Urinary Kallikrein and Plasma Renin Activity as Determinants of Renal Blood Flow

THE INFLUENCE OF RACE AND DIETARY SODIUM INTAKE

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ABSTRACT We investigated the relationship of the kallikrein-kinin system and the renin-angiotensin system in the regulation of blood pressure, salt and water excretion, and renal blood flow. Normotensive and hypertensive black and white men were studied during unrestricted sodium intake as well as on a 10-meq/day sodium intake; potassium intake was held constant throughout the study (80 meq/day).

During unrestricted sodium intake, urinary kallikrein activity was greater in white normotensives than white hypertensives or black normotensives. There was no difference ($P > 0.05$) between white and black hypertensives or between black normotensives and black hypertensives. All groups had greater urinary kallikrein activity on low sodium vs. unrestricted sodium intake, but the increase in black hypertensives was small, and they excreted significantly less kallikrein than the other groups on the low sodium diet. Plasma renin activity showed similar increments after sodium restriction in all groups. Urinary kallikrein activity correlated with renal blood flow in all groups except the black normotensives on low sodium intake. Renal blood flow could be correlated uniformly with log (urinary kallikrein activity/supine plasma renin activity) in all groups on either diet. Urinary sodium and potassium excretion and urine volume were not different among the groups. We conclude: (*a*) important racial differences exist in urinary kallikrein activity that are unrelated to sodium or potassium excretion or urine volume; (*b*) dietary sodium restriction further delineates racial differences and suggests alternative pathophysiologic mechanisms for human hypertension; (*c*) urinary kallikrein activity correlates with renal blood flow; and (*d*) our data support the concept that the kallikrein-kinin system and the renin-angiotensin system contribute to the regulation of renal blood flow and may account for racial differences in renal vascular resistance.

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INTRODUCTION

Kallikrein is an enzyme that catalyzes the formation of the vasodilator hormones, lysyl-bradykinin (kallidin) and bradykinin, from a plasma alpha-2 globulin substrate, kininogen (1). Urinary kallikrein appears to be derived from the kidney and differs from the enzyme that circulates in plasma (2). It has been proposed that kallikrein exists in the renal cortex and may occupy a location that is juxtaposed to the enzyme renin (3). Although a physiologic function for lysyl-bradykinin, the enzymatic product, has not been established, pharmacologic infusion studies have shown that the peptide is a natriuretic and vasodilatory compound (4).

A number of studies in humans and experimental animals have attempted to determine the relationship between urinary kallikrein activity and blood pressure regulation (5–9). Although most investigators feel that kallikreins could influence blood pressure, the meaning of these findings is controversial. Based on provocative preliminary observations (10) and the aforementioned studies, it seemed appropriate to examine the simultaneous influence of race and blood pressure on urinary kallikrein activity. In addition, we have examined the association between kallikrein in urine and factors that have been related to blood pressure regulation including urinary volume, sodium excretion, plasma renin activity, and renal blood flow. These latter studies were designed to determine a possible physiologic role for the kallikrein-kinin system in blood pressure control.

METHODS

Patients. Four groups of men (38 white and 21 black) with differing blood pressure patterns were studied at the Diagnostic and Treatment Unit of the San Diego Veterans Administration Hospital. The subjects included 25 hypertensive patients (18 white, 7 black) and 34 apparently healthy men (20 white, 14 black). All subjects were screened several times before hospitalization, and all hypertensive patients were required to exhibit the arbitrary criteria consisting of a supine mean arterial pressure (diastolic blood pressure plus one-third pulse pressure) that was at least 110 mm Hg on each determination. Apparently healthy normotensive men were paid volunteers who fulfilled the arbitrary criteria of a supine mean arterial pressure that was consistently less than 95 mm Hg during at least three separate measurements. Every subject gave his informed written consent, and the Committee on Human Experimentation of the University of California, San Diego, approved the protocol.

Each subject was admitted to the hospital and the evaluation consisted of a complete history and physical examination; multiple determinations of blood pressure (*vide infra*); routine blood chemical studies including determinations of the blood urea nitrogen concentration and the serum concentrations of sodium, potassium, chloride, bicarbonate, magnesium, calcium, and creatinine; a hemogram, a complete urinalysis including microscope examination of the urinary sediment and urine culture, a roentgenogram of the chest, and an electrocardiogram. While ingesting a diet with defined quantities of sodium and potassium (*vide infra*), each subject provided urine for two consecutive 12-h measurements of kallikrein activity, creatinine, sodium and potassium concentrations. In addition, samples of peripheral venous blood were obtained for the determination of plasma renin activity (PRA).[1]

Patients who met our arbitrary criteria for hypertension were evaluated further for known secondary causes of blood pressure elevation. These studies included an intravenous urogram, selective renal angiography with measurements of PRA in the renal venous effluent from the two kidneys, and the 24-h urinary excretion of 17-hydroxycorticosteroids, catecholamines, and vanillylmandelic acid. Only two patients had received previous therapy for hypertension, and in both instances medication was omitted for 3 wk before enrollment in the study.

