Effects of frusemide on the renal kallikrein–kinin system of the rat

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Summary

1. In male Wistar rats, three doses of frusemide (0.5, 5.0 and 50.0 mg/kg) were injected subcutaneously. A dose-related increase in urine flow and natriuresis occurred, whereas there was a biphasic response in kallikrein excretion with an initial, dose-related transient increase and a secondary reduction. When the urine losses were replaced by the infusion of 0-9% NaCl solution, the biphasic response of urinary kallikrein excretion was maintained.

2. In all experiments, urinary excretion of kallikrein correlated with the excretion of potassium. Frusemide enhanced the excretion of kinins, which correlated with the urine volume and the natriuresis, but not with kallikrein excretion.

3. In contrast to the initially increased excretion of kallikrein, kallikrein activity in the renal cortex remained unchanged or was even reduced. Kininogen content of the perfused tissue of the renal cortex did not vary throughout the experiment, but decreased markedly in the non-perfused tissue of the cortex 30 min after the injection of frusemide.

4. It is concluded that forced diuresis induced by frusemide causes a ‘wash out’ of renal kallikrein in urine, probably not indicating the true changes in the activity of the renal kallikrein–kinin system within the kidney. Kinin excretion in urine was not correlated with kallikrein excretion but with changes in diuresis. Thus it might be suggested that the renal kallikrein–kinin system could be involved by kinins in the regulation of renal water and sodium excretion as well as of renal plasma flow.

Key words: diuretics, frusemide, kallikrein–kinin system, kininogen, kinins, natriuresis, renal plasma flow.

Abbreviations: BK, bradykinin; PAH, p-aminohippurate.

Introduction

It has been suggested that the renal kallikrein–kinin system might be involved in the regulation of the excretion of sodium and water, since kinins cause a natriuretic and diuretic effect when injected into the renal artery. Furthermore, the natriuretic response to saline loading was reduced by antibodies against bradykinin and by the kallikrein inhibitor aprotinin [1, 2].

Frusemide induces natriuresis and diuresis and increases renal blood flow. These effects are associated with an enhanced excretion of kallikrein, and have been described in chronic experiments in rats with free access to salt and water [3], in acute experiments in anaesthetized rats [4] and in the isolated perfused rat kidney [5]. In man, frusemide caused a short-lasting increase in urinary kallikrein excretion [6, 7], which was not always correlated with the increased urine volume [8]. In other studies in rats (G. Bönner, D. Ganten & F. Gross, unpublished work) and in man [9, 10] no significant change in urinary kallikrein excretion was observed during frusemide-induced diuresis. In view of these discrepancies we investigated the acute effects of this diuretic on kallikrein excretion as well as on the concentration of renal cortical kallikrein and kininogen.
Materials and methods

Male Wistar rats weighing 190–210 g were fed ad libitum on a standard chow (ssniff), containing 100 mmol of Na+/kg and 200 mmol of K+/kg, and were placed in individual cages for at least 3 days before the experiment. The rats were then anaesthetized with pentobarbital sodium (Nembutal, 30 mg/kg intraperitoneally), and polyethylene catheters (PP 50) were introduced through the femoral vessels into the inferior vena cava and the aorta. The bladder was also cannulated, and the urethra was ligated. During the experiment, 37.5 μl of 0.9% NaCl solution/min was infused intravenously. Every 15 min, mean arterial blood pressure was recorded (Statham P23Db, Gold-Brush 220). After an equilibration period of 30 min, urine was collected for periods of 15 min each for a total of 150 min. After two control periods (60 min after surgery), frusemide (Lasix), dissolved in 1 ml of 0.9% NaCl solution (saline), was injected subcutaneously at 0.5 (n = 5), 5.0 (n = 6), and 50.0 (n = 6) mg/kg. Control rats (n = 6) received an equal volume of the solvent.

In an additional experiment, 0.5 ml of saline was injected intravenously into six rats given frusemide (5 mg/kg subcutaneously), each time they excreted this volume of urine. To study the initial effects of frusemide on kallikrein excretion, the urine of two rats was collected for periods of 3 min each during 30 min.

The influence of frusemide on the kallikrein activity of the renal cortex was investigated in five groups of six rats each. Nephrectomy was performed under pentobarbital anaesthesia 5, 30, 120, and 180 min after the injection of frusemide (5 mg/kg). The rats of the control group were nephrectomized 30 min after the injection of 1 ml of saline. The kidneys were perfused with saline (65 ml/min) through the thoracic aorta.

