The Role of Renal Kallikrein and Prostaglandin in the Control of Renin Release

Shin Suzuki, M.D., Masao Kobayashi, M.D., and Kunitake Hashiba, M.D.

SUMMARY

Renal prostaglandins and kallikrein are considered to play an important role in the control of renin release. Recently, we have shown that aprotinin, a kallikrein inhibitor, inhibits the stimulation of plasma renin activity (PRA) by either furosemide or a low sodium diet. However, the mechanisms of action of kallikrein are unknown. Since kallikrein may stimulate bradykinin and prostaglandin production, the present study examines the relationship of renal kallikrein and renal prostaglandins in the control of renin release. Furosemide and a low sodium diet stimulated PRA, urinary kallikrein excretion and urinary prostaglandin E₂ excretion. Aprotinin and indomethacin inhibited both furosemide and low sodium diet stimulation of PRA. When maximum doses of both aprotinin and indomethacin were given, PRA was more strongly suppressed than by indomethacin alone. The stimulation of urinary kallikrein excretion by furosemide and by a low sodium diet was not inhibited by indomethacin. These results suggest that both renal kallikrein and prostaglandins play an important role in the control of renin release under sodium depletion. Renal kallikrein may also have a direct action on the kidney to release renin.

Additional Indexing Words:
Plasma renin activity Aprotinin Indomethacin Urinary kallikrein excretion Urinary prostaglandin E₂ excretion

RECENT reports suggest that renal prostaglandins play an important role in the control of renin release. Prostaglandin (PG) E₂, PGI₂ and PGD₂ stimulate renin release in vitro¹⁾⁻⁴⁾ and in vivo.⁵⁾⁻⁷⁾ Indomethacin, an inhibitor of prostaglandin synthesis, can inhibit the renin release evoked by isoproterenol,⁸⁾⁻⁹⁾ hydralazine,¹⁰⁾ insulin-induced hypoglycemia,¹¹⁾ furosemide,
a low sodium diet, a reduced renal perfusion pressure or aortic constriction. Furthermore, renal kallikrein has been proposed as an intrarenal activator of inactive renin. Our previous reports suggested that renal kallikrein stimulates active renin release from renal cortical slices and that aprotinin, an inhibitor of kallikrein, inhibits stimulation of plasma renin activity (PRA) by furosemide and by a low sodium diet. However, the mechanisms of this kallikrein action are still not clear. It is possible that kallikrein can stimulate renin release via bradykinin and prostaglandin production. The present study was designed to clarify the roles of renal kallikrein and prostaglandins in the control of renin release.

**Materials and Methods**

Male Wistar rats (10 rats in each experiment) weighing 200–250 Gm were used for the experiments. Aprotinin (trasylol, Bayer, West Germany; 10,000 U/ml), and/or indomethacin (Sigma, St Louis, MI; 4 mg/ml) were administrated intraperitoneally (i.p.) at 9:00 and 10:00 a.m. in a volume of 1 ml/Kg. Control rats (no aprotinin, no indomethacin) were injected with the same volume of saline (1 ml/Kg, i.p.) at 9:00 and 10:00 a.m. Furosemide (Hoechst, Somerville, NJ) was given at 11:00 a.m. at a dose of 5 mg/Kg. A low sodium diet (Oriental, Tokyo, Japan) containing 0.02% sodium was given ad libitum for 2 weeks before the experiments. A normal sodium diet (Oriental, Tokyo, Japan) containing 0.39% sodium was given ad libitum for the other experiments. Immediately after decapitation at 12:00 a.m., blood samples were collected from the neck vessels in tubes containing 5 mg of ethylenediaminetetraacetic acid disodium salt. The samples were immediately placed in an ice bath and centrifuged at 4°C. Urine samples were collected using metabolic cages from 9:00 to 12:00 a.m. PRA was measured by a modification of Haber’s method using a CEA-IRE-SORIN angiotensin I radioimmunoassay kit. Plasma samples (1 ml) were incubated with 10 µl of 8-hydroxyquinoline, 10 µl of dimercaprol and 100 µl of 4 M tris (hydroxymethyl) aminomethane chloride buffer (pH 7.2). After incubation for 3 hours at 37°C, the samples were transferred to an ice bath and 50 µl aliquots were taken for radioimmunoassay of generated angiotensin I. Urinary sodium excretion was determined from 24-hour urine collections from rats fed for 2 weeks either with the normal sodium diet or with the low sodium diet. Urinary kallikrein excretion was measured by the esterase method of Moriya et al. Urinary prostaglandin E, excretion was measured by radioimmunoassay. Statistical analyses were performed using an unpaired Student’s t-test, and differences of the mean values with p<0.05
were considered to be significant.