Procedures

Each hypertensive patient was studied during two separate balance periods of dietary salt ingestion. During the initial phase (5 days) of hospitalization, subjects were fed a diet without restriction of sodium content (>100 meq/24 h) and which contained approximately 80 meq/24 h of potassium. Repeat urinary investigations were performed on the 11th hospital day after 5 days of restricted sodium ingestion (<10 meq/24 h) with supplementary oral potassium to a total intake of 80 meq/24 h, and further plasma samples for renin determination were obtained during the final hospital day (day 12). Normotensive volunteers were studied on a similar protocol but were, of necessity, different subjects during each extreme of dietary salt ingestion.

On the day before the end of each phase of dietary sodium balance a 24-h urine collection was obtained to document the achievement of a stable sodium balance. On the following day, two consecutive 12-h urine collections were obtained for the measurement of kallikrein activity, volume, and sodium and potassium concentration. Urine was stored in a refrigerator at 4°C immediately after each voiding. After the completion of a 12-h collection, the volume was measured in a graduated cylinder and an aliquot was frozen at −30°C for later analysis.

On the day after the final urine collection of each phase of the balance study, renal blood flow was estimated by the method of constant infusion of *p*-aminohippurate without urine collection (11). After this test blood was obtained for supine PRA, the patient was given 80 mg of oral furosemide and ambulated for 5 h (12), and an aliquot of blood was obtained for determination of stimulated PRA.

Chemistry

Routine chemistries were performed in the clinical laboratories of the San Diego Veterans Administration Hospital. Blood for renin determination (PRA) was drawn without a tourniquet into chilled EDTA tubes which were kept on ice until the samples could be centrifuged and frozen. PRA was measured with the method of Haber et al. (13) via the radioimmunoassay of angiotensin I generated after 1 h of incubation at pH 5.5. The reagents for the assay were purchased from New England Nuclear, Boston, Mass. Single incubations and duplicate immunoassays were performed resulting in two values of generated angiotensin I per plasma sample. Values were required to fall within 15% of the average of the two values. Reproducibility of the method was determined by replicate analysis of a single plasma sample ($n = 20$), and the interassay coefficient of variation (expressed as SD/mean) was 14%. All results are expressed as nanograms of angiotensin I (A_I) generated per milliliter plasma per hour.

Kallikrein activity was measured for *p*-tosyl arginine methylester (TAME) hydrolysis by using the radiochemical method of Beaven et al. (14) and modified by Margolius et al. (15). Urine aliquots were assayed immediately after thawing. Each assay reaction mixture contained 0.05 ml 0.2 M Tris-HCl buffer at pH 8.5, 0.027 μCi [^{3}H]TAME in 0.02 ml with a specific activity of 50 mCi/mmol (Calatomic Inc., Los Angeles, Calif.), and 0.03 ml urine or standard enzyme solution. Incubation of the reaction mixture was performed at 37°C for 30 min in a 1.5-ml micro-test tube (Eppendorf) placed in a glass vial containing 10 ml scintillation fluid (32.2 g Beckman Fluoralloy TLA, [Beckman Instruments, Fullerton, Calif.] in 3.75 liter toluene) and 0.05 ml of a stop solution consisting of 0.01 M TAME (unlabeled), 50% dimethylformamide and 10% glacial acetic acid. After incubation, the vials were shaken vigorously for 15–30 s to quench the reaction. Unhydrolyzed [^{3}H]TAME remained in the aqueous phase and [^{3}H]methanol was extracted into the organic phase. The vials were counted in a Beckman LS-230 liquid scintillation counter (Beckman Instruments), at 35% efficiency. A standard curve of esterase activity was constructed for each assay by using a preparation of human urinary kallikrein purified partially according to the method of Hial et al. (16) to a specific activity of 3 esterase units (EU)/mg protein, as determined by the colorimetric assay of Roberts (17). EU is defined as that amount of enzyme which catalyzes the hydrolysis of 1 μmol TAME/min under the conditions of the assay, pH 8.5 and 37°C. This EU is equivalent to 12.5 Frey units (15). Similar to previous investigations (18), we have found that the hydrolysis of TAME by urinary kallikrein at pH 8.5 and 37°C proceeds by a factor of 1.6 faster than the TAME hydrolytic activity at pH 8.0 and 30°C, standard conditions

[1] *Abbreviations used in this paper*: A_I, angiotensin I; EU, esterase units; PRA, plasma renin activity; TAME, *p*-tosyl arginine methylester.

TABLE I
Subject Characteristics

Experimental group	Diet*	Age	MAP‡	Serum sodium	Serum potassium	Serum creatinine
		yr	*mm Hg*	*meq/liter*	*meq/liter*	*mg/dl*
Normotensives						
White	$UnNa^{+}$*	36.9±3.2	80.3±1.6	139.0±0.6	4.1±0.1	1.1±0.04
	LNa^{+}*	32.0±4.3	83.4±2.4	139.9±1.4	4.2±0.1	1.0±0.05
Black	$UnNa^{+}$	36.9±4.3	79.9±4.0	139.0±1.3	4.0±0.1	1.1±0.03
	LNa^{+}	35.9±5.0	88.0±2.7	139.5±2.1	4.2±0.1	1.2±0.04
Hypertensives						
White		37.8±2.8	114.5±1.4	139.0±0.6	4.2±0.1	1.0±0.04
Black		41.4±4.7	121.6±3.6	139.6±0.9	4.2±0.1	1.2±0.08

* Diet: UnNa, unrestricted sodium intake; LNa, low sodium intake.
‡ MAP, mean arterial pressure.

used by some other workers (14, 15). We have adopted the former conditions for compliance with the sample assay run at optimal sensitivity. The assay was linear to 0.0006 EU and the maximum urinary kallikrein activity in an assay sample was 0.0004 EU. Blank values were never more than 5% of the total counts. Each sample was assayed in duplicate and the data were corrected for recovery by including an internal standard for each sample. Recovery corrected values measured in raw or desalted urine were the same consistently (14), and urine samples were not desalted routinely for assay. Final results are expressed in esterase units per 12-h time period.

