 0.5			L	33.3 ± 7.3	
C_{PAH}	R	199 ± 37	<.02	U_KV	R	16.2 ± 3.0	<.05
	L	94 ± 10			L	9.4 ± 1.9	
C_{IN}	R	44 ± 3	<.005	$U_{Na}V$	R	21.3 ± 5.3	N.S.
		22 ± 3			L	19.2 ± 9.7	

[a] V, urine volume (ml/min); C_{PAH}, clearance of PAH (ml/min); C_{IN}, clearance of inulin (ml/min); $U_{Ka}V$, kallikrein excretion (mEU/min); U_KV, potassium excretion (μEq/min); $U_{Na}V$, sodium excretion (μEq/min).
[b] Calculated from paired t test.

1.13 to 4.34). The renal clearance of inulin by the left kidney, 22 ± 3 ml/min, was significantly ($P < .005$) less than that of the right kidney, 44 ± 3 (Table I). The ratio of inulin clearance of the right kidney to that of the left kidney was 2.46 ± 0.61 (range 1.18 to 7.12).

In association with these changes kallikrein excretion was significantly ($P < .005$) lower from the left kidney, 33.3 ± 7.3 mEU/min, than that from the right kidney, 61.9 ± 8.1 (Fig. 1 and Table I). The ratio of kallikrein excretion for the right to that of the left kidney was 2.41 ± 0.54 (range, 1.20 to 6.01). The excretion of potassium from the left kidney, 9.4 ± 1.9 mEq/min, was significantly lower ($P < .05$) than that from the right kidney, 16.2 ± 3.0. Sodium excretion from the two kidneys was not significantly different.

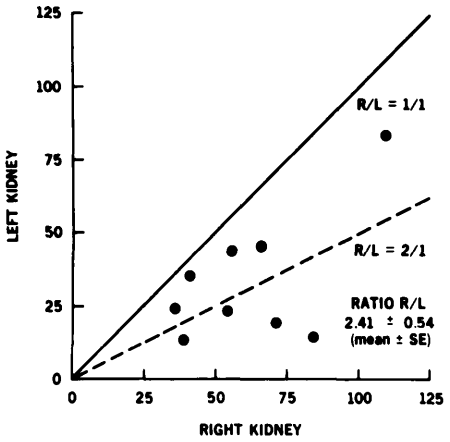

FIG. 1. Excretion of kallikrein (mEU/min) by right kidney (abscissa) and left kidney (ordinate) in nine dogs with chronic constriction of left renal artery.

There was no direct correlation between kallikrein excretion and PAH clearance when all pairs of such data were plotted. However, there was a highly significant ($P < .005$) correlation ($r = 0.882$) between the reduction of PAH clearance produced by the ligature (i.e., C_{PAH} for R kidney–C_{PAH} for L) and the reduction of urinary kallikrein (Fig. 2). There was also a highly significant ($P < .001$) correlation ($r = 0.899$) between the reduction of inulin clearance produced by the ligature and the reduction of urinary kallikrein. There were no significant correlations between the reduction of urinary kallikrein and the reductions of urinary volume ($r = 0.556$, $P > .10$) or potassium ($r = 0.342$, $P > .30$).

Discussion. In this study we constricted one renal artery in dogs and produced an expected persistent decrease in renal blood flow (as measured by PAH clearance) and concomitant decreases in urinary volume and inulin clearance on the side with the constriction. Accompanying these changes is a decrease in kallikrein excretion on the side with the constriction. These data are in agreement with earlier work from this laboratory in which we found a significant decrease in urinary kallikrein in rats with a clip on one renal artery (7) and with the work of Croxatto and San Martin (6), who found that total urinary kallikrein in rats with a ligature around one renal artery was reduced and about equal to that of rats after unilateral nephrectomy. However, the present study for the first time provides data about urinary kallikrein along with other aspects of renal function in any animal model.

