The Kallikrein-Kinin System and Prostaglandins in the Kidney
Their Relation to Furosemide-Induced Diuresis and to the Renin-Angiotensin-Aldosterone System in Man

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SUMMARY The relations among the renin-angiotensin-aldosterone system, renal prostaglandin E, the renal kallikrein-kinin system, and furosemide diuresis were studied in 16 healthy volunteers. The diuretic and natriuretic effects of furosemide were accompanied by an increase in the excretion rates of urinary prostaglandin E (UPCEV), urinary kallikrein (UkaiiV), and urinary kinin (UkininV), and in plasma renin activity (PRA) and plasma aldosterone concentration (PAC). However, the time courses of the increase in PRA and PAC following furosemide administration and assumption of an upright posture were different from those of UPCEV, UkaiiV, UkininV, urine flow (UV), and urinary sodium output (UNV). In comparison with the early increase in UPCEV, UkaiiV, UkininV, UV, and UNV, the increases in PRA and PAC were delayed. The augmentation of UPCEV, UkaiiV, and UkininV was closely related to the diuretic and natriuretic effects of furosemide. Highly significant correlations also were found between UPCEV and UkaiiV, UPCEV and UkininV, and UkaiiV and UkininV. On the contrary, there were no significant correlations between UPCEV and PRA or PAC, between UkaiiV and PRA or PAC, or between UkininV and PRA or PAC before and after the furosemide injection. These results indicate that the augmentation of urinary prostaglandin E and urinary kallikrein-kinin system following furosemide administration is independent of the renin-angiotensin-aldosterone system but directly dependent on the effects of furosemide. The possibility that renal prostaglandin E and the renal kallikrein-kinin system are involved in the diuretic and natriuretic effects of furosemide also is suggested by these experiments.

Furosemide has been shown to increase plasma renin activity (PRA) and plasma aldosterone concentration (PAC). In our recent study in man, the urinary excretion of prostaglandin E (PGE) was increased significantly after the intravenous injection of furosemide.1 Another renal vasodepressor substance, kallikrein, also was found to increase following furosemide administration in rats and in man.2 Margolius and his co-workers3-4 have suggested that urinary kallikrein excretion is regulated by aldosterone or other sodium-retaining steroid hormones. It also has been reported by McGiff and co-workers6 that there is a close interrelationship between the renal kallikrein-kinin system and renal prostaglandins. Thus possibilities exist that the augmentation of urinary PGE and the urinary kallikrein-kinin system following furosemide administration is dependent on the renin-angiotensin-aldosterone system. To the contrary, several laboratories have found that an inhibitor of prostaglandin synthetase, indomethacin, suppresses the natriuresis and increase in PRA and PAC produced by the administration of furosemide.7-9 These results suggest the involvement of renal prostaglandins in the mechanism of diuresis and stimulation of renin and aldosterone secretion by furosemide. To investigate this hypothesis, urinary excretion of immunoreactive PGE, the main urinary metabolite of prostaglandin E2 (PGE2-MUM), urinary kallikrein and kinin, PRA and PAC were measured in normal volunteers before and after furosemide administration. In addition, we also determined whether the augmentation of urinary kallikrein-kinin system and urinary PGE following furosemide administration is dependent on the renin-angiotensin-aldosterone system.

Methods

Studies were carried out in 16 healthy volunteers (12 men and four women) ranging in age from 19 to 47 with an average of 27.7 ± 2.3 (mean ± se) years. Physical examination and routine laboratory tests were normal. The subjects were allowed to take unrestricted diets ad libitum. The study was started at 8 a.m., and the subjects, fasted overnight, were kept supine in bed for at least 1 hour. After sampling peripheral venous blood and obtaining urine during the control period for measurement of plasma electrolytes, PRA, PAC, urinary electrolytes, urinary PGE, urinary PGE2-MUM, urinary kallikrein and urinary kinin, furosemide (1 mg/kg) was injected into the antecubital vein and the subjects were asked to assume...
an upright posture for 120 minutes. At 30 and 120 minutes after furosemide administration, blood and urine samples were taken. Blood pressure and pulse rate were checked before each sampling. Institutional rules for the protection of human subjects were followed in this study.

