Effect of Alterations in Extracellular Fluid Volume on Urinary Kallikrein in the Conscious Rat

ERIC S. MARKS, M.D., MARIAN FRECH, B.A., DAVID PROUD, PH.D., AND HARRY R. KEISER, M.D.

SUMMARY The effect of alterations in extracellular fluid volume (ECV) and solute concentration on excretion of urinary kallikrein was examined in conscious Sprague-Dawley rats. Animals were given infusions of either dextrose and water, saline, or albumin according to a variety of protocols. These were designed to evaluate possible relationships between excretion of kallikrein, volume, sodium, and potassium. A reproducible pattern of kallikrein excretion was noted in all volume expanded groups. This consisted of a short lived increase during the initial hour of expansion with a subsequent fall to lower levels than baseline and a gradual recovery. To define the role of aldosterone in these studies, an adrenalectomized group and a group of appropriately prepared sham controls were expanded with saline. Adrenalectomy did not effect this pattern. We postulate a tubular "wash-out" phenomenon as the etiology of these observations. Results of these studies fail to demonstrate a consistent relationship between urinary volume, sodium, or potassium and the simultaneous amount of kallikrein found in the urine. (Hypertension 4: 625-633, 1982)

KEY WORDS • expansion • sodium excretion • potassium excretion • kidney

THE renal kallikrein-kinin system has been proposed as a contributor to control of volume homeostasis. Urinary kallikrein originates from the kidney so the amount of kallikrein present in urine has been used as a marker of the renal activity of the system. Despite a variety of experiments in man and laboratory animals a clear relationship between urinary kallikrein, volume, and solute excretion has not been defined.1-6 We used a standardized conscious animal model and sequential sampling techniques to provide data to clarify this issue. Our experiments were designed to determine if acute changes in extracellular fluid volume (ECV) and composition affected urinary kallikrein and if a related pattern of solute excretion and urine volume existed. A variety of expansion protocols was employed which allowed evaluation of specific aspects of excretory function.

Materials and Methods

Female Sprague-Dawley rats weighing 200 to 290 g (Taconic Farms) were randomized into seven groups of eight animals each and two groups of six animals each. Each group received a different infusion as noted below (fig. 1). A standard operative sequence began approximately 14 hours prior to the infusion protocol. The rats were anesthetized with sodium pentobarbital (50 mg/kg intraperitoneally) and the right external jugular vein cannulated with polyethylene tubing (PE50) which was brought through a subcutaneous tunnel that existed in the midback. The urinary bladder was catheterized with PE50 tubing which was externally sutured to prevent leakage. In one group adrenalectomy was accomplished through a midline abdominal incision under sodium pentobarbital 36 to 40 hours preceding the infusion experiment. Sham-operated animals had manipulation of both adrenals and kidneys under anesthesia to stimulate the stress of gland removal. Adrenalectomized animals received dexamethasone (1 µg/kg/day intraperitoneally). After venous catheterization rats were placed in specially designed cylindrical plexiglass restraining holders which minimized external stimuli and allowed limited movement of the front half of the body. All experiments were conducted at the same time each day to avoid circadian changes in the measured variables.10

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Overnight infusions can be divided into three types based on the infusate composition and rate of administration. Group 1 (control) received 5% dextrose and saline (150 mEq/liter) at 0.6 ml/hr, groups 2 through 7 received 5% dextrose and water at 0.2 ml/hr, while groups 8 and 9 received 5% dextrose and water at 0.4 ml/hr. Urine was collected in glass tubes and the overnight collection was discarded. Urine was then collected in hourly samples and after volume determination, divided into two aliquots: one for determination of kallikrein and the other for assay of electrolytes. For kallikrein, 10 μl of sodium azide (0.025%) was added to samples and all urines were quick frozen and stored at -20°C until analyzed. Kallikrein was measured by the radioimmunoassay method of Lawton et al. which recognizes total immunoreactive antigen.