There was an excellent correlation between the change in renal blood flow (as measured by C_{PAH}) produced by the ligature and the decrease in kallikrein excretion. This was true whether the data were ex-

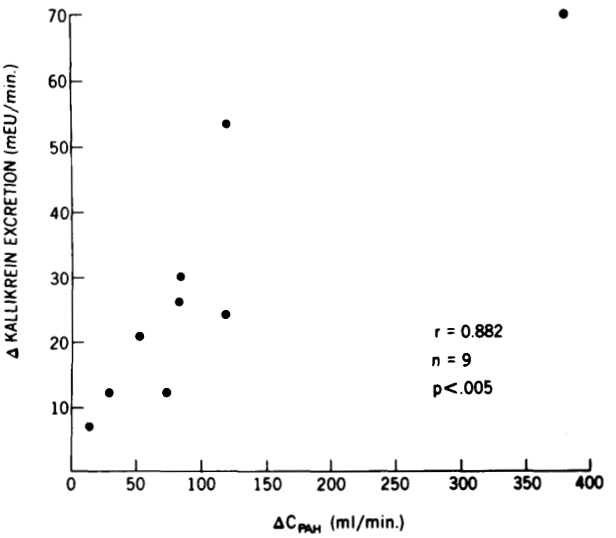

FIG. 2. Plot of difference in clearance of *p*-aminohippurate (right kidney minus left) versus difference in kallikrein excretion (R–L) in nine dogs with chronic constriction of left renal artery.

pressed as the amount of change or the ratio of the normal side to the stenotic side. There was a similar highly significant correlation between the change in glomerular filtration rate (as measured by C_{IN}) and the change in kallikrein excretion regardless of the way the data were expressed. This is expected since at low levels of renal blood flow glomerular filtration rate has been shown to be dependent on renal blood flow (15). Urinary volume and potassium excretion were also reduced on the stenotic side. However, while urinary volume and potassium excretion tended to move in the same direction in response to renal arterial constriction as renal blood flow and kallikrein excretion, the degree of change was not of equal magnitude.

Bradykinin or kallidin (16, 17) injected into the renal artery causes renal vasodilation, an increase in renal blood flow and natriuresis and diuresis. Thus, it would be tempting to say that the changes in renal blood flow were due to the changes in kallidin and kallikrein. However, in this study the changes in renal blood flow were due to the ligature on the renal artery. Thus, the changes noted in urinary kallikrein are most probably secondary to the changes in renal blood flow. While other workers have reported that urinary kallikrein excretion correlated well with urine volume and sodium (5, 18), there were no such correlations found in this study. In fact, in none of our studies in either man (10, 11) or animals (19) have we found any direct correlation between urinary kallikrein and urine volume or sodium.

We have previously reported that urinary kallikrein in man and rat increases in response to a low-sodium diet or administration of sodium-retaining steroids and is increased in patients with primary aldosteronism. The increase in urinary kallikrein in response to a low-salt diet or in patients with primary aldosteronism is abolished by the aldosterone antagonist, spironolactone (10, 11, 19). Thus, urinary kallikrein appears to be regulated by the effective circulating level of sodium-retaining steroid. The present data showing that urinary kallikrein is also correlated with renal blood flow may seem confusing. However, there is abundant evidence that more than one mechanism may control the release of a biologically active substance, e.g., renin (20) or aldosterone (21). Thus, our proposal of two different mechanisms for controlling urinary kallikrein is not without precedent.

Note added in proof: Unlike human urine in which virtually all TAME esterase activity at pH 8.0 is due to kallikrein, in dog urine about 25% of TAME ester-

ase activity is probably due to urokinase. This non-kallikrein TAME esterase activity disappears during the filtration through Sephadex G-25 that precedes our radiochemical assay for kallikrein (11). TAME esterase activity from gel-filtered dog urines was shown to be equal to that from human urine in its ability to liberate kinin (as determined by radioimmunoassay) from human plasma kininogen.

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