Urinary Kinin

Urinary kinin was measured by Carretero’s method. Collected urine was stored immediately at −20°C until the assay. The radioimmunoassay was performed in 0.1 m Tris-HCl buffer, pH 7.4, containing 0.2% of gelatin and 0.1% of neomycin (buffer A). The incubation system consisted of 125I-8-tyrosine-bradykinin, 3000 counts/min (specific radiological activity, 800–1000 mCi/μμ, Daiichi Radioisotope Corp.), urine, 0.01–0.02 ml, and 0.1 ml of antiserum (1:16,000) adjusted to a final volume of 0.8 ml with buffer A. The mixture was incubated for 24 hours at 4°C and free kinin was separated with dextran-coated charcoal. After counting radioactivity, kinin content was calculated. This method is sensitive to 10 pg of kallidin. The recovery rate of added kallidin (50–500 pg) was 97 ± 4% (mean ± se, n = 15). The metabolic fragments which are produced by incubating bradykinin, kallidin and methionyl-lysyl-bradykinin with chymotrypsin showed 0.5% cross-reaction with kinin antiserum. The values of urinary kinin determined by the present method in 32 subjects showed a highly significant correlation with values determined by a bioassay consisting of extraction and assay using the autoperfused dog femoral arterial blood flow (r = 0.71, P < 0.001).

Urinary Kallikrein

Urinary kallikrein activity was measured as kininogenase activity. The urine was incubated with low molecular weight bovine serum kininogen. The incubation system consisted of 0.05 ml of urine and 4 μg of kininogen dissolved in 0.4 ml of 0.1 m phosphate buffer, pH 8.4, containing 0.1% neomycin, 3 mM 8-hydroxy-quinoline, and 30 mM disodium ethylenediaminetetraacetic acid. The mixture was incubated at 37°C for 20 minutes. After the incubation, the mixture was diluted 5-fold with cold water, heated to 80°C for 15 minutes to stop the enzymatic reaction, and stored at −20°C until the radioimmunoassay. With the present method, the extraction procedure of kinin was not necessary, because bovine serum low molecular weight kininogen did not cross-react with the kinin antibody. The kinin present in the urine before incubation was measured as described above; urinary kallikrein activity was calculated by subtracting the preincubation kinin value from that obtained postincubation. In the present study, kallikrein activity was expressed as total kinin generated during an incubation of 20 minutes.

Urinary Prostaglandin E

Urinary prostaglandin E was measured radioimmunologically with a commercial kit (CA 501, Clinical Assay). A sample (5–10 ml) of urine was lyophilized. After the residue had been dissolved in 1 ml of 0.05 M phosphate buffer, pH 7.4, urinary PGE was converted to prostaglandin B (PGB) by alkaline treatment according to Zasman’s method. Then, the sample was acidified to pH 3 to 4 with hydrochloric acid and extracted with ethyl acetate. The organic phase was dried, the residue was applied to a silicic acid column, and PGB was eluted by a mixture of benzene-ethyl acetate (60:40) according to the method of Jaffe. The PGB fraction was dried and measured radioimmunologically using PGB antiserum which does not distinguish PGB1 from PGB2. The endogenous PGB also was measured by the same procedure without alkaline treatment. The urinary PGE values was calculated by subtracting the PGB value before alkaline treatment from that after alkaline treatment. The ratio of the endogenous PGB to PGE was 8–25%. Prior conversion of PGE to PGB precluded dehydration of PGE to PGA during the extraction procedure. The overall recovery rate of added PGE (1 to 3 ng) was 54.8 ± 0.7% (mean ± se, n = 15). The estimated value was corrected for this loss.

Main Urinary Metabolite of Prostaglandin F2α

Main urinary metabolite of prostaglandin F2α,17,18-dihydroxy-11-keto tetrostan-1,16-dioic acid was measured by Ohki’s method. Diluted urine (corresponding to 0.01 to 0.05 ml of original urine) was directly measured radioimmunologically using PGF2α-MUM antiserum and PGF2α urinary metabolite-125I-tyrosine methyl ester amide. This antiserum did not cross-react with the main PGE, urinary metabolites, 15-keto PGF2α, 15-keto PGE1, and 15-keto PGE2.

Plasma Aldosterone Concentration

PAC was measured with a commercial radioimmunoassay kit (Cer Ire Sorin). This method is sensitive to 10 pg of aldosterone. Serum and urinary Na+ and K+ were measured with an autoanalyzer. All results were expressed as mean ± SEM. The significance of differences between mean values were evaluated by Student’s t-test.