In addition to kallikrein, the antibody reacts with the inactive zymogen, prokallikrein, and enzyme inhibitor complexes. The radioimmunoassay method was selected in preference to either esterase or kininogenase assays to avoid interference from rat urinary esterase A. This enzyme does not crossreact in the radioimmunoassay but is responsible for approximately 50% of the esterase activity in unfractionated rat urine and is known to possess some kinin-generating activity. Serial dilutions of rat urine produced a radioimmunoassay displacement curve which was parallel to the standard. Intra- and interassay coefficients of variation (SEM/SEM) were 2% and 4% respectively (n = 6 in each case). Recovery of purified kallikrein added to urine (n = 4) was 102% ± 2% (X ± SEM). Flame photometry was used for sodium and potassium determinations.

Venous catheter placement was verified at the conclusion of each experiment and rats were discarded if evidence of infusate extravasation from blood vessel damage was noted. In addition, data from animals that developed hematuria were not used.

### Statistical Analysis

Results are reported as mean values ± the standard error. Bartlett’s test for the homogeneity of variance-covariance matrices was applied to all groups followed by the chi-square test for the uniformity of this variance-covariance matrix. All groups conformed to those tests and were both homogenous and uniform. An analysis of variance for repeated measures with the Greenhouse-Geisser profile analysis procedure was then performed. Linear regression and paired Student’s t tests were applied when appropriate. The null hypothesis was rejected at the 0.05 level.

### Experimental Protocols

Nine infusion protocols were performed (fig. 1). Group 1 (control) received 0.9% saline in 5% dextrose and water at 0.06 ml/hr throughout the entire experiment, commencing immediately after catheter placement.

### Table 1. Urinary Kallikrein, Volume Sodium, and Potassium in Rats Receiving a Continuous Infusion of 5% Dextrose and Saline at 0.6 ml/hr (Group 1)

<table>
<thead>
<tr>
<th>Period</th>
<th>Kallikrein (μg)</th>
<th>Volume (ml)</th>
<th>Sodium (mEq)</th>
<th>Potassium (mEq)</th>
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<tr>
<td></td>
<td>1.3 ± 0.4</td>
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<td>0.11 ± 0.01</td>
<td>0.04 ± 0.01</td>
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<td>2</td>
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<td>3</td>
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<td>1.2 ± 0.1</td>
<td>0.16 ± 0.02</td>
<td>0.04 ± 0.01</td>
</tr>
<tr>
<td>4</td>
<td>1.3 ± 0.2</td>
<td>1.2 ± 0.1</td>
<td>0.15 ± 0.02</td>
<td>0.04 ± 0.01</td>
</tr>
<tr>
<td>5</td>
<td>1.3 ± 0.2</td>
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<td>0.16 ± 0.04</td>
<td>0.04 ± 0.01</td>
</tr>
<tr>
<td>6</td>
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<td>0.9 ± 0.1</td>
<td>0.14 ± 0.02</td>
<td>0.03 ± 0.004</td>
</tr>
<tr>
<td>7</td>
<td>1.2 ± 0.1</td>
<td>0.9 ± 0.1</td>
<td>0.15 ± 0.03</td>
<td>0.03 ± 0.003</td>
</tr>
</tbody>
</table>

Values are mean ± SEM.
ment and ending after the seventh collection period. Groups 2 and 3 (limited expansion) received infusions of 0.9% saline or 5% dextrose and water respectively to provide a 20% expansion of the calculated extracellular fluid (ECF) volume over 1 hour. This was followed in both groups by an infusion of 5% dextrose and water at 0.24 ml/hr for 5 additional hours. In Group 4, a 15% increase in total body sodium without a concomitant significant volume expansion was accomplished with a 1-hour infusion of 3% saline. The figure of 15% was derived from the amount of sodium infused during the 20% expansion of ECF volume with 0.9% saline (based on a total body sodium in the rat of 45 mEq/kg body weight). Groups 5 and 6 (maintained diuresis) were expanded for 1 hour as were Groups 2 and 3 and this was followed by hourly replacement of urine volume with saline or dextrose and water respectively. In Group 7 the initial 20% expansion was followed by 3 hours of replacement of urine volume with 0.9% saline, at which point the infused composition was changed to 0.45% saline-12.5% albumin with continued replacement of urine volume. In this group, urine collection continued for an additional 5 hours. Groups 8 and 9 (sham operation or adrenalectomy) received similar treatment as Group 2 with two differences; the baseline infusion rate was 0.4 ml/hr and the collection periods were continued only 3 hours following expansion. In all groups a 1-hr preexpansion urine collection was obtained at the baseline infusion rates noted in the methods section.

