Effect of Renal Nerve Stimulation on Urine and Tissue Kininogenase Activity in Cats

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SUMMARY The effect of electrical stimulation of efferent renal nerves on urine and renal tissue kininogenase activity was studied in cats. Handling renal nerves before crushing produced a significant increase in mean blood pressure (BP) in intact animals. After crushing, stimulation of efferent fibers (15 V, 0.5 msec, and 25 Hz) by 15-second train duration and at 2.45-minute intervals did not alter the average renal blood flow (RBF) or BP over 30 minutes. Glomerular filtration rate, water, and potassium excretion rates did not change significantly in either kidney. Sodium excretion in the ipsilateral kidney decreased significantly (p < 0.05) in both intact and adrenalectomized cats. In intact animals, the kininogenase activity of urine (UK) also decreased significantly in both kidneys during nerve stimulation. In adrenalectomized animals it decreased significantly (p < 0.05) only in the ipsilateral kidney, and UK of the ipsilateral kidney was significantly lower than in the contralateral (p < 0.05). In both groups of cats, UK returned to control values during the recovery period of 30 minutes after stimulation. Adrenergic blockade abolished the effect of nerves stimulation on sodium and UK. Renal tissue kininogenase activity (RK) per gram of wet tissue was significantly lower in adrenergically blocked animals. No differences were detected when comparing RK content of ipsilateral vs contralateral kidney. These results suggest that renal nerve stimulation may produce a decrease in UK through a release of adrenal and peripheral nerve ending catecholamines.

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KEY WORDS • electrical stimulation • kininogenase activity • renal blood flow • catecholamines • sympathetic nervous system • adrenal glands • renal tissue kininogenases

Materials and Methods

Cats of either sex, weighing 2.5 to 3.5 kg, were anesthetized with sodium pentobarbitone (Nembutal Abbott, 40 mg/kg i.p.). Extra doses were administered whenever necessary. Rectal temperature was maintained at 37° ± 0.5°C. Mean arterial pressure (MAP) was monitored from a catheter in the femoral artery connected to Statham P 23 AC pressure transducer and recorded, together with other hemodynamic variables, on a Model 7B Grass polygraph. Each kidney was exposed retroperitoneal through a dorsal flank incision and both ureters catheterized with a PE 10 catheter, 4 to 5 cm rostral to their bladder insertion.

All cats received mannitol (15 μmol/hr) in saline, infused with a pump at the rate of 12 ml/hr, to compensate for fluid loss and baseline urine output. Urine was collected separately at 30-minute periods; its volume was recorded, and sodium, potassium, creatinine, and kininogenases contents measured. For measuring exogenous creatinine clearance, creatinine was injected intravenously in a priming dose of 100 mg/kg and then diluted in the mannitol-saline solution, so that the infusion of 12 ml/hr would maintain a constant rate of 100 mg creatinine/hr. In all cases, a period of at least 1 hour of equilibration was allowed.
The instantaneous rate of renal blood flow (RBF) was measured in both renal arteries with a Statham electromagnetic flowmeter model SP2202, and SP probe (1.5–2 mm i.d.) integrated by a Grass 7P108 integrator, reset at 10-second intervals and recorded on a Grass 7B polygraph.

First Experiment

In the first series of 10 control animals at 30 minutes after surgery, urine samples were taken from both kidneys for three periods of 30 minutes each, while BP and RBF were continuously recorded. Five of them were maintained without any manipulation for the 90 minutes of the experiment. In the other five cats, urine was collected throughout 30 minutes, then the renal nerves on one side were isolated, positively identified by verifying a small decrease in blood flow in the ipsilateral kidney and an increase in BP by short electrical stimulation, and then crushed. The second period of urine collection of 30 minutes included renal nerve handling and crushing time, and was followed by a recovery period.

Second Experiment

In a second series of six animals, the renal nerves on one side were isolated and placed on a stainless steel bipolar sleeve electrode. The nerves were then crushed to allow selective stimulation of different fibers. Rectangular pulses were delivered to a Grass Isolation Unit from a S 4 Grass stimulator (15 V, 0.5 msec, 25 Hz). The stimulation period was 30 minutes, with a train duration of 15 seconds and 2.45-minute intervals. Urine was collected from each kidney throughout 30 minutes in each period as in the first series.

