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Urinary Kallikrein and Urinary Prostaglandin E_2 in Genetically Hypertensive Mice (40746)

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Kallikreins are proteinases that liberate kinin peptides from α-globulin substrates (kininogens). Renal kallikrein is produced in the distal nephron and there catalyzes kinin formation. In the kidney, kinins are vasodilators that may play a role in blood pressure regulation through influences on renal functions (1). Close interactions apparently occur between the renal kallikrein–kinin and prostaglandin systems. The principal interaction involves stimulation by kinins of prostaglandin E production (2, 3). Prostaglandin synthesis in the kidney takes place almost exclusively in the medulla. Prostaglandin E_2 (PGE_2) is the major prostaglandin of the E series produced in the renal medulla, and, like kinins, it is an intrarenal vasodilator that could affect renal mechanisms associated with blood pressure control (4). Kallikrein and PGE_2 in urine originate intrarenally (5, 6). Humans with essential hypertension have reduced urinary kallikrein activity (7) and urinary PGE_2 levels (8) compared to normotensive control subjects, suggesting a possible involvement of the renal kallikrein–kinin and prostaglandin vasodepressor systems in the pathogenesis of essential hypertension. The objective of the present study was to evaluate urinary kallikrein activity and urinary PGE_2 levels in the genetically hypertensive mouse model developed by Schlager (9).

Materials and methods. The high blood pressure (HBP) and low blood pressure (LBP) mice used in this study were first generation progeny of animals sampled from the 19th and 20th generations of a selective breeding program designated BPI (9). Mice were maintained in standard fashion in an animal room at 24°C, with a 12 hr of light and 12 hr of darkness cycle. Water and food (Wayne Lab Blox) were provided *ad libitum*.

Twenty four-hr urine samples were collected under toluene from HBP and LBP mice of both sexes on three occasions, 5 to 7 days apart. During collections, mice were housed individually in metabolism cages and deprived of food, but allowed free access to tap water containing 5% sucrose (addition of sucrose to drinking water was undertaken to increase urine sample volumes, via increased fluid consumption, for facilitation of quantitative recovery and practical handling of the samples). The first two 24-hr urine samples from each mouse were stored under toluene at 4°C until the third sample was obtained, at which time the volume of each sample was measured and the three samples were pooled. Toluene was then removed and the pool was divided into several aliquots which were stored at −20°C. Systolic blood pressure measurements were made for each animal 2 to 3 days after the second urine collection and again 2 to 3 days after the third collection. These measurements were carried out indirectly by a tail cuff method utilizing a Narco-Biosystems physiograph to simultaneously monitor distal pulse and cuff pressure (9). During measurements, mice were unanesthetized, but restrained in a small holder mounted on a 38°C warming plate. Body weight determinations were made for each animal following the second blood pressure measurement. At the start of experimental manipulation, all mice were at

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least 90 days old, and no mouse had reached an age greater than 120 days by the time of the final experimental handling.

Kallikreins, as well as other trypsin-like proteinases, have the ability to hydrolyze synthetic arginine esters at an alkaline pH. Mouse urinary kallikrein arginine esterase activity was measured by a colorimetric method (10). The method employs the synthetic substrate α-*N*-*p*-tosyl-L-arginine methyl ester HCl (TAME) and incubations are carried out at 37°C and pH 8.6. We have performed studies which suggested that TAME esterase activity in the urine of HBP and LBP mice was due only to urinary kallikrein (see *Results and discussion.*).

Urinary PGE_2 immunoreactivity was determined by a method involving ethyl acetate extraction, silicic acid chromatography, and radioimmunoassay (RIA) (11, 8). Briefly, to 1 ml of mouse urine was added 1200 cpm of [^{3}H]PGE_2 (for recovery estimation) and 4 ml of distilled H_2O. The pH was adjusted to 3.5 with 1 *N* HCl and then 8 ml of ethyl acetate was added. Following 30 min of mechanical shaking, the organic phase was removed and evaporated under an air stream at room temperature. The dried organic extract was subjected to silicic acid chromatography for separation of the PGE fraction from the PGA and PGF fractions. The PGE fraction was evaporated under an air stream at room temperature and the dried residue was reconstituted in RIA buffer. Duplicate aliquots of the reconstituted PGE fraction were then taken for both recovery estimation and RIA. The RIA was carried out using a highly specific anti-PGE_2 antibody obtained from Dr. F. Dray of the Pasteur Institute, Paris, France. For the HBP and LBP urine samples, the mean recovery of added tracer through the entire procedure was 64.9 ± 1.85% (standard error) (n = 39).