Urinary kininogenase activity was measured as described previously [11, 12] and expressed as μg of bradykinin released min⁻¹ of incubation ml⁻¹ of urine (μg min⁻¹ ml⁻¹). Kallikrein excretion was calculated for each period as (urine volume x kallikrein activity). Kinin concentration in the urine was determined by a radioimmunoassay for bradykinin after ethanol extraction, as reported previously [13]. To minimize kinin formation and inactivation, urine was collected on ice and frozen immediately at the end of the collection period. When stored at −20°C, the urinary kinin content remained unchanged for at least 60 days.

The kallikrein activity of the renal cortex was measured with the chromogenic tripeptide substrate D-Val-Leu-Arg-p-nitroanilide (Kabi GmbH, Munich, Germany), as described previously [14], and expressed as milliunits/mg of protein. Kininogen in the renal cortical tissue was measured by means of a bradykinin radioimmunoassay as described previously [13].

p-Aminohippurate clearance was used as a measure of renal plasma flow [15]. Urinary sodium and potassium were determined with an

![FIG. 1. Effect of frusemide on urine volume (UV) and urinary excretion of sodium (U_{Na}V), potassium (U_{K}V) and kallikrein (U_{kall})V). Three doses of frusemide were injected subcutaneously into three groups of rats: 0.5 mg/kg (O---O), 5.0 mg/kg (▲—▲) and 50.0 mg/kg (x—x). Control rats received an equal volume of saline (●—●). Values are x ± SEM; significance: *P < 0.05; **P < 0.005; ***P < 0.001. BK, Bradykinin.](image-url)
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internal-standard flame photometer (Klina, Beckman).

Protein concentration of the renal cortex was determined by the method of Lowry et al. [16], with bovine serum albumin used as standard.

All values are given as means ± SEM. The significance of differences of the means was estimated by Student’s t-test for paired and unpaired samples. The significance of correlations was tested against zero.

Results

Frusemide caused a dose-dependent increase in urine volume and in sodium and potassium excretion. Initially, kallikrein excretion rose in a dose-dependent manner, reaching a peak 15 min after frusemide administration, and subsequently decreased rapidly (Fig. 1). Only the initially enhanced excretion of kallikrein correlated with the urine flow (r = 0.56, n = 28, P < 0.01).

However, in all experiments, the peak of urinary kallikrein excretion preceded that of urine flow (Fig. 1). When urine was collected for periods of 3 min, kallikrein excretion was found to be elevated only in the second period (3–6 min) after frusemide injection, when urine flow had slightly increased (Fig. 2). The initial rise in total kallikrein excretion was not due to an increase in urinary kallikrein activity. It rather seems to be the consequence of the initial rise in urine volume by frusemide, since urinary kallikrein activity was found to be reduced at this time.

Renal plasma flow was elevated during the first 30 min of diuresis and diminished by the end of the experiment (Table 1). Mean arterial blood pressure remained unchanged in all experiments.

The diuretic, natriuretic and kaliuretic effects of frusemide were more pronounced in rats in which fluid and sodium loss was replaced (Fig. 3). In this experiment, the secondary reduction of renal plasma flow was prevented, but kallikrein had a biphasic course similar to that observed without fluid replacement.

Potassium excretion was the only variable found to correlate with kallikrein excretion in all experimental groups. In the experiments in which various doses of frusemide were given, renal plasma flow correlated with urinary kallikrein excretion, but after substitution of the excreted volume, this correlation was no longer demonstrable (Table 2).

Kinin excretion rose after frusemide (5 mg/kg) and remained elevated until the end of the experiment (Fig. 4). It correlated with urine flow (r = 0.92, n = 17, P < 0.001), but not with the excretion of kallikrein.

At 30 min after the injection of frusemide (5 mg/kg) kidney weight was elevated (P < 0.01)

![Graph](image)

**Fig. 2.** Urinary excretion of kallikrein ($U_{kal}$, unbroken line) and of water ($U_{W}$, broken line) in two rats after subcutaneous injection of 5 mg of frusemide/kg. Urine was collected at intervals of 3 min each over a 30 min period.