**Results**

**Group 1**

These animals demonstrated stability of all measured parameters through all collection periods (table 1). Baseline kallikrein was not significantly different among any of the groups of nonlaparotomized animals.

**Groups 2 and 3**

In these groups which received a limited expansion of 20% of ECV, there was an average 200% increase in urine volume during the expansion period which persisted for at least one additional period. Sodium excretion was increased 500% with the 0.9% saline and unchanged with dextrose and water. Potassium excretion during the initial dextrose infusion, then fell to baseline, while with the saline infusion potassium excretion was unchanged. Urinary kallikrein rose in both groups during the hour of expansion although this rise was not statistically significant. In the 4 hours following expansion, kallikrein excretion was significantly lower than during expansion in Group 2, and lower than baseline in Group 3. Kallikrein returned to basal levels by the seventh hour in both groups (figs. 2 and 3).

**Group 4**

Infusion with 3% saline increased urinary sodium 316% with no change in urinary volume. A fall in kallikrein began during the infusion period and this reached significance during the following two hours, returning to baseline by four hours. Potassium excretion was unchanged throughout the experiments (fig. 4).

**Groups 5 and 6**

The maintained diuresis experiments demonstrated similar patterns of increase in urinary volume (three- and four-fold respectively). Sodium excretion, however, was unchanged with water diuresis but increased nine-fold with saline. Potassium behaved as in Groups 2 and 3. Urinary kallikrein rose insignificantly during the hour of initial expansion, followed by a fall in both groups. In Group 5, postinfusion kallikrein remained significantly lower than during the primary expansion but unchanged from baseline (figs. 5 and 6).
FIGURE 3. Urinary kallikrein, volume, sodium, and potassium in rats receiving a 20% ECV expansion with dextrose and water. (Mean, n = 8.)

FIGURE 4. Urinary kallikrein, volume, sodium, and potassium in rats receiving a 15% expansion of total body sodium with 3% saline. (Mean, n = 8.)

FIGURE 5. Urinary kallikrein, volume, sodium, and potassium in rats receiving a 20% ECV expansion and maintained diuresis with normal saline. (Mean, n = 8.)
Group 7

This group received 0.9% saline with the later addition of hyperoncotic albumin and demonstrated two separate changes in urinary volume and sodium excretion. The initial expansion provided an immediate six-fold increase in the excretion of volume and a 24-fold increase in sodium followed by a typical diuresis with stabilization of both variables over time. Addition of albumin produced a pronounced and significant 76% further increase in urine volume and a 52% further increase in sodium which persisted for two hours before returning to prealbumin levels. Urinary potassium rose with expansion and remained elevated throughout, showing no further evidence of any effect from the addition of albumin. An insignificant increase in kallikrein was noted with the initial expansion followed by a fall and then return to basal levels. The addition of albumin produced no significant changes in kallikrein to correspond with the marked changes in urinary sodium and volume noted above (fig. 7).
Groups 8 and 9
Sham and adrenalectomized rats followed the same pattern of sodium, potassium, and volume excretion with 20% ECF limited expansion as did Group 2. Baseline kallikrein levels were 50% lower in the adrenalectomized group when compared to the sham controls. In both groups kallikrein rose with expansion and then immediately fell, in the hour following the expansion period, to a value that was about 50% lower than the baseline value. No significant differences existed in sodium, volume, or potassium excretion between the two groups, with both experiencing a 2-fold increase in sodium excretion (figs. 8 and 9).