Measurements of total protein in HBP and LBP urine samples were performed by the method of Lowry *et al.* (12). Bovine serum albumin was used as a protein standard.

Results and discussion. Urine samples were obtained in a single collection from adult HBP and LBP mice of both sexes as described above (see Materials and Methods). All samples from mice of a given sex and selected line were pooled, toluene was removed, and the pool was extensively dialyzed against distilled H_2O at 4°C. The four dialyzed urine pools were then lyophilized. Each lyophilized pool was chromatographed on a DEAE-Sephadex ion exchange column and column fractions were assayed for TAME esterase activity by the colorimetric method. Details of the chromatography procedure are given in the legend of Fig. 1. Results for HBP and LBP urine pools were identical and were as illustrated in Fig. 1 for HBP female urine. Only one peak of TAME esterase activity was distinguishable, eluting at a K^+ concentration of 0.27 *M*. These results are in sharp contrast to those obtained for male Sprague–Dawley rat urine collected, processed, and chromatographed in the same fashion (Fig. 2). Three peaks of male rat urinary TAME esterase activity were separable by DEAE-Sephadex chromatography. The last peak to be eluted, at a K^+

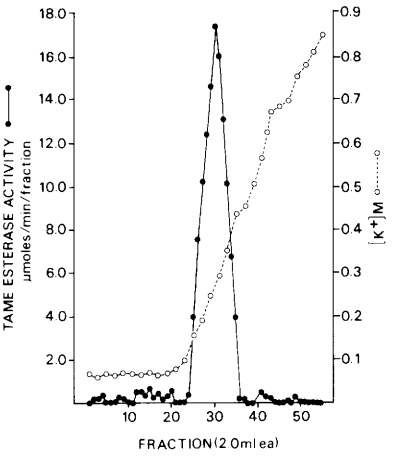

FIG. 1. DEAE-Sephadex chromatography of HBP female urine. The column was run at 4°C. Column dimensions were 1.8 × 7.5 cm. Lyophilized sample, containing approximately 125 μmole/min TAME esterase activity, was dissolved in 6 ml column-equilibrating solution (0.05 *M* KCl in 0.01 *M* potassium phosphate buffer, pH 7.0). After sample application, column was washed with 30 ml equilibrating solution and then eluted with 80 ml of a linear KCl gradient to 1 *M* in the potassium phosphate buffer. Flow rate was 10 ml/hr. Potassium concentrations were measured by flame photometry.

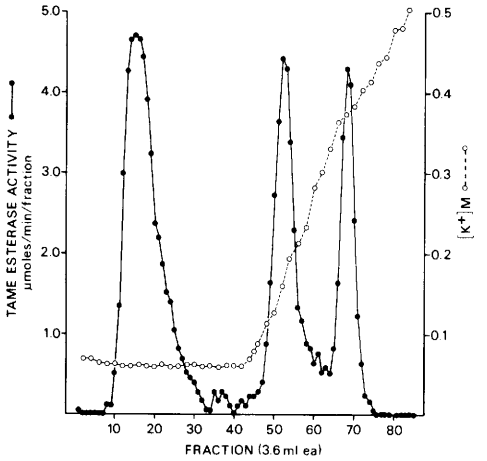

FIG. 2. DEAE-Sephadex chromatography of male rat urine. The column was run at 4°C. Column dimensions were 2 × 23 cm. Lyophilized sample, containing approximately 165 μmole/min TAME esterase activity, was dissolved in 12 ml column-equilibrating solution (0.05 *M* KCl in 0.01 *M* potassium phosphate buffer, pH 7.0). After sample application, column was washed with 70 ml equilibrating solution and then eluted with 230 ml of a linear KCl gradient to 0.7 *M* in the potassium phosphate buffer. Flow rate was 25 ml/hr. Potassium concentrations were measured by flame photometry.

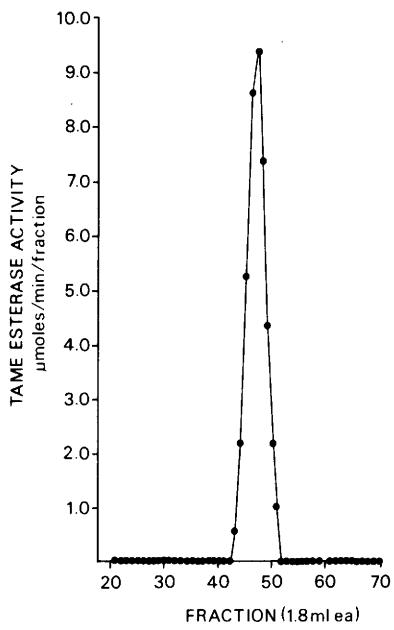

FIG. 3. Sephacryl S-200 chromatography of HBP female urinary TAME esterase. Column was run at 4°C. Column dimensions were 1.5 × 95 cm. Lyophilized sample, containing approximately 50 μmole/min TAME esterase activity, was dissolved in 1 ml chromatography solution (0.05 *M* NaCl, 0.2% NaN_3 in 0.05 *M* Tris–HCl buffer, pH 8.0) and applied to the column. Flow rate was 8 ml/hr.