To validate the relationship of TAME esterolytic activity measurements to human urinary kallikrein activity, we performed a bioassay with an anesthetized rat as previously described (18). The catheterized femoral artery of an anesthetized Munich-Wistar rat was connected to a Statham blood pressure transducer (Statham Instruments Div., Gould Inc., Oxnard, Calif.) and solutions were injected via a catheter in the jugular vein. The preparation was standardized via incremental injections of bradykinin, and the hypotensive response was noted to be linear. A proportional decrease in blood pressure was observed with increasing amounts ($n = 15$) of partially purified human urinary kallikrein preparation ($r = 0.99$). Samples ($n = 20$) of desalted urine from normotensive and hypertensive black and white men assayed by the TAME esterolytic method produced a similar response over the range of esterase activities observed in this study ($r = 0.88$). TAME esterase activity of the same raw urines assayed with an internal standard exhibited a correlation ($r = 0.95$) that approximated the purified enzyme preparation. Pancreatic trypsin inhibitor blocked the hypotensive response and other proteolytic enzymes such as trypsin had no effect.

Statistics

Student's t test and linear regression analysis were performed using standard techniques (19). The Wilcoxon signed rank test was used to compare changes between the same group at different levels of sodium intake. All values are expressed as the mean±SEM unless otherwise stated. Since it did not seem appropriate, we did not compare any racial group with normal blood pressure to the opposite racial group with a differing blood pressure pattern (e.g., white normotensives were not contrasted with black hypertensives).

RESULTS

All groups of men exhibited similar characteristics (Table I). White hypertensives ranged in age from 20 to 57 yr (mean 37.8±2.8 yr) and did not differ ($P > 0.10$) from black hypertensives who averaged 41.4±4.7 yr (range 28–57 yr). In addition, hypertensive patients did not differ in age from any group of normotensive subjects ($P > 0.10$). Analysis of automated blood pressure patterns revealed the expected differences between those subjects previously and arbitrarily classified as hypertensive and those defined as normotensive (Table I). The average automated mean arterial pressure of white hypertensive men was 114.5 ±1.4 mm Hg (range 108–126 mm Hg) and was similar ($P > 0.10$) to black hypertensive men (mean 121.6 ±3.6 mm Hg, range 113–141 mm Hg). Regardless of race or dietary salt intake, all normotensive subjects exhibited blood pressures that were similar to each other ($P > 0.10$) but differed ($P < 0.01$) from all hypertensive men. Serum sodium and potassium concentrations were similar ($P > 0.10$) in all groups of subjects, and all exhibited normal renal function by the criteria of serum creatinine concentrations (Table I) and urinary examination. Results of all other diagnostic procedures were within the normal range for all hypertensive patients, and thus they were considered to have primary (essential) hypertension. Dietary salt intake or race did not influence these results in any group (Table I). Urinary sodium excretion is summarized in Table II. There were no differences between sodium excretion on the day before urinary kallikrein measurement and the 24-h period during

TABLE II
Summary of Urinary Sodium Excretion

Experimental group	Diet*	24-h $U_{Na+}V$‡ Before kallikrein determination	24-h $U_{Na+}V$‡ During kallikrein determination
		meq	*meq*
Normotensives			
White	$UnNa^+$	161±12	149±11
	LNa^+	16±4	18±5
Black	$UnNa^+$	176±26	178±20
	LNa^+	15±4	13±3
Hypertensives			
White	$UnNa^+$	168±21	177±20
	LNa^+	15±3	19±2
Black	$UnNa^+$	159±15	165±16
	LNa^+	19±4	19±3

* Diet: $UnNa^+$, unrestricted sodium intake; LNa^+, low sodium intake.
‡ $U_{Na+}V$, 24-h urinary sodium excretion.

which kallikrein was measured in any of the four groups. Additionally, every subject had decreased his urinary sodium excretion to less than 40 meq/24 h by the 4th day of the low sodium phase and in no instance was this value more than 5 meq greater than the 5th day, documenting that stable sodium balance was attained.