RESULTS

Furosemide and a low sodium diet both elicited increases in PRA (Fig. 1) and urinary kallikrein excretion (Fig. 2). Furosemide stimulated PRA from $7.07 \pm 0.29$ to $21.86 \pm 2.24$ ng/ml/hr ($p < 0.005$), and the low sodium diet also stimulated PRA to $18.75 \pm 0.64$ ng/ml/hr ($p < 0.005$). Similarly, furosemide stimulated urinary kallikrein excretion from $0.170 \pm 0.016$ to $0.443 \pm 0.030$ E.U./hr ($p < 0.005$) and the low sodium diet stimulated urinary kallikrein excretion to $0.420 \pm 0.027$ E.U./hr ($p < 0.005$). There was no difference between the furosemide and low sodium diet effects on either PRA or kallikrein.

The effects of indomethacin and aprotinin on PRA stimulation are shown in Fig. 3. Indomethacin, at doses from 1 mg/Kg to 4 mg/Kg, suppressed the increase of PRA elucidated by furosemide. Aprotinin at doses from 2,500 to

![Fig. 1. Effects of furosemide treatment and low sodium diet on PRA. Data represent the mean ± SE of 10 experiments. Furosemide and low sodium diet stimulated PRA significantly.](image-url)
Fig. 2. Effects of furosemide treatment and low sodium diet on urinary kallikrein excretion. Furosemide and low sodium diet stimulated urinary kallikrein excretion significantly.

Fig. 3. Effects of indomethacin and aprotinin on PRA stimulated by furosemide treatment and a low sodium diet.

10,000 U/Kg, had the same effect, and the effects of 5,000 U/Kg and 10,000 U/Kg of aprotinin were identical.

Fig. 4 shows the effects of both separate and combined administration of aprotinin and indomethacin on the furosemide-induced increase in PRA.
Fig. 4. Effects of aprotinin and indomethacin on PRA stimulated by furosemide treatment. Aprotinin and indomethacin significantly inhibited PRA stimulated by furosemide. When aprotinin was used with indomethacin it suppressed PRA further.

Fig. 5. Effects of aprotinin and indomethacin on PRA stimulated by a low sodium diet. Aprotinin and indomethacin significantly inhibited PRA stimulated by a low sodium diet. The effects were additive.
Aprotinin inhibited the furosemide effect on PRA from 21.86±2.24 to 15.22±1.30 ng/ml/hr (p<0.01). Indomethacin also inhibited PRA stimulation by furosemide to 13.86±0.57 ng/ml/hr (p<0.01). When aprotinin (10,000 U/Kg, the maximum dose as shown in Fig. 3) was used in combination with indomethacin (4 mg/Kg, the maximum dose as shown in Fig. 3) a summation effect was observed (p<0.01). The same effects were observed with a low sodium diet (Fig. 5). Slightly different results were observed for urinary kallikrein excretion (Figs. 6 and 7). Aprotinin inhibited furosemide-induced urinary kallikrein excretion from 0.443±0.030 to 0.175±0.015 E.U./hr (p<0.005). However, indomethacin was ineffective even at a dose of 4 mg/Kg (Fig. 5). Aprotinin inhibited stimulation of PRA by the low sodium diet from 18.75±0.64 to 9.64±0.41 ng/ml/hr (p<0.005). The same effect was observed for indomethacin (9.85±0.53 ng/ml/hr, p<0.005). When aprotinin (10,000 U/Kg) was used in combination with indomethacin (4 mg/Kg) the effects summated (PRA: 6.79±0.27 ng/ml/hr, p<0.01).