Third Experiment

In a third experimental series, six animals were given a bolus injection of phentolamine (0.5 mg/kg) and propranolol hydrochloride (1 mg/kg). They were then infused with a mixture of phentolamine (10 μg/min) and propranolol (1 μg/min). The response of BP to norepinephrine and isoproterenol was checked before and after blocking.

Fourth Experiment

The fourth series consisted of eight adrenalectomized animals. The measurements were begun about 3 hours after bilateral adrenalectomy.

General

At the end of each experiment, cats were bled and both kidneys were removed, weighed, and stored at −40°C. Urine and renal kallikrein were determined by their kininogenase activity. Kinins were measured by bioassay in cat jejunum. Mean values of kininogenase activity in the urine drawn in each period and in the renal tissue extracts were obtained by testing at least 10 samples per animal. Urinary sodium and potassium were measured with an Eppendorf flame photometer. Data were expressed as means ± sem. Values of BP and RBF were derived three times a minute from the integrator tracing, and submitted to a two-way analysis of variance. Control vs experimental periods were compared using the Student's paired t test, while contralateral and ipsilateral kidneys were contrasted by Student's t test.

Results

The control groups of animals did not show any changes in BP, RBF, glomerular filtration rate (GFR), water and sodium excretion rate in the periods tested. GFR ranged from 20% to 22% of total blood flow.

Kininogenase activity in the urine of both kidneys in animals only submitted to surgery, without manipulation of the renal nerves, did not undergo any change during the three periods analyzed. The group with unilateral renal denervation showed a tendency toward a decrease of kininogenase activity of the urine of the ipsilateral kidney during the period of renal nerve handling. The values returned close to the basal ones in the recovery period. These values, in urine of the ipsilateral kidney were 9.2 ± 0.7; 7.2 ± 0.9 (p < 0.1) and 8.7 ± 0.7 μg bradykinin (BK)/2 min incubation in the three periods mentioned; and 9.4 ± 0.6; 8.2 ± 0.4 and 8.9 ± 0.8 μg BK/2 min incubation in urine of the contralateral kidney.

Values of renal tissue kininogenase activity were similar in both groups and in both kidneys. In cats only submitted to surgery, renal tissue kininogenase was 50 ± 7.5 μg BK in the left kidney and 51 ± 8.0 in the right kidney.

Table 1 shows BP and RBF values of the experimental series. BP increased significantly (p < 0.05) during the unilateral renal nerve handling in the intact animals of the first series. After adrenergic blockade BP decreased significantly (p < 0.001) and remained at a constant level. Before α and β blockade with phentolamine and propranolol, the BP response (∆BP) to 1 μg norepinephrine was 41 ± 7 mm Hg and a response to 1 μg of isoproterenol was −38 ± 5 mm Hg. After injection of the α and β blockers, ∆BP response to 1 μg norepinephrine was 5 ± 2 mm Hg; and to 1 μg isoproterenol it was −2 ± 1.5 mm Hg. Similar low values of ∆BP were observed at the end of the experiment. In the group of bilateral adrenalectomized cats, BP values were significantly lower than in the intact experimental animals, and they remained constant throughout the experiment. RBF decreased during renal nerve handling and stimulation. However the changes observed were not statistically significant. Differences in RBF observed in table 1 between contralateral and ipsilateral kidneys were not statistically significant under any experimental situation. Table 2 summarizes the mean values of water and sodium excretion. Values for water excretion of each experimental period when compared with the previous period or of the ipsilateral kidney when com-
Table 1.  Mean Blood Pressure (MAP) and Renal Blood Flow (RBF) of the Kidney with Untouched Nerves (C = Contralateral) or Dissected Nerves Under Electrical Stimulation (I = ipsilateral)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Kidney</th>
<th>Control 1 (after surgery)</th>
<th>After α and β blockade</th>
<th>Control 2 (renal nerve handling)</th>
<th>Unilateral renal nerve stimulation</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>BP (mm Hg)</td>
<td>C</td>
<td>107.7 ± 4.9</td>
<td>114.2 ± 6.1*</td>
<td>108.7 ± 8.3</td>
<td>103.6 ± 7.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>I</td>
<td>20.0 ± 3.7</td>
<td>18.5 ± 3.2</td>
<td>19.9 ± 2.1</td>
<td>20.3 ± 4.7</td>
<td></td>
</tr>
<tr>
<td>RBF (ml/min)</td>
<td>C</td>
<td>19.2 ± 2.0</td>
<td>17.5 ± 2.5</td>
<td>17.2 ± 4.6</td>
<td>18.9 ± 1.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>I</td>
<td>21.0 ± 4.0</td>
<td>23.5 ± 4.0</td>
<td>20.7 ± 0.5</td>
<td>18.1 ± 0.6</td>
<td></td>
</tr>
</tbody>
</table>