Urinary kallikrein activity differed among groups according to race and blood pressure pattern, and these differences showed distinct changes after dietary salt restriction (Tables III and IV). During unrestricted dietary sodium intake, 24-h urinary kallikrein activity (the sum of the two 12-h collections) was 18.7±3.1 EU in white normotensives and differed ($P < 0.025$) from that of white hypertensive men who averaged 9.2±2.3. Black normotensives excreted 3.8±0.9 EU, not significantly different from black hypertensives (3.0±1.1 EU) at this amount of dietary salt intake. The difference between black and white normotensives is significant ($P < 0.01$), while black hypertensives do not differ ($P > 0.1$) from the white hypertensive group (Fig. 1). After dietary salt restriction (Table IV, Fig. 1) there was a significant increment ($P < 0.05$) of urinary kallikrein activity in all groups. White normotensive men excreted 38.8±12.1 EU and did not differ ($P > 0.05$) from white men with hypertension (30.3±6.8 EU) or healthy black subjects who averaged 20.9±5.7 EU. Black hypertensive men exhibited urinary kallikrein activity which averaged 4.9±1.9 EU and was significantly less ($P < 0.01$) than all other groups on low sodium diets. The aforementioned differences among subjects were observed despite similar ($P > 0.05$) urinary volumes and urinary sodium and potassium excretions among all groups (Tables III and IV).

Urine was collected in two 12-h aliquots (8:00 a.m. to 8:00 p.m. and 8:00 p.m. to 8:00 a.m.) as an index

TABLE III
Summary of Urinary Kallikrein Activity, $U_{Na}V$, UV, Renal Blood Flow, and Supine and Stimulated PRA on Unrestricted Sodium Intake (Mean±SEM)

Experimental group	$U_{Na}V$ 8 a.m.–8 p.m.	$U_{Na}V$ 8 p.m.–8 a.m.	UV 8 a.m.–8 p.m.	UV 8 p.m.–8 a.m.	UKa 8 a.m.–8 p.m.	UKa 8 p.m.–8 a.m.	RBF/BSA	PRA Supine	PRA Stimulated
	meq/12 h		*ml/12 h*		*EU/12 h*		*ml/min/m²*	*ng A_I/ml/h*	
Normotensives									
White (n = 13)	84±9	65±5	878±130	818±113	10.6±2.1	8.1±1.4	*570±13	1.6±0.3	6.2±0.9
Black (n = 8)	100±19	82±12	951±154	683± 87	2.2±0.8	1.6±0.5	582±12	0.8±0.3	4.3±1.0
Hypertensives									
White (n = 18)	110±14	67±10	1,025±108	747±88	5.9±1.6	3.3±0.7	*499±17	2.8±1.1	4.6±2.0
Black (n = 7)	87±9	78±8	686±64	691±83	1.3±0.5	1.7±0.6	470±39	0.7±0.2	1.0±0.4

$U_{Na}V$, urinary sodium excretion; UV, urinary volume; PRA, plasma renin activity; UKa, urinary kallikrein activity; RBF/BSA, renal blood flow/body surface area.
Supplemental material for Tables III and IV has been deposited with the National Auxiliary Publications Service (NAPS) as NAPS document 03007. This information may be ordered from ASIS/NAPS. c/o Microfiche Publications, 305 East 46th Street, New York 10017. Remit with order for each NAPS document number $1.50 for microfiche or $5.00 for photocopies for up to 30 pages. Checks should be made payable to Microfiche Publications.
* n = 12 for this determination.

TABLE IV

Summary of Urinary Kallikrein Activity, $U_{Na}V$, UV, Renal Blood Flow, and Supine and Stimulated PRA on Low Sodium Intake (Mean±SEM)

Experimental group	$U_{Na}V$		UV		UKa			PRA	
	8 a.m.–8 p.m.	8 p.m.–8 a.m.	8 a.m.–8 p.m.	8 p.m.–8 a.m.	8 a.m.–8 p.m.	8 p.m.–8 a.m.	RBF/BSA	Supine	Stimulated
	meq/12 h		*ml/12 h*		*EU/12 h*		*ml/min/m²*	*ng A_I/ml/h*	
Normotensives									
White (n = 7)	12±4	6±2	783±222	656±181	19.4±4.4	19.4±10.4	518±19	3.7±0.6	10.2±0.9
Black (n = 6)	7±3	6±2	402±96	432±78	10.6±4.6	10.3±2.8	509±15	1.9±0.7	7.1±1.3
Hypertensives									
White (n = 18)	11±2	8±1	815±63	896±126	17.3±3.6	13.0±3.5	*449±23	8.2±1.4	13.4±3.1
Black (n = 7)	9±1	9±2	577±123	601±108	2.7±1.0	2.1±0.9	360±35	6.9±2.1	10.7±3.8

$U_{Na}V$, urinary sodium excretion; UV, urinary volume; PRA, plasma renin activity; UKa, urinary kallikrein activity; RBF/BSA, renal blood flow/body surface area.
For supplemental material, see legend of Table III.
* n = 12 for this determination.

of day-night variation of urinary kallikrein excretion (Tables III and IV). White hypertensive patients exhibited significantly higher ($P < 0.05$) urinary kallikrein activity during the day, and this was accompanied by a parallel variation of urinary volume and urinary sodium concentration. We observed these differences among white hypertensive men irrespective of sodium intake, but we could not observe this phenomenon in any other group during either dietary manipulation.