Fig. 6. Effects of aprotinin and indomethacin on urinary kallikrein excretion stimulated by furosemide treatment. Aprotinin but not indomethacin inhibited urinary kallikrein excretion stimulated by furosemide.
Fig. 7. Effects of aprotinin and indomethacin on urinary kallikrein excretion stimulated by a low sodium diet. Aprotinin, but not indomethacin, inhibited the urinary kallikrein excretion stimulated by a low sodium diet. This same pattern of responses was observed for the low sodium diet group. Aprotinin inhibited urinary kallikrein excretion stimulation by a low sodium diet from $0.420 \pm 0.027$ to $0.146 \pm 0.014$ E.U./hr ($p < 0.005$). Indomethacin was ineffective.

Fig. 8 shows the effects of indomethacin on the urinary PGE$_2$ excretion stimulated by furosemide and by a low sodium diet. With a normal sodium diet, furosemide stimulated urinary PGE$_2$ excretion from $2.37 \pm 0.24$ to $4.08 \pm 0.17$ ng/hr ($p < 0.005$). Indomethacin inhibited this effect. Indomethacin also inhibited urinary PGE$_2$ excretion stimulated by a low sodium diet from $4.17 \pm 0.27$ to $2.74 \pm 0.07$ ng/hr ($p < 0.005$).

These data, then, suggest that the effects of indomethacin are specific to PRA and PGE$_2$ excretion increments. It does not affect kallikrein excretion.
Both the renin-angiotensin-aldosterone system and the kallikrein-kinin-prostaglandin system are inter-related and play important roles in the control of blood pressure, sodium-water balance, and renal hemodynamics. Aldosterone stimulates renal kallikrein. It has been reported that renal kallikrein and prostaglandin production are stimulated by furosemide and by a low sodium diet. It has also been reported that renin release is stimulated by sodium depletion via prostaglandin production. However, it is not clear if the action of kallikrein is via prostaglandin production. It was previously reported that rat urinary kallikrein stimulates renin release from rat renal cortical slices, that this action is not blocked by indomethacin, and that bradykinin does not stimulate renin release. These data suggest that kallikrein acts directly on the kidney to release renin. Furthermore, we have recently observed that aprotinin inhibits the increases PRA elicited by furosemide and by a low sodium diet. This result agrees with the findings of Shimoda et al who showed that furosemide-induced renin release is suppressed by aprotinin.
The results of this study clearly show that both renal kallikrein and prostaglandins play an important role in the control of the renin release elicited by furosemide and by a low sodium diet. Aprotinin can inhibit both plasma kallikrein and renal kallikrein. However, since soy-bean trypsin inhibitor, which inhibits plasma kallikrein, trypsin and chymotrypsin but not renal kallikrein, does not affect PRA in sodium depleted conditions, it is clear that plasma kallikrein, trypsin and chymotrypsin do not play an important role in the control of renin release in sodium depleted rats.

Rat urinary kallikrein excretion was measured in the present study by the esterase method which was established by Moriya et al. They reported that their method was 100 times more sensitive than the hydrozamate-ferric complex method and 10 times more sensitive than chromotroplc acid method. They also showed that the data which was obtained by their method are in agreement with bioassay data. They concluded that their method is useful for the measurement of small amounts of kallikrein in physiological or pathological investigations. It has also been reported that rat urinary kallikrein measured by esterolytic activity is correlated with radioimmunoassay results. The possibility that esterase A plays an important role in the control of renin release can be excluded, since soy-bean trypsin inhibitor inhibits esterase A and does not affect the renin release stimulated by furosemide and by a low sodium diet.

Urinary kallikrein excretion and urinary prostaglandin E2 excretion were increased by furosemide treatment and by a low sodium diet. Since aprotinin inhibited this increment; furosemide and a low sodium diet probably stimulate renin release via kallikrein and prostaglandin production.

However, the lack of efficacy of indomethacin in blocking kallikrein excretion effects suggests that kallikrein and prostaglandin mechanisms are independent. This is supported by the finding that effects of aprotinin and indomethacin against furosemide- and low sodium diet-induced PRA increments were additive. Thus, kallikrein actions are not mediated solely through increased prostaglandin production. Although the mechanisms of action of kallikrein are not clear, kallikrein may activate inactive renin in the kidney, since it is a proteolytic enzyme and can activate inactive renin in plasma.

References