*Significant at p < 0.05 between this and the preceding column.
†Significant at p < 0.001 between this and the preceding column.

pared with the contralateral kidney were not statistically significant.

Sodium excretion of the ipsilateral kidney during the stimulation period of both intact and bilaterally adrenalectomized cats was significantly lower than in the previous period. In the intact animals, sodium excretion of the ipsilateral kidney was also significantly lower than in the contralateral. Sodium excretion of the ipsilateral kidney returned to control levels during the recovery period of 30 minutes.

In the intact experimental cats of the second series, the initial GFR was 4.4 ± 0.4 ml/min in the contralateral and 4.0 ± 0.6 ml/min in the ipsilateral kidney. In the series of α and β blockade animals, it was 4.1 ± 0.5 and 4.3 ± 0.3 ml/min respectively. The group of bilaterally adrenalectomized cats showed a similar GFR: 3.9 ± 0.6 in contralateral and 4.2 ± 0.5 ml/min in ipsilateral kidney. The GFR values of the three series did not change throughout the experiment.

Renal nerve handling produced a decrease of kininogenase in the urine of both kidneys of intact and adrenalectomized animals. This decrease was only significant in the urine of the ipsilateral kidney (figs. 1A and 2B). A further and significant decrease in kininogenases was observed during renal nerves stimulation in urine of both kidneys of the intact animals (fig. 1A) and of the ipsilateral kidney of adrenalectomized cats (fig. 2B). The average kininogenases in the urine from ipsilateral and contralateral kidneys of adrenalectomized cats during renal nerves stimulation were 84.9 ± 4.1 and 83.6 ± 3.4, respectively. The GFR values of the three series did not change throughout the experiment.

Table 2.  Water and Sodium Excretion from the Kidney, with Intact Nerves (C) or Dissected Nerves after Electrical Stimulation (I)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Kidney</th>
<th>Control 1 (after surgery)</th>
<th>After α and β blockade</th>
<th>Control 2 (renal nerve handling)</th>
<th>Unilateral renal nerve stimulation</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>H₂O (ml/h)</td>
<td>C</td>
<td>3.0 ± 0.4</td>
<td>2.1 ± 0.4</td>
<td>2.6 ± 0.9</td>
<td>3.1 ± 1.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>I</td>
<td>2.0 ± 0.4</td>
<td>1.9 ± 0.5</td>
<td>3.3 ± 1.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Na⁺ (mEq/l)</td>
<td>C</td>
<td>337 ± 157</td>
<td>331 ± 152</td>
<td>140 ± 25**</td>
<td>479 − 174*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>I</td>
<td>327 ± 152</td>
<td>341 ± 47</td>
<td>342 ± 43</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Significant at p < 0.05 between this and the preceding column.
†Significant at p < 0.01 between C and I kidney.

All values are means ± SEM; numbers in parenthesis indicate number of cats.

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stimulation were significantly different \( p < 0.05 \). The effects of renal nerve handling and renal nerve stimulation were completely blocked by phentolamine plus propranolol (fig. 1B). Urinary kininogenase activity in adrenalectomized cats was about four times lower than in the other groups of animals (fig. 2).

Values of kininogenase activity of ipsilateral and contralateral kidneys were not statistically different. Both the ipsilateral and contralateral kidneys of cats which had received adrenergic blockade showed a significantly lower amount of kininogenase activity per gram of wet tissue when compared with the ipsilateral \( p < 0.01 \) and contralateral \( p < 0.02 \) kidney of the intact animals (fig. 3).