Discussion
Our experiments demonstrate that acute limited volume expansion with water, saline, or albumin, produces a tendency for kallikrein to rise during the first hour, followed by an acute fall. In none of the groups did this increase reach statistical significance when compared to preexpansion values. No correlation could be found between urinary kallikrein and either urinary sodium, potassium, or volume. This was most apparent in the groups in which a diuresis was maintained (Groups 5 and 6) and where solute and water excretion remained at elevated levels while kallikrein decreased after the initial expansion period. To our knowledge no studies have been performed in which a diuresis was maintained for a prolonged period and kallikrein measured in sequential hourly samples. The acute fall in kallikrein in the third hour occurred in all groups, with a return to baseline in all but the animals that had a laparotomy. Adrenalectomy did not alter the pattern of urinary kallikrein from acute limited volume expansion with saline. Kallikrein excretion observed after hypertonic saline infusion was unique among the groups, as no initial rise was noted. Changes in kallikrein observed in these experiments were both abrupt and short-lived. The absolute increase in kallikrein excretion could with less frequent collection (periods of longer duration) be misinterpreted as the new steady state situation. In addition, the data chosen as basal values are crucial in determining whether changes of kallikrein are, or are not, correlated with changes in urinary sodium, volume, or potassium. We used as baseline the 1 hour period that preceded expansion, 14 hours after surgery.

The development of a suitable model required stability and minimal physical manipulation of the animal over the course of the experiment. A conscious rat avoided the potential and demonstrable alteration in renal function induced by anesthesia\textsuperscript{15,16} and still allowed sequential measurement of urinary kallikrein, electrolytes, and volume. The stability of the model is evident from the consistent urine values obtained in the control group. The preinfusion value of urinary kallikrein did not differ between the control and any of the nonlaparotomy groups.

The infusion protocols were designed to provide a variety of physiologic manipulations of renal function. Acute limited expansion with saline (Group 2) pro-
duced the expected increase in urine volume and sodium. These increases were of short duration as the solute and volume loads were excreted rapidly. Urinary kallikrein tended to rise during the expansion period but fell to a level significantly lower than the basal level in the hour immediately following expansion (third hour) and then gradually returned to baseline. The fall occurred while sodium and volume were still elevated. This apparent dissociation of kallikrein from volume and sodium was confirmed in the “maintained saline diuresis” experiments (Groups 5 and 7). In these groups volume and solute excretion were maintained at high levels while kallikrein demonstrated the same pattern as observed with limited expansion.

The renal responses to volume expansion with saline include an increase in blood flow, a redistribution of plasma flow, and an associated decrease in proximal tubular reabsorption of sodium. One of the proposed mechanisms for the resultant natriuresis and diuresis is a fall in peritubular oncotic pressure. This would increase delivery of sodium to the distal nephron and collecting duct which play an important role in volume regulation and solute excretion. Kallikrein has been localized to these areas by both histochemical and functional “stopflow” techniques. Changes in distal tubular function could be related to both the vasodilatory and permeability alterations that kinins can effect. Data obtained with micropuncture techniques show that infusion of hyperoncotic albumin into the renal vasculature can return proximal tubular reabsorption close to preexpansion levels. Albumin expansion also affects proximal function to a lesser degree than saline; and may modify distal function.

In view of these reports a combined saline-albumin experiment was performed to determine if alteration of distal nephron activity induced by changes in solute load was correlated with urinary kallikrein. Our data failed to show a significant acute change in kallikrein associated with this shift in sodium handling sites. Kallikrein remained at baseline levels in the face of a marked increase in urinary volume and sodium.

The effect of water loading on kallikrein excretion was evaluated with both limited expansion and maintained water diuresis (Groups 3 and 6). The pattern of kallikrein excretion was similar to the results seen with limited saline expansion providing additional evidence for a complete dissociation of sodium excretion from kallikrein. Data from animal studies have found water expansion to be a less potent stimulator of kallikrein than saline. Expansion in our animals was accompanied intravenously as compared to the oral approach used by others, a difference which could be important in terms of the results. Oral loading may not provide the acute ECF expansion seen when renal perfusion pressure is lowered and associated with this shift in sodium handling sites. Kallikrein remained at baseline levels in the face of a marked increase in urinary volume and sodium.