Results

Blood Pressure and Pulse Rate

Average systolic blood pressure in the subjects was 111 ± 2.2 mm Hg. The systolic blood pressure measured 30 minutes after the administration of furosemide (F30) was
lower in nine of the 16 subjects and elevated in five. In the remaining two, it was not changed. On the average, the blood pressure at F30 (108 ± 3.0 mm Hg) was not significantly changed. The systolic blood pressure measured 120 minutes after furosemide injection (F120) was lowered in nine subjects, elevated in one, and unchanged in the remaining six. The average value at F120 (103 ± 2.9 mm Hg) was significantly lower (P < 0.05) than the preinjection value. Diastolic blood pressure was increased from 69 ± 1.9 mm Hg to 74 ± 2.7 mm Hg at F30 and to 71 ± 2.4 mm Hg at F120, but these changes were not significant. As opposed to blood pressure, pulse rate rose gradually during the 120-minute period after the administration of furosemide. Average pulse rates increased from 69 ± 2 to 83 ± 3 at F30 (P < 0.005) and to 91 ± 4 at F120 (P < 0.001).

Serum Concentrations of Na⁺ and K⁺

No significant change was noted in serum Na⁺ concentration after the administration of furosemide. The average concentrations were 139.0 ± 0.4 mEq/liter in the control period, 139.0 ± 0.5 mEq/liter at F30, and 138.0 ± 0.6 mEq/liter at F120. On the other hand, the serum K⁺ concentration was significantly increased at F30 but not at F120. The values were 4.1 ± 0.1 mEq/liter during the control period, 4.4 ± 0.1 mEq/liter at F30 (P < 0.05), and 4.2 ± 0.1 mEq/liter at F120 (P > 0.05).

Urine Flow and Urinary Excretion of Na⁺ and K⁺

Urine flow was increased in all subjects after furosemide injection. The diuretic effect was greater in the first 30 minutes after the administration of furosemide than in the subsequent 90 minutes. The average flow rates were increased significantly from 1.0 ± 0.1 ml/min to 14.6 ± 1.1 ml/min in the former (P < 0.001) and to 6.6 ± 0.6 ml/min in the latter (P < 0.001) period. Increases in urinary excretion of Na⁺ and K⁺ also were found in all subjects after the furosemide injection. The peak of natriuresis and kaliuresis appeared in the first 30 minutes after the drug administration. The average excretion rate of Na⁺ was increased from 169 ± 16 μEq/min to 1928 ± 135 μEq/min in the first 30 minutes (P < 0.001) and 812 ± 93 μEq/min in the subsequent 90 minutes (P < 0.001) after furosemide administration. The excretion of urinary K⁺ was also increased from 39 ± 5 μEq/min to 136 ± 10 μEq/min (P < 0.001) and to 90 ± 8 μEq/min (P < 0.05).

Plasma Renin Activity and Plasma Aldosterone Concentration

The changes of PRA and PAC following furosemide administration and assumption of the upright posture are demonstrated in Figures 1 and 2. After the injection, a significant increase in PRA and PAC was found in all subjects. In five of the 16 subjects, the PRA value was maximum at F30, and subsequently it did not change. On the contrary, in the remaining 10 subjects, PRA values continued to increase for up to 120 minutes after furosemide injection, and a maximum value was found at F120. The average value of PRA increased significantly from 1.93 ± 0.47 ng/ml per hr to 5.76 ± 0.76 ng/ml per hr at F30 (P < 0.01) and to 7.57 ± 0.70 ng/ml per hr at F120 (P < 0.001). PAC values continued to increase up to 120 minutes after the administration of furosemide and a peak value was found at F120 in all subjects except two. The average value of PAC was significantly increased from 4.3 ± 0.5 ng/100 ml to 8.2 ± 1.1 ng/100 ml at F30.
Urinary Excretion of Prostaglandin E and a Prostaglandin F\(_2\alpha\)-Metabolite

The changes in urinary excretions of PGE and PGF\(_{2\alpha}\)-MUM following the furosemide injection and assumption of an upright posture are illustrated in Figures 2 and 3. Urinary PGE excretion was increased in 13 of 16 subjects in the first 30 minutes after the furosemide injection; subsequently it decreased and returned to the control level at 120 minutes. In the remaining three subjects, urinary excretion of PGE did not change in the first 30 minutes and then it decreased. The average excretion rates of PGE were 0.49 ± 0.07 ng/min in the control period, 1.30 ± 0.18 ng/min in the first 30 minutes (P < 0.05), and 0.52 ± 0.05 ng/min in the subsequent 90 minutes [not significant (NS)]. Urinary excretion of PGE and urine flow were highly correlated with each other before and during furosemide administration (r = 0.72, P < 0.001). Urinary excretion of PGE and urinary excretion of Na\(^+\) (r = 0.71, P < 0.001) also were correlated significantly. In contrast, there was no correlation between urinary PGE excretion and PRA values or PAC levels. Urinary excretion of PGF\(_{2\alpha}\)-metabolite was decreased

\(P < 0.01\) and to 15.9 ± 2.4 ng/100 ml at F120 (\(P < 0.001\)).