Determinations of PRA during unrestricted sodium intake and supine posture averaged 2.8±1.1 ng A_I/ml per h among white hypertensives and was similar ($P > 0.05$) to black hypertensives and white normotensives, who exhibited measurements of 0.7±0.2 and 1.6±0.3 ng A_I/ml per h, respectively (Tables III–V). However, stimulated PRA on unrestricted sodium diet for black hypertensive men (mean, 1.0±0.4 ng A_I/ml per h) was significantly less ($P < 0.025$) than that of apparently healthy black men who averaged 4.3 ±1.0 ng A_I/ml per h. There were no other differences ($P > 0.10$) among the compared groups during unlimited salt intake. A comparison of groups on restricted sodium intake indicated that there were no significant differences ($P > 0.05$) among subjects irrespective of race or blood pressure pattern (Table V). We also contrasted supine vs. stimulated PRA at both extremes of salt intake (Tables III–V). It is noteworthy that black hypertensive men alone did not exhibit an increase of PRA ($P > 0.05$) after our standard stimulation during either dietary manipulation. All other groups on either diet increased PRA consistently after the stimuli of furosemide and ambulation. However, dietary salt restriction resulted in a significant increment ($P < 0.05$) of PRA in all groups regardless of position (Table V).

Renal blood flow was determined from the formula: renal blood flow = p-aminohippurate clearance/(1-hematocrit). Data for renal blood flow are expressed as square meters of body surface area (Tables III and IV). On unrestricted sodium intake white normotensives had a renal blood flow of 570± 13 ml/min per m²,

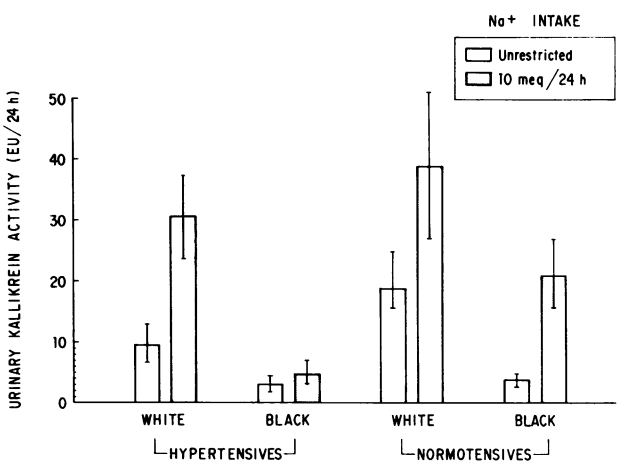

FIGURE 1 Urinary kallikrein response to sodium restriction. On unrestricted sodium intake white normotensives have greater urinary kallikrein activity than white hypertensives or black normotensives. There is no difference between white and black hypertensives or between black normotensives and black hypertensives. All groups had greater urinary kallikrein activity on low sodium vs. high sodium intake, with black hypertensives excreting less kallikrein than the other compared groups.

TABLE V
Summary of 24-H $U_{Na}V$, UV, UKa, U_KV, and Supine and Stimulated PRA

Experimental group	Diet	UV	$U_{Na}V$	U_KV	UKa	PRA	
						Supine	Stimulated
		ml/24 h	*meq/24 h*	*meq/24 h*	*EU*	*ng A_I/ml/h*	
Normotensives							
White	$UnNa^+$	1,701±206	149±11	70±5	18.7±3.1	1.6±0.3	6.2±0.9
	LNa^+	1,499±363	18±5	66±4	38.8±12.0	3.7±0.6	10.2±0.9
Black	$UnNa^+$	1,633±173	178±20	64±5	3.8±0.9	0.8±0.3	4.3±1.0
	LNa^+	833±168	13±3	68±4	20.9±5.7	1.9±0.7	7.1±1.3
Hypertensives							
White	$UnNa^+$	1,768±183	177±20	63±3	9.2±2.3	2.8±1.1	4.6±2.0
	LNa^+	1,710±157	19±2	68±5	30.3±6.8	8.2±1.4	13.4±3.1
Black	$UnNa^+$	1,377±144	165±16	65±6	3.0±1.1	0.7±0.2	1.0±0.4
	LNa^+	1,181±227	19±3	71±4	4.9±1.9	6.9±2.1	10.7±3.8

$U_{Na}V$, urinary sodium excretion; UV, urinary volume; UKa, urinary kallikrein; PRA, plasma renin activity; $UnNa^+$, unrestricted sodium intake; LNa^+, low sodium intake; U_KV, urinary potassium excretion.

not different from black normotensives who averaged 582±12 ml/min per m^2. At this level of sodium intake, white and black hypertensives had similar renal blood flow, 499±17 and 470±39 ml/min per m^2, respectively. For both racial groups normotensives had greater renal blood flow than hypertensives ($P < 0.025$). Renal blood was significantly ($P < 0.05$) lower in each of the four groups on the low sodium diet. At this level of sodium intake black normotensives were found to have a renal blood flow of 509±15 ml/min per m^2, similar to white normotensives (518±19 ml/min per m^2). Renal blood flow for white hypertensives (449 ±23 ml/min per m^2) was significantly ($P < 0.05$) greater than for black hypertensives (360±35 ml/min per m^2). Black hypertensives had significantly ($P < 0.01$) lower renal blood flow than black normotensives, whereas the difference between white normotensives and hypertensives did not reach significance ($P > 0.05$).