Hypertonic saline was used to separate the effect of sodium from that of volume on urinary kallikrein. Kallikrein excretion fell with the initiation of the expansion and gradually returned to baseline over the remainder of the periods. This primary fall was accompanied by an increase in sodium excretion and no significant change in urinary volume, with both observations unique to this Group 4. It has been demonstrated in the rat that infusion of hypertonic saline decreases both whole kidney and single nephron glomerular filtration rates. We believe that acute changes in urinary kallikrein are related to acute changes in renal tubular flow rates. The fall that we observed with hypertonic saline may be related to the fall in tubular flow rates expected with the autoregulatory decrease in glomerular filtration rate. This may also be the case with the reported fall in urinary kallikrein seen when renal perfusion pressure is lowered below the autoregulatory range.

An apparent stimulatory action of aldosterone on kallikrein synthesis and/or release has been demonstrated both in vitro and in vivo. In addition, it has been previously shown that adrenalectomy or administration of an inhibitor of aldosterone significantly reduce urinary levels of kallikrein. In view of the immediate fall in kallikrein observed after expansion in our experiments, it was postulated that acute aldosterone suppression may have been a causative factor. This hypothesis was tested in groups 8 and 9. Kallikrein levels in adrenalectomized animals were approximately 50% of the sham-operated controls, but there was no significant difference in the response to expansion between the two groups. Thus our results do not support a role for aldosterone in the acute response of kallikrein to saline expansion. The basal level of kallikrein in the sham-operated rats was at least twice the level in other groups of nonlaparotomized rats, while the kallikrein in adrenalectomized rats did not demonstrate this increase. The reason for this increase is not known, however, surgery with its effects on ECF volume may have stimulated aldosterone production with the subsequent increase in kallikrein. The lack of adrenals in group 9 would prevent this rise. These two groups (8 and 9) are comparable only with each other in regard to the absolute level of kallikrein.

In all “volume” related expansion experiments (Groups 2, 3, 5, 6, 7, 8, and 9), a consistent pattern of kallikrein excretion was observed with an initial rise during the acute expansion followed by an immediate decrease (table 2). The significant and early fall in kallikrein after the initial period of expansion appears to be a new finding. We postulate that the initial rise is due to a “washout” phenomenon of kallikrein from renal tubular cell membrane. Kallikrein has been described as an ectoenzyme and its anatomical location on the luminal surface of distal tubule cells has been confirmed. We propose that an acute increase in tubular flow would result in an acute increase in the amount of enzyme in the urine. Continued high tubular flow rates would “wash-out” accessible membrane-bound material and levels in the urine would decrease to reflect basal cellular synthesis and release. This concept is supported by the experiments in which we
maintained a diuresis and the fall in kallikrein occurred while urine volume and sodium excretion were increasing. The addition of albumin caused a secondary increase in volume and sodium excretion which was not accompanied by a similar increase in kallikrein excretion seen with the initial expansion. Correlation between kallikrein in urine and in the kidney have been unsuccessful, with the urine levels significantly higher. This is additional support for the existence of active synthesis and release by the renal tubular cells.

Renal and urinary kallikrein are immunologically identical, but this fact does not support the assumption that changes in urinary excretion reflect physiologically significant activity in the kidney. Our studies show that acute changes in urinary volume, sodium, and potassium are essentially unrelated to kallikrein activity as measured by excretion. The design of the experimental protocols provides for conclusions about acute expansion only. We feel that there is sufficient evidence to show that chronic manipulation of the kallikrein-kinin system as seen, for example, with sodium deprivation or fludrocortisone administration does affect enzyme synthesis rate. It is therefore important that a clear distinction between acute and chronic observations be made in assigning a role for the kallikrein system in volume homeostasis. Further work into the control of kallikrein synthesis, release and metabolism requires a more fundamental approach than just the measurement of its urinary excretion.

References

22. Brenner BM, Falchuk KH, Keimowitz RI, Berliner RW: The relationship between peritubular capillary protein concentra-

### Table 2. Urinary Kallikrein in Experimental Groups 2-9, Reported as Total Antigen in Micrograms

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<th>Group</th>
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<th>4</th>
<th>5</th>
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</table>

Values are means ± sd.


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