### Table 1. Course of renal plasma flow (ml/min) after subcutaneous injection of frusemide (0.5, 5.0, and 50.0 mg/kg) in male Wistar rats

Significances were calculated against the control group; values are given as $\bar{x} \pm$ SEM. N.S., Not significant.

<table>
<thead>
<tr>
<th>Time (min) after injection...</th>
<th>Renal plasma flow (ml/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group</td>
<td>0</td>
</tr>
<tr>
<td>Control (n = 5)</td>
<td>6.17 ± 0.65</td>
</tr>
<tr>
<td>0.5 mg/kg (n = 6)</td>
<td>5.65 ± 0.47</td>
</tr>
<tr>
<td>5.0 mg/kg (n = 6)</td>
<td>6.06 ± 0.39</td>
</tr>
<tr>
<td>50.0 mg/kg (n = 6)</td>
<td>6.42 ± 0.22</td>
</tr>
</tbody>
</table>
Discussion

Furosemide induced an initial steep increase in kallikrein excretion, followed by a secondary decrease. The diuresis and natriuresis were dose-dependent, but not related to the changes in kallikrein excretion. The diuretic peak was reached when the kallikrein excretion had returned to control levels. A similar effect of furosemide on kallikrein excretion was found by Cinotti et al. [8] in man. The measurable changes in urinary kallikrein excretion induced by furosemide are probably only the consequence of 'wash out' of kallikrein from the tubules as a result of increased urine flow. A similar effect could be expected by an enhanced lymph flow in the kidney, reducing the kallikrein content in the renal interstitial space [17]. Such a wash-out mechanism by urine and lymph would also explain the decrease in kallikrein content of the renal cortex observed during furosemide-induced diuresis. The restoration of the renal kallikrein content found 3 h after the injection of furosemide could simply be the consequence of the normalization of the urine and lymph flow. How-

and kallikrein activity in the renal cortex was diminished ($P < 0.001$) (Fig. 5). Subsequently, renal kallikrein activity returned to control values. An inverse correlation was found between cortical kallikrein activity and both urine flow and kidney weight ($r = -0.95, P < 0.05; r = -0.56, P < 0.001$) respectively.

After the injection of furosemide, the kininogen content of the renal cortex remained unchanged (Fig. 5). However, in an additional experiment, in which blood was not washed out from the renal cortex, the kininogen concentration fell by $28 \pm 3\%$ ($n = 6, P < 0.001$).
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**TABLE 2. Correlations (r) between renal plasma flow (RPF), urine volume (UV), sodium excretion ($U_{Na}V$), potassium excretion ($U_{K}V$) and urinary kallikrein excretion ($U_{Kal}V$)**

The significances of correlation (s) and the number of observation pairs (n) are also given. N.S., Not significant.

<table>
<thead>
<tr>
<th>Correlation (x/y)</th>
<th>Group...</th>
<th>Control</th>
<th>Frusemide injection</th>
<th>Frusemide injection with volume replacement</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>0-5 mg/kg</td>
<td>5-0 mg/kg</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>50 mg/kg</td>
<td>50-0 mg/kg</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>0-0 mg/kg</td>
<td>5-0 mg/kg with volume replacement</td>
</tr>
<tr>
<td>$UV/U_{Kal}V$</td>
<td>$r$</td>
<td>0-45</td>
<td>0-12</td>
<td>0-05</td>
</tr>
<tr>
<td></td>
<td>$n$</td>
<td>60</td>
<td>50</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td>$s$</td>
<td></td>
<td>P &lt; 0-001</td>
<td>N.S.</td>
</tr>
<tr>
<td>$U_{Na}V/U_{Kal}V$</td>
<td>$r$</td>
<td>0-28</td>
<td>0-03</td>
<td>0-04</td>
</tr>
<tr>
<td></td>
<td>$n$</td>
<td>60</td>
<td>50</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td>$s$</td>
<td></td>
<td>P &lt; 0-05</td>
<td>N.S.</td>
</tr>
<tr>
<td>$RPF/U_{Kal}V$</td>
<td>$r$</td>
<td>0-85</td>
<td>0-94</td>
<td>0-98</td>
</tr>
<tr>
<td></td>
<td>$n$</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>$s$</td>
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<td>P &lt; 0-05</td>
<td>P &lt; 0-01</td>
</tr>
<tr>
<td>$U_{K}V/U_{Kal}V$</td>
<td>$r$</td>
<td>0-44</td>
<td>0-41</td>
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<td></td>
<td>$n$</td>
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<td>50</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td>$s$</td>
<td></td>
<td>P &lt; 0-001</td>
<td>P &lt; 0-01</td>
</tr>
</tbody>
</table>