TABLE VI
Correlations between Urinary Kallikrein Activity and Renal Blood Flow/Body Surface Area

Experimental group	Diet	r	P
White normotensives	$UnNa^+$	0.68	<0.01
	LNa^+	0.80	<0.01
White hypertensives	$UnNa^+$	0.58	<0.05
	LNa^+	0.56	<0.05
All whites	$UnNa^+$	0.66	<0.01
	LNa^+	0.58	<0.01
Black normotensives	$UnNa^+$	0.67	<0.05
	LNa^+	−0.16	>0.10
Black hypertensives	$UnNa^+$	0.71	<0.05
	LNa^+	0.69	<0.05
All blacks	$UnNa^+$	0.60	<0.05
	LNa^+	0.57	<0.05

$UnNa^+$, unrestricted sodium diet; LNa^+, low sodium diet.

To investigate a possible relationship among urinary kallikrein activity, PRA (supine and stimulated), mean arterial pressure, urinary sodium and urinary potassium excretion, renal blood flow, and urine volume, we performed a linear regression analysis using these measurements. These associations were evaluated for each of the four groups (white hypertensives, white normotensives, black hypertensives, and black normotensives). In addition, we analyzed each racial group regardless of blood pressure pattern (Table V). All blacks on restricted salt exhibited a significant negative correlation ($r = -0.52$, $P < 0.05$) between urinary kallikrein activity and supine PRA. While ingesting an unlimited sodium diet, black normotensives exhibited a significant correlation ($r = 0.67$, $P < 0.05$) between urinary kallikrein activity and stimulated PRA, and black hypertensive men had a significant inverse correlation ($r = -0.85$, $P < 0.01$) between urinary kallikrein activity and a 24-h sodium excretion. For all 12 comparisons that were made we found significant correlations between urinary kallikrein activity and renal blood flow in all groups except black normotensives on the low sodium diet (Table VI).

To evaluate the hypothesis that the renin-angiotensin system may oppose the kallikrein-kinin in regulating renal blood flow we correlated log (urinary kallikrein/PRA supine) and renal blood flow for both normotensives and hypertensives at both levels of

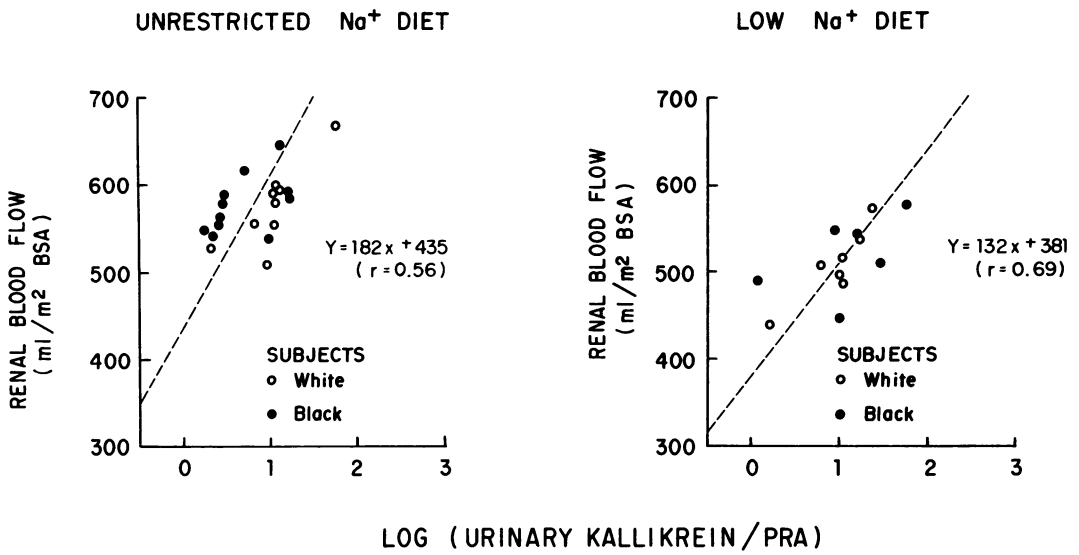

FIGURE 2 A linear relationship is demonstrated between renal blood flow (milliliters per minute per square meters of body surface area) and log urinary kallikrein/supine PRA for all normotensive subjects (regardless of race). A significant correlation was found on either extreme of dietary sodium intake.

dietary sodium intake. These data are depicted graphically in Figs. 2 and 3 for normotensives and hypertensives, respectively. Significant ($P < 0.01$) correlation coefficients were found in all cases.