**Figure 2** Mean changes in plasma renin activity (PRA), plasma aldosterone concentration (PAC), and urinary excretion of prostaglandin E (U\(_{PGE}\)), prostaglandin F\(_{2\alpha}\)-main urinary metabolite (U\(_{PGF_{2\alpha}-MUM}\)), kallikrein (U\(_{Kal}\)) and kinin (U\(_{Kin}\)) (mean ± SEM). Upper panel shows PRA (○) and PAC (●) (n = 16). Middle panel shows U\(_{PGE}\) (○) and U\(_{PGF_{2\alpha}-MUM}\) (●) (n = 12). Lower panel shows U\(_{Kal}\) (○) and U\(_{Kin}\) (●) (n = 16). C = control period, F\(_{30}\) = 30 minutes after the furosemide injection and F\(_{120}\) = 120 minutes after furosemide injection. *Values significantly different from control values (\(P < 0.05\)); **\(P < 0.01\); ***\(P < 0.005\).

**Figure 3** Changes in urinary excretion of prostaglandin E (A) following furosemide injection and upright posture in 16 healthy subjects and of prostaglandin F\(_{2\alpha}\)-main urinary metabolite (PGF\(_{2\alpha}-MUM\)) (B) following furosemide injection and upright posture in 12 healthy subjects. C = control period, F\(_{30}\) = 30 minutes after the furosemide injection and F\(_{120}\) = 120 minutes after furosemide injection.
after the administration of furosemide. In eight of 12 subjects, the excretion rate of urinary PGF₂₀-MUM was decreased and in the remaining four it was increased or not changed during the first 30 minutes after furosemide injection. During the subsequent 90 minutes, however, the excretion rate of PGF₂₀-MUM was decreased in all subjects. The average excretion values were significantly decreased from 17.1 ± 2.9 ng/min to 10.7 ± 1.3 ng/min in the first 30 minutes (P < 0.05) and 8.2 ± 0.9 (P < 0.01) ng/min in the subsequent 90 minutes after the furosemide injection.

Urinary Excretion of Kallikrein and Kinin

The changes in urinary excretion of kallikrein and kinin following furosemide injection and upright posture are illustrated in Figures 2 and 4. Urinary kallikrein excretion was increased after furosemide injection. A maximum excretion rate was found in the first 30 minutes in all subjects, after which the excretion rate was decreased in all except three subjects in whom it remained unchanged. Average excretion rates of urinary kallikrein were significantly increased from 44.9 ± 6.7 ng/min to 143.0 ± 21.4 ng/min at 30 minutes (P < 0.01) and were 70.4 ± 15.3 ng/min at 120 minutes (NS) following the administration of furosemide. The changes of urinary kinin excretion were similar to those of kallikrein. Urinary kinin excretion was increased in 14 subjects after the furosemide injection and maximum excretion was noted in the first 30 minutes. Then the excretion rate decreased in 10 subjects and continued to increase in four. In the remaining two, urinary kinin output was decreased in the first 30 minutes and then it increased in one. The average excretion rate was increased from 10.5 ± 1.9 ng/min to 40.6 ± 8.6 ng/min at 30 minutes (P < 0.01) and to 23.0 ± 5.4 ng/min (P < 0.05) at 120 minutes. Urinary kallikrein excretion correlated with urinary kinin excretion in 48 samples from 16 subjects (r = 0.71, P < 0.001). Urinary excretion of prostaglandin E also correlated highly with the excretion of urinary kallikrein (r = 0.65, P < 0.001). There was also significant correlation between urinary kallikrein excretion and urine flow (r = 0.61, P < 0.001) or urinary Na⁺ excretion (r = 0.62, P < 0.001). However, urinary kallikrein excretion was not significantly correlated with PRA values or PAC levels.