**FIG. 5.** Kidney weight, kallikrein activity ($R_{Kal}$) and kininogen content of perfused renal cortex ($R_{Kininogen}$) after subcutaneous injection of frusemide (5-0 mg/kg) in male rats. Values are $\bar{x}$ ± SEM; significance: ***$P < 0-01$; **$P < 0-001$. Control; ■, frusemide.

However, it also could be caused by an enhanced secretion rate of aldosterone [18]. The hypothesis that renal kallikrein is washed out by a forced diuresis is further supported by the observation that a similar biphasic response of kallikrein occurred during osmotic diuresis induced by mannitol [14], during increased diuresis by triamterene [19] and also during by pressure change-enhanced diuresis in the isolated perfused rat kidney [19]. Similar changes have been observed in the secretion of kallikrein into saliva and by studies of the content of the enzyme in the submandibular gland of the rat after cervical nerve stimulation or the infusion of noradrenaline [20, 21].

In contrast to the biphasic response to frusemide of kallikrein excretion, kinin excretion remained elevated during the whole experiment. The course of kinin urinary excretion was correlated with sodium excretion and urine volume. The latter observation has also been reported for other forms of diuresis [22]. In the experiments without volume substitution, urinary kinin excretion was not correlated with renal plasma flow, since renal plasma flow was reduced secondary to volume depletion. However, after volume replacement, changes in renal plasma flow by frusemide showed a parallel course with kinin excretion in urine. Similar observations were obtained after injection of dihydralazine [13] and after application of triamterene [23]. Parallel to urinary kinin excretion an increase in
renal kinin content was observed during dihydralazine-induced increment in renal plasma flow [13]. These changes in kinins, too, were unrelated to renal kallikrein.

It is unlikely that kinins originated in plasma, since in recent experimental investigations even pharmacological doses of bradykinin infused intra-arterially into the kidney were not able to increase urinary kinin excretion [24]. All filtered kinins were inactivated immediately by kininase II in the renal tubular system [25, 26] and kininase II activity could not be exhausted by even unphysiological doses of bradykinin. Furthermore, enhanced kinin excretion is not caused by changed activity of intrarenal kininase of the renal tubular cells, but also is found on the kininogen in the kidney rather than in the urine. This hypothesis is supported by the findings that the kinin content in the kidney increased at the same time as urinary kinins increased [13] and the kininogen content of the unperfused kidney with intact kininogen from plasma and interstitial fluid decreased markedly. The release of kinins might play an important role in the pharmacological action of frusemide, since they could antagonize locally the frusemide-enhanced activity of the renin–angiotensin system, producing unchanged or even increased renal blood flow.

It has been suggested that extracellular fluid volume and the activity of the renal kallikrein–kinin system are correlated [28–30]. However, this is probably not the case, since the kallikrein response to frusemide is similar, whether or not greater depletion of the extracellular fluid volume is prevented by infusion of saline. Volume replacement reverses the correlation between renal plasma flow and kallikrein excretion seen after all three doses of frusemide. Hence, changes in volume may be of significance for maintaining this casual correlation.

We found no correlation between kallikrein excretion and urine volume or sodium excretion. However, we regularly observed a correlation between the excretion of kallikrein and that of potassium, thus confirming previous observations [28, 31–33].

In conclusion, after injection of frusemide, a dissociation between the excretion of kinins and kallikrein appears. The initial rise in kallikrein excretion is possibly due to a ‘wash out’ of intrarenally stored kallikrein by the markedly enhanced urine flow. Hence, measurement of kallikrein in urine might not be representative of the activity of the renal kallikrein–kinin system. It is more probable that after acute stimulation kallikrein left the kidneys in lymph or blood rather than in urine [5, 17]. Kinin excretion seemed to be a better measure of the role of the renal kallikrein–kinin system. The kinins are well correlated with renal plasma flow and it is possible that they play an important role in the regulation of renal blood flow, probably by antagonizing the renal renin–angiotensin system.

Acknowledgments

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References