DISCUSSION

The observed differences of urinary kallikrein excretion in black and white men indicate a distinct racial influence on the excretion of the renal enzyme. Furthermore, after defining the subjects according to prevalent blood pressure pattern, we have demonstrated racial differences regardless of dietary sodium intake and the observed racial similarities of urinary volume, sodium, and potassium excretion. While ingesting an unrestricted sodium diet, 24 h urinary kallikrein activity was highest (average: 18.7±3.1 EU) in white subjects who were felt to exhibit normal

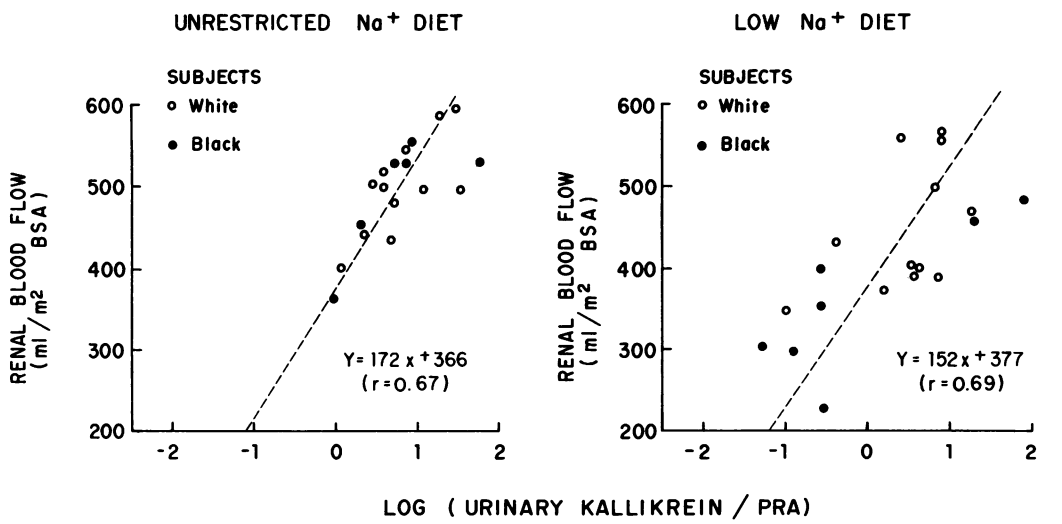

FIGURE 3 A linear relationship is demonstrated between renal blood flow (milliliters per minute per square meters of body surface area) and log urinary kallikrein/supine PRA for all hypertensive subjects (regardless of race). On low dietary sodium intake black hypertensives have urinary kallikrein activity and renal blood flow significantly lower than white hypertensives and tend to cluster nearer to the origin of the regression line.

blood pressure, whereas it was significantly ($P < 0.05$) lower in white hypertensive (mean: 9.2±2.3 EU) and black hypertensive (mean 3.0±1.1 EU) patients (Fig. 1). Urinary kallikrein activity was also low in black normotensive men (average: 3.8±0.9 EU). After dietary salt restriction, urinary kallikrein activity increased ($P < 0.05$) in all subjects irrespective of blood pressure (Fig. 1). All white subjects and black normotensives exhibited urinary enzyme activities that were similar ($P > 0.05$) to each other. However, kallikrein activity was significantly less ($P < 0.05$) in hypertensive black men than in any other group. Thus, urinary kallikrein activity appears to reflect a fundamental difference between black and white men with essential (primary) hypertension.

The demonstrations of biologic differences between racial groups is not without precedent. Several examples include the higher prevalence of decreased intestinal lactase activity in blacks (20), the increased percentage of blacks with erythrocyte glucose-6-phosphate dehydrogenase deficiency (21), and the variable prevalence rates of many types of neoplasia between races (22). More relevant to our own results, there are experimental and epidemiologic reports that suggest fundamental differences between blacks and whites with primary hypertension. The latter studies have noted racial variability regarding possible pathophysiology, sequela of untreated disease, and response to antihypertensive therapy. We have observed previously, in a limited number of patients, that black hypertensive men exhibit greater plasma volumes than white hypertensives after correction for body surface areas (10). In addition, one type of "low renin" hypertension appears to be more common among blacks (23), and another study suggests that hypertensive whites exhibit a greater decrement of blood pressure after the administration of propranalol (24).

Previous investigations of the kallikrein-kinin system in man have not been analyzed for a racial influence. Margolius et al. (25) reported that 21 patients with essential hypertension (unspecified race and dietary salt intake) excreted only half the quantity of urinary kallikrein as apparently normal controls. Subsequently, the same group (7) observed diminished urinary kallikrein activity in 11 patients with essential hypertension (eight blacks and three whites). Hypertensives exhibited a blunted but significant rise in urinary kallikrein excretion after dietary sodium restriction. Although our kallikrein assay procedures (including bioassay correlations) are similar, our differing criteria for hypertension and separate racial analysis do not allow a detailed comparison with these studies.