Discussion

Our results show that increase in efferent renal nerve activity by manipulation or electrical stimulation produces a significant decrease in kininogenase activity of the urine from the ipsilateral kidneys. During electrical stimulation, the drop of kininogenases in urine was associated with a decrease in sodium excretion in both intact and bilaterally adrenalectomized

![Figure 1](image1.png)

**Figure 1.** Mean and standard error of the mean (SEM) of kininogenase in urine of intact (A) and \( \alpha \) and \( \beta \) adrenergic blocked animals (B). The symbol \( c_1 \) corresponds to control period urine collection; \( c_2 \) to a period in which renal nerves were handled; \( e \) renal nerve stimulation period; \( r \) recovery period; and \( in \) after injection of adrenergic blockers. Open circles are values for ipsilateral kidneys; closed circles, for contralateral kidneys. Results of the statistical analysis between this and the preceding period are indicated by asterisks \((^* p < 0.05; (** p < 0.01; (***) p < 0.001)\). No differences were found between values of ipsilateral and contralateral kidneys.

![Figure 2](image2.png)

**Figure 2.** Entries represent individual values of kininogenases in urine obtained in eight bilaterally adrenalectomized cats and the horizontal bars represent the average values. Left Graph: Values of the contralateral kidneys. Right Graph: Values of the ipsilateral kidneys. All explanations are the same as those in figure 1.
animals. In cats with intact adrenal glands, kininogenases in urine also decreased in contralateral kidneys without changes in sodium excretion. Both effects, the decrease in sodium and kininogenase in urine, were completely abolished by simultaneous \( \alpha \) and \( \beta \) adrenergic blockade.

Data on interaction of renal adrenergic system and the kallikrein-kinin system are scarce, and they are related to exogenous catecholamine infusion. It has been described that maximum amounts of catecholamines released by the kidney into urine and venous effluent are obtained with high level renal nerve stimulation, which is the one we used in our experiments. It has also been reported that renin release increases with elevated sympathetic renal nerve activity.

The interpretation of the kallikrein response to arterial infusion of norepinephrine and to the nerve stimulation used in this study is complex owing to the many factors involved: renin-angiotensin, prosta-glandins, and catecholamines, either released from adrenal medulla or locally formed, which were not measured. The simultaneous decrease in sodium and kininogenase in ipsilateral kidneys during nerve stimulation is in accordance with Mills and Newport's study, and it may be related to catecholamines' effect on sodium reabsorption and the effect of sodium tubular loading on kallikrein excretion. The effect of renal nerve handling on urine kininogenases of the ipsilateral kidney and the effect of renal nerve stimulation on the contralateral kidney without changes in sodium excretion suggest a threshold response mechanism, which may be different for sodium and for kininogenases. A release of catecholamines from the adrenal medulla during renal nerve stimulation is probable, because of the close vicinity of adrenal glands and kidney. These catecholamines may reach the contralateral kidney in an amount that does not produce detectable hemodynamic changes and may be effective in inhibiting kininogenase formation without altering sodium reabsorption. Such threshold response mechanism, or the use of different species, may explain Mills and Obija's results with norepinephrine infusion at low doses in dog kidneys, which evoked an increase in sodium excretion, followed by an elevated kallikrein excretion during the recovery period. The possibility that surgery including nerve handling and crushing may produce a decrease in kininogenase activity of urine, which may have extended into the renal nerve stimulation period, is ruled out. Animals submitted only to surgery did not show changes in kininogenases excretion for 90 minutes after the operation. A decreased kininogenase activity was found in the urine drawn from ipsilateral kidney in acute renal denervation; however, it was of short duration. In these animals kininogenases returned to basic values 30 minutes after.

In the adrenalectomized animals, the basic amount of kininogenases in urine was 4 times lower than in control animals. The low values of the urine kininogenase activity are consistent with the idea that adrenal corticoids have an important effect on urine kallikrein excretion. Finally, we have also shown that kininogenase activity of the renal tissue of \( \alpha \) and \( \beta \) blocked animals, in intact and stimulated kidneys, was significantly lower than in all the other groups. This might indicate that the mechanism of synthesis and storage of kininogenases by the kidneys might be under nerve control, but further experimental trials are required to clarify the problem.

References

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