concentration of 0.37 *M*, has been identified as rat urinary kallikrein; the other two activity peaks are composed of nonkallikrein TAME esterases (Ref. (10) and unpublished data). The presence of nonkallikrein urinary TAME esterases in some species, as illustrated here for the rat, demonstrates the need for caution before equating TAME esterase activity with kallikrein activity in urine.

Samples of HBP and LBP urinary TAME esterases, isolated by DEAE-Sephadex chromatography, were dialyzed against distilled H_2O at 4°C, lyophilized, and further analyzed by Sephacryl S-200 gel filtration chromatography. The same results were obtained for HBP and LBP TAME esterases and were as shown in Fig. 3 for HBP female TAME esterase. Only one peak of activity was discernable, eluting at a volume characteristic of protein molecules with a molecular weight of about 40,000 (reference standards used were cytochrome C, ovalbumin, lysozyme, bovine serum albumin, and chymotrypsinogen A). This is within the molecular weight range of glandular kallikreins from other species (1).

Samples of HBP and LBP urinary TAME esterases, isolated by DEAE-Sephadex chromatography and then dialyzed and lyophilized, were further analyzed by polyacrylamide slab gel electrophoresis (13). Assays of TAME esterase activity in 1-mm gel slices were carried out by a highly sensitive technique employing radioactive TAME (14). In this assay, incubations are performed at room temperature and pH 8.0. The HBP and LBP TAME esterases each showed a single peak of activity after electrophoresis, as illustrated in Fig. 4 for HBP female TAME esterase. The HBP and LBP TAME esterases had equivalent electrophoretic mobilities.

We have thus analyzed HBP and LBP urinary TAME esterase activity by three

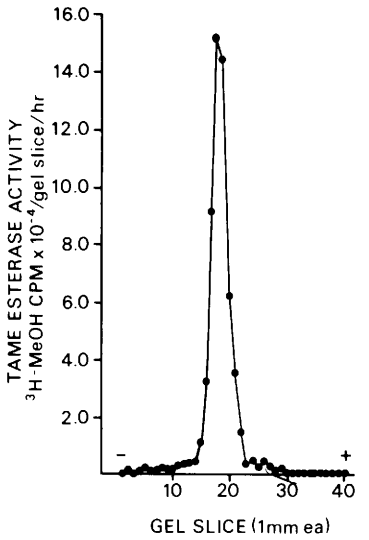

FIG. 4. Polyacrylamide slab gel electrophoresis of HBP female urinary TAME esterase. Electrophoresis carried out at 4°C for 1 hr at 200 V and 1.5 hr at 400 V. Running gel acrylamide concentration was 13% and pH was 8.9. Lyophilized sample, containing approximately 5 μmole/min TAME esterase activity, was dissolved in 1 ml distilled H_2O. A 50-μl volume of this solution, in which about 1.5 μg bromophenol blue tracking dye was dissolved, was applied to the gel. After electrophoresis, gel slices were eluted overnight in 200 μl 0.2 *M* Tris–HCl buffer, pH 8.0, at 4°C. TAME esterase activity was expressed as cpm [^{3}H]methanol (^{3}H-MeOH) generated per hour. Tracking dye band peak was contained mainly in gel slice 40.

different methods of protein molecule fractionation. By all three methods only one peak of TAME esterase activity was evident. Although these results did not prove that urinary TAME esterase activity in these mice was due to a single enzyme, they strongly suggested that this was the case.

