The Kallikrein-Kinin System and Prostaglandins in the Kidney

Their Relation to Furosemide-Induced Diuresis and to the Renin-Angiotensin-Aldosterone System in Man

KEISHI ABE, NOBUO IROKAWA, MINORU YASUJIMA, MASAHIDE SEINO, SATORU CHIBA, YUTAKA SAKURAI, KAORU YOSHINAGA, AND TETSUO SAITO

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 rats2 and in man.3 Margolius and his co-workers4-5 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 F2α (PGF2α-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 PGF2α-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/μM, 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 kinogenase 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 of human subjects were followed in this study.

Main Urinary Metabolite of Prostaglandin F₂α

Main urinary metabolite of prostaglandin F₂α,5α,7α-dihydroxy-11-keto tetroran-prostan-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.
KALLIKREIN, PGE, RAA, AND FUROSEMIDE DIURESIS/Abe et al.

257

Urinary Excretion of Prostaglandin E and a
Prostaglandin F\(_2\alpha\) Metabolite

The changes in urinary excretions of PGE and PGF\(_\alpha\)-
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 min-
etes [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

\[
\begin{align*}
P & < 0.01 \\
& \text{and to } 15.9 \pm 2.4 \text{ ng/100 ml at } F120 (P <
0.001).
\end{align*}
\]

\[
\begin{align*}
\text{Urinary Excretion of Prostaglandin E and a} \\
\text{Prostaglandin F\(_\alpha\) Metabolite}
\end{align*}
\]

The changes in urinary excretions of PGE and PGF\(_\alpha\)-
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 min-
etes [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

\[
\begin{align*}
P & < 0.01 \\
& \text{and to } 15.9 \pm 2.4 \text{ ng/100 ml at } F120 (P <
0.001).
\end{align*}
\]

\[
\begin{align*}
\text{Urinary Excretion of Prostaglandin E and a} \\
\text{Prostaglandin F\(_\alpha\) Metabolite}
\end{align*}
\]

The changes in urinary excretions of PGE and PGF\(_\alpha\)-
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 min-
etes [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

\[
\begin{align*}
P & < 0.01 \\
& \text{and to } 15.9 \pm 2.4 \text{ ng/100 ml at } F120 (P <
0.001).
\end{align*}
\]

\[
\begin{align*}
\text{Urinary Excretion of Prostaglandin E and a} \\
\text{Prostaglandin F\(_\alpha\) Metabolite}
\end{align*}
\]

The changes in urinary excretions of PGE and PGF\(_\alpha\)-
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 min-
etes [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

\[
\begin{align*}
P & < 0.01 \\
& \text{and to } 15.9 \pm 2.4 \text{ ng/100 ml at } F120 (P <
0.001).
\end{align*}
\]
after the administration of furosemide. In eight of 12 subjects, the excretion rate of urinary PGF$_{2\alpha}$-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 PGF$_{2\alpha}$-MUM was decreased in all subjects. The average excretion values were significantly decreased from $17.1 \pm 2.9$ ng/min to $10.7 \pm 1.3$ ng/min in the first 30 minutes ($P < 0.05$) and $8.2 \pm 0.9$ (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 $44.9 \pm 6.7$ ng/min to $143.0 \pm 21.4$ ng/min at 30 minutes ($P < 0.01$) and were $70.4 \pm 15.3$ 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 $10.5 \pm 1.9$ ng/min to $40.6 \pm 8.6$ ng/min at 30 minutes ($P < 0.01$) and to $23.0 \pm 5.4$ 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 kalli-

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 esti-

![Figure 4](http://circres.ahajournals.org/doi/figure/10.1161/01.RES.43.2.258)
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 in 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 reported 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 PGE, increased urinary sodium excretion by an inhibition of net sodium transport in rabbit isolated collecting tubules. It also has been suggested that the renal prostanoid PGE and kallikrein-kinin system and PGE excretion following furosemide injection is independent of the renin-angiotensin-aldosterone system. 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.

Acknowledgments
We thank Dr. Oscar A. Carreterro of Henry Ford Hospital, Detroit, for the supply of kinin antisera, Dr. H. Kato of Protein Research Institute, Osaka, for the supply of bovine serum low molecular weight kininogen, and Dr. F. Hirata of Oono Pharmaceutical Company, Osaka, for the supply of antisera of PGE2-MUM.

References

KalliKREIN, PGE, RAA, AND FUTUREMIDE DIURESISA46e

259
The kallikrein-kinin system and prostaglandins in the kidney: their relation to forosomide-induced diuresis and to the renin-angiotensin-aldosterone system in man.
K Abe, N Irokawa, M Yasujima, M Seino, S Chiba, Y Sakurai, K Yoshinaga and T Saito

doi: 10.1161/01.RES.43.2.254

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/43/2/254.citation