A similar relationship was observed for the urinary excretion of kinin. There was a significant correlation between urinary excretion of kinin and urinary prostaglandin E excretion before and after the furosemide injection (r = 0.46, P < 0.005). Urinary kinin excretion correlated significantly with urine flow (r = 0.57, P < 0.001) and urinary sodium excretion (r = 0.44, P < 0.005). However, no correlation was found between urinary excretion of kinin and PRA values or PAC levels.

Discussion

Primary prostaglandins have a very short half-life in circulating blood, because PGE is removed almost completely during a single passage through the pulmonary circulation. Therefore, it is very difficult to evaluate the pathophysiological roles of renal prostaglandins by estimating plasma PGE concentrations. Recently, Frolich et al. reported that renal prostaglandins are excreted in urine and their excretion rates reflect the synthesis of renomedullary prostaglandins or their release. Therefore,
the urinary PGE excretion rate was measured as an indicator of renal PGE synthesis in the present experiment.

In the present study, the potent diuresis and natriuresis caused by furosemide were accompanied by a concomitant increase in urinary excretion of prostaglandin and kallikrein-kinin, PRA and PAC. According to McGiff and his co-workers, there is a coupling between the kallikrein-kinin system and PGE within the kidney. In our study, the time sequence of urinary PGE excretion following furosemide administration was parallel to those of urinary kallikrein and kinin, and a highly significant correlation between the two systems was found. These data support McGiff's hypothesis. The previous observations by Margolius and his co-workers indicated that aldosterone or other sodium-retaining steroid hormones regulate urinary excretion of kallikrein in humans and animals. These reports suggest that the augmentation of the renin-angiotensin-aldosterone system induced by furosemide can mediate an overproduction of renal kallikrein-kinin and PGE. In the present experiments, however, the time courses of changes in PRA and PAC after the furosemide administration were different from those of urinary output of kallikrein-kinin and PGE. In comparison with the early increase in the latter, there was a delay in the rise of the former. These results indicate that the augmentation of the urinary kallikrein-kinin system and PGE following furosemide injection is independent of the renin-angiotensin-aldosterone system.

The previous reports of the role of renal PGE in renal sodium handling have been conflicting. That an intrarenal infusion of PGE induces natriuresis indicates that renal PGE may be involved in renal sodium output. However, the observation by Tobian and his co-workers that renal PGE content in the rat decreased after sodium loading suggests that renal PGE may act as an antinatriuretic hormone. Recently, Stokes and Kokko reported that PGE2 increased urinary sodium excretion by an inhibition of net sodium transport in rabbit isolated collecting tubules. It also has been suggested that the renal kallikrein-kinin system acts as a natriuretic factor. In the present study, an augmentation of urinary kallikrein-kinin and PGE excretion following furosemide injection was closely related to the diuretic and natriuretic effects of furosemide. These results suggest that renal PGE and kallikrein-kinin might be involved in the natriuretic or diuretic effects of furosemide.

Recently, Weber and his co-workers reported that furosemide increased free arachidonic acid, a main precursor of prostaglandins, in man. In the present experiment, however, urinary output of PGF2α-MUM was decreased after furosemide injection. This result is contradictory to the concept of stimulation of prostaglandin synthesis by furosemide. Paulrud and Miller reported that furosemide inhibits 15-hydroxy prostaglandin dehydrogenase of human placenta. On the other hand, Stone and Hart found that furosemide suppressed the activity of renal prostaglandin-9-ketoreductase. Therefore, the augmentation of urinary PGE by furosemide may not only be induced by an overproduction of renal PGE, but also by an inhibition of 15-hydroxy prostaglandin dehydrogenase and/or of prostaglandin-9-ketoreductase. Weber and co-workers have reported that renin release can be stimulated by the prostaglandin precursor, arachidonic acid, or prostaglandin endoperoxides. Several investigators have also demonstrated that indomethacin pretreatment prevents the increase of renin secretion following furosemide injection or other stimuli. These reports suggest that there is an intrarenal mechanism of prostaglandin-mediated renin release. From the present experiment, it is not clear that the augmented PGE induced by furosemide mediates the hypersecretion of renin.

In conclusion, the augmentation of the urinary kallikrein-kinin system and PGE following furosemide administration is independent of the renin-angiotensin-aldosterone system. The present data that the increases in urinary kallikrein-kinin system and urinary PGE after the furosemide injection were highly correlated with the diuretic and natriuretic effects, suggest that furosemide facilitates the formation and the liberation of kallikrein-kinin and PGE in the kidney.

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