Urinary TAME esterase from HBP female mice and urinary kallikrein from adult female Sprague–Dawley rats, which had each been chromatographed on DEAE-Sephadex and then on Sephacryl S-200, were tested for kinin generating activity by incubations with a kininogen substrate of acid-treated dog plasma (15) and measurements of released kinins by bioassay on an isolated rat uterus (5). Incubations were performed at room temperature and pH 8.0. Synthetic bradykinin was used as a standard in the bioassay. The mouse urinary TAME esterase, rat urinary kallikrein, and dog plasma kininogen preparation, at the amounts employed, showed no kininase or endogenous uterine contracting activity. For rat urinary kallikrein, the ratio of kinin-generating activity (μg bradykinin equivalents generated/min) to TAME esterase activity in the colorimetric assay (μmole TAME hydrolyzed/min) was 2.18. The same ratio for the mouse TAME esterase was 1.66. Bovine pancreatic trypsin, which was free of kininase activity and which was used at a TAME esterase activity concentration 15 times higher than that of the rat urinary kallikrein or mouse urinary TAME esterase, was inactive in the generation of kinins from the dog plasma kininogen substrate employed.

The ratios of kinin-generating activity to TAME esterase activity were thus quite comparable between rat urinary kallikrein and the mouse urinary TAME esterase. The dog plasma kininogen preparation used was not susceptible to kinin-liberating action by the nonkallikrein TAME esterase trypsin and therefore might be considered a specific substrate for kallikreins. From these observations, it was concluded that the mouse urinary TAME esterase was in all probability mouse urinary kallikrein.

Table I shows means and standard errors and analysis of variance results for body weight, blood pressure, urinary volume, and urinary protein in HBP and LBP mice. The HBP mice had significantly greater body weights than LBP mice. This finding differs from previous findings of equivalent body weights in the two groups (9, 16). Blood pressures in HBP and LBP mice were significantly different, with the HBP males and females having mean pressures which were 60.3 and 53.3 mm Hg higher than mean pressures in LBP males and females, respectively. Similar blood pressure differences have recently been reported for these mice (16). Twenty four-hr urinary volumes were significantly greater in HBP than in LBP mice. Preliminary studies revealed that this difference exists regardless of whether sucrose is added to the drinking water supplied during urine collections. Larger urinary volumes in HBP mice could possibly be due to pressure

TABLE I. MEANS AND STANDARD ERRORS AND ANALYSIS OF VARIANCE RESULTS FOR BODY WEIGHT, BLOOD PRESSURE, URINARY VOLUME, AND URINARY PROTEIN IN HBP AND LBP MICE[a]

	LBP		HBP		P		
	♂♂ (9)	♀♀ (10)	♂♂ (10)	♀♀ (10)	Line	Sex	Interaction
Body weight (g)	25.1 ±0.85	22.3 ±0.86	29.0 ±0.78	24.5 ±0.99	<0.005	<0.001	NS
Blood pressure (mm Hg)	87.2 ±4.53	95.0 ±5.03	147.5 ±7.40	148.3 ±7.04	<0.001	NS	NS
Urinary volume (ml/24 hr)	5.20 ±0.868	6.71 ±0.714	8.26 ±0.661	9.32 ±0.400	<0.001	NS	NS
Urinary protein (mg/ml)	4.59 ±0.636	1.48 ±0.170	2.68 ±0.387	1.07 ±0.092	<0.005	<0.001	NS
Urinary protein (mg/24 hr)	20.43 ±2.627	9.49 ±1.075	20.54 ±1.405	9.66 ±0.799	NS	<0.001	NS

[a] Values in parentheses indicate sample sizes. Probabilities (P) were obtained from two-way analyses of variance. Analyses for urinary protein were performed on log-transformed data since variances were heterogeneous and related to magnitudes of means. NS = not significant (i.e., $P > 0.05$). The blood pressure of each animal was the average of two measurements and the 24-hr urinary volume of each animal was the average of three measurements.

diuresis. Urinary protein concentrations (mg/ml of urine) were significantly lower in HBP compared to LBP mice. This was undoubtedly a reflection of differing 24-hr urinary volumes between HBP and LBP lines, since urinary protein daily outputs (mg/24 hr) were nearly identical for the two groups. Both urinary protein concentrations and daily outputs were significantly higher in males than in females. A sex difference in proteinuria is well known in mice (17).

Results of urinary kallikrein TAME esterase activity and urinary PGE_2 immunoreactivity determinations for HBP and LBP mice are presented in Fig. 5. Urinary kallikrein activity, whether expressed as amount per milliliter of urine or amount excreted per day, was significantly elevated in HBP compared to LBP mice. Urinary PGE_2 immunoreactivity was significantly reduced in HBP compared to LBP mice, both in amount per milliliter of urine and amount excreted per day.