To the extent that multiple factors may be associated with the etiology of primary or essential hypertension, we have examined renal blood flow and PRA in our subjects. This further investigation would be supported by the proposal that kallikrein originates in the renal cortex near the distal tubule similar to renin (26). In addition, kallikrein and renin appear to occupy the same anatomic location in the submaxillary glands of the mouse (27, 28). Finally, animal and human experiments are consistent with an interrelated antagonism and modulation (29, 30). The significant correlations that were found between urinary kallikrein activity and renal blood flow in all groups (Table VI), except black normotensives on low sodium intake, are compatible with the concept that the kallikrein-kinin system may contribute to the regulation of renal vascular resistance. We have no explanation for our failure to document this relationship in the black normotensives except that it may represent a chance occurrence based on the size of this subject group. This conclusion does not preclude the possibility that other factors are more important in the regulation of renal blood flow in normotensive black men on restricted salt diets. The black hypertensive group had a significantly lower renal blood flow on the low sodium diet when compared to white hypertensives even though a difference did not exist on the unrestricted sodium intake. An attempt was made to explain further the observed racial variation in renal blood flow by relating this measurement to the proposed antagonism between the vasodilatory kallikrein-kinin system and the vasoconstricting renin-angiotensin system. This was done by correlating renal blood flow (per square meters of body surface area) with log (urinary kallikrein/PRA supine) for normotensives and hypertensives on both diets (Figs. 2 and 3). Significant correlations were found in all cases, and these observations provide support for the concept that both the kallikrein-kinin system and renin-angiotensin system are important regulators of renal blood flow in man. Thus, the lower urinary kallikrein activity in black hypertensives (compared to white hypertensives with similar blood pressures) on the low sodium diet is paralleled by a lower average renal blood flow. This is seen graphically in Fig. 3 by the tendency for the black hypertensives to cluster closer to the origin of the regression line. To the extent that diminished renal blood flow reflects increased renal vascular resistance in black hypertensives on low salt, we have demonstrated another pathophysiologic explanation for hypertension in the black population. A more prospective investigation will be required to define the exact relationship between our enzyme measurements and renal hemodynamics. However, it should be noted that our interpretation of these results is consistent with reports that renal vascular resistance contributes importantly to the genesis of hypertension, and we have provided evidence for a biochemical basis for this phenomenon (31).

We have not provided information regarding the

influence of kinins as important systemic vasodilatory substances in normotensive subjects or patients with systemic hypertension, and there is little available information concerning the access of renal kallikrein or the related kinins to the systemic circulation. Previous experimental data indicates that kallikrein enzymatically cleaves a plasma alpha-2 globulin to produce a potent systemic and local vasodilatory peptide. In addition, one human study reported changes in circulating plasma kinin concentration during experimental volume manipulations that appeared most consistent with an important regulatory role for tissue (e.g. renal) kallikrein (30). Relative increases of blood kinin concentration have been reported in such diverse hypotensive conditions as experimental dumping syndrome in dogs (32) and familial orthostatic hypotension in man (33). In any event, to the extent that decreased urinary kallikrein activity may reflect deficiency of a vasodepressor substance, the detection of lower values in white hypertensive patients (vs. white normotensives) on unrestricted sodium diets does suggest that impaired renal kallikrein activity may play a role in certain forms of primary hypertension. Furthermore, the lower urinary kallikrein activities of black hypertensive patients after dietary salt restriction could be explained in a similar manner. Thus, the present observations would appear to offer ample justification for suspecting that renal kallikrein may well contribute to systemic hypertension in both racial groups.

A proposal that the renal killikrein-kinin system (as reflected by urinary enzyme activity) is natriuretic in human subjects remains controversial. It has been suggested (34) and denied (15) that a positive relationship exists between urinary sodium excretion and kallikrein activity. In an attempt to reconcile apparent differences, Nasjletti and Colina-Chourio have proposed a reduced natriuretic potential for the kallikrein-kinin system after plasma volume contraction (35). However, the present and previous observations (15) of incremental urinary kallikrein excretion in normal subjects after documented steady-state volume contraction seem incompatible with the concept that kallikrein is an important chronic natriuretic substance in normotensive humans. Yet, our present study does not enable us to draw conclusions regarding the importance of the kallikrein-kinin system in the more acute or even day-to-day regulation of salt excretion and control of extracellular fluid volume. It is attractive to propose that the previously reported volume expansion of black hypertensive men (10) may be related to deficient renal kallikrein and that this could be of pathophysiologic importance in this racial group. The observed negative correlation ($r = -0.85$, $P < 0.01$) between urinary sodium concentration and kallikrein activity in black men on unrestricted salt intake would be consistent with a deficiency of kallikrein, a potentially natriuretic substance. In black hypertensives, this latter defect might be synergistic with the previously proposed deficiency of a renal or systemic vasodilatory hormone.

Diurnal rhythms exist for a number of biologic systems including cortisol and aldosterone (36). Based on the proposed mineralcorticoid influence on urinary kallikrein excretion (15) we have attempted to measure a possible broad day-night variation of the urinary enzyme. We observed day-night variation among white hypertensive patients but were unable to demonstrate a similar phenomenon in other studied groups (Tables III and IV). Interestingly, white hypertensives had similar variability of urinary volume and sodium. This observation might be related to the larger number of hypertensive white subjects or may have physiologic meaning regarding blood pressure and volume regulation. Larger numbers of subjects and more frequent urine collections will be required to elucidate this observation further.

In conclusion, we have demonstrated racial and dietary influences on urinary kallikrein excretion in normotensive and hypertensive subjects. Additionally, racial variations in renal blood flow have been documented. We have observed a direct relationship between renal blood flow and urinary kallikrein excretion in all subjects except black normotensive men on a low sodium diet, and have shown a broad day-night variation of urinary kallikrein activity in white hypertensive patients. Finally, we have proposed several possible pathophysiologic mechanisms, based on these measurements, to explain differences in human hypertension which exist between the black and white races. Although further study is necessary, it appears that measurements of urinary kallikrein activity may provide a means to further our understanding of the physiologic basis for human hypertension.

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