Renal kallikrein, via kinin generation, and renal PGE_2 could be involved in the regulation of blood pressure through influ-

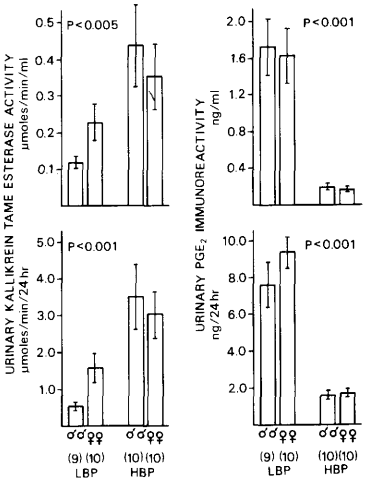

FIG. 5. Means and standard errors and analysis of variance results for urinary kallikrein TAME esterase activity and urinary PGE_2 immunoreactivity in HBP and LBP mice. Values in parentheses indicate sample sizes. Probabilities (P) are those associated with line effects obtained from two-way analyses of variance. Analyses were performed on log-transformed data since variances were heterogeneous and related to magnitudes of means. No significant sex or interaction effects were observed (i.e., $P > 0.05$).

ences on renal blood flow and the excretion of salt and water (1, 4). Humans with essential hypertension have lower urinary kallikrein activity (7) and lower urinary PGE_2 levels (8) than normotensive control subjects. In contrast to this, humans with hypertension due to primary aldosteronism possess elevated urinary kallikrein activity (7) and unchanged urinary PGE_2 levels (18) compared to normotensive control subjects. Several strains of genetically hypertensive rats show reduced urinary kallikrein activity in comparison to appropriate control strains (19–23). Urinary PGE_2–levels have been measured in only one of the genetically hypertensive rat models and were found to be unaltered compared to controls (24).

The genetically hypertensive rats are thus similar to human essential hypertension in having low urinary kallikrein activity. Schlager HBP mice appear to be unique among the genetically hypertensive animal models in that urinary kallikrein activity is elevated. This is similar to the situation in human primary aldosteronism. That high urinary kallikrein activity is a direct result of the increased mineralocorticoid activity in primary aldosteronism is suggested by the observations that urinary kallikrein activity is enhanced in rats (25) and humans (14) by low sodium diet or mineralocorticoid administration, and that mineralocorticoid stimulates kallikrein release by kidney cell suspensions (26). The Schlager hypertensive mouse might possibly be a model for a genetically mediated hypermineralocorticoid state.

Urinary PGE_2 immunoreactivity is markedly low in HBP compared to LBP mice, which of course resembles the situation in human essential hypertension. The Schlager hypertensive mouse is thus not only unique in being a genetically hypertensive animal model with elevated urinary kallikrein activity, it is also unique in that it appears to provide the only genetically hypertensive animal model with a known reduction in urinary PGE_2 levels.

With regard to the interrelationship of the renal kallikrein–kinin and prostaglandin systems, one might expect that higher urinary kallikrein activity would be associated with higher urinary PGE_2 levels (2, 3). In HBP and LBP mice, this is definitely not the case. These mice have an inverse relationship between urinary kallikrein activity and urinary PGE_2 levels. One possible explanation for this apparently altered relationship is that HBP mice could have a physiological block preventing stimulation of renal prostaglandin synthesis by the renal kallikrein–kinin system.

Summary. We have evaluated urinary kallikrein activity and urinary prostaglandin E_2 (PGE_2) immunoreactivity in Schlager high blood pressure (HBP) and low blood pressure (LBP) mice. Urinary kallikrein activity was measured by a colorimetric method employing α-*N*-*p*-tosyl-L-arginine methyl ester HCl (TAME) as substrate. It was shown that urinary TAME esterase activity in HBP and LBP mice was probably due to a single enzyme and that this enzyme appeared to be urinary kallikrein. Urinary PGE_2 immunoreactivity was determined by a specific radioimmunoassay. Urinary kallikrein activity was significantly elevated and urinary PGE_2 immunoreactivity was significantly reduced in HBP compared to LBP mice. High urinary kallikrein activity in HBP mice resembles situations such as human primary aldosteronism, where hypertension is produced by mineralocorticoid excess. Low urinary PGE_2 levels in HBP mice, however, compares to the situation observed for human essential hypertension. The HBP mouse thus has alterations in physiological parameters believed to reflect renal blood pressure regulating mechanisms. These alterations are unique among genetically hypertensive animal models and appear to be analogous to alterations seen in certain human hypertensive states. One possible explanation for the inverse relationship between urinary kallikrein activity and urinary PGE_2 levels in HBP and LBP mice is that HBP mice could have a physiological block preventing stimulation of renal prostaglandin synthesis by the renal kallikrein–kinin system.

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