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Short communication

Involvement of the renal kallikrein–kinin system in K^+-induced diuresis and natriuresis in anesthetized rats

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Abstract

Intravenous infusion of a high-K^+ solution (67.5 mM KCl, 67.5 mM NaCl) to anesthetized rats increased urine volume by 47.6% after 60 min, compared with infusion of a Na^+ solution (135 mM NaCl). This treatment also increased urinary excretion of Na^+ by 32.2%, in parallel with an increase in excretion of K^+ or Cl^-. Urinary excretion of kallikrein increased within 60 min after the start of K^+ infusion. A bradykinin B_2 receptor antagonist, 8-[3-[*N*-[(E)-3-(6-acetamidopyridin-3-yl)acryloylglycyl]-*N*-methylamino]-2,6-dichlorobenzyloxy]-2-methylquinoline (FR173657; 1.0 mg/kg, i.v.), inhibited the K^+-induced diuresis and natriuresis by 41.0% and 26.7%, respectively. These results indicate that K^+ load induces diuresis and natriuresis through the renal kallikrein–kinin system in rats.

Keywords: Kallikrein secretion; K^+; Diuresis; Natriuresis; Bradykinin B_2 receptor antagonist; FR173657

1. Introduction

A study on large Utah pedigrees showed that low levels of urinary kallikrein excretion were related to a positive family history of hypertension in adults and youths (Berry et al., 1989). It was suggested that in this Utah population, dietary K^+ intake had associations with urinary kallikrein levels in individuals inferred to be heterozygous at a locus associated with kallikrein (Hunt et al., 1993). Potassium administration has been shown to lower blood pressure in both hypertensive subjects (Smith et al., 1992; Svetkey et al., 1987; Valdes et al., 1991) and hypertensive animal models (Suzuki et al., 1981; Fujita and Sato, 1983; Barden et al., 1988). There have been several studies that showed an increase in urinary kallikrein excretion and bradykinin B_2 receptor mRNA levels in the kidney after K^+ loading (Barden et al., 1988; Valdes et al., 1991; Jin et al., 1999). We have recently reported that the increase in urinary kallikrein excretion by K^+ loading was not occurring through a washout phenomenon, but might be mediated by ATP-sensitive K^+ channels (Fujita et al., 1999; Hayashi et al., 1999). Jin et al. (1999) proposed that vascular smooth muscle relaxation, caused by increases in release of nitric oxide and prostacyclin, reflected as increases in urinary levels of cGMP and cAMP, followed by activation of the tissue kallikrein–kinin system, may involve in the hypotensive effects of K^+. On the other hand, urinary kinin is reported to have a direct inhibitory effect on reabsorption of Na^+ and Cl^- at the cortical collecting duct (Tomita et al., 1985). Our previous report showed that administration of a urinary kininase inhibitor, ebelactone B, suppressed the development of deoxycorticosterone acetate-salt hypertension, accompanied by prevention of Na^+ retention in rats (Ito et al., 1999). Therefore, acceleration of urinary Na^+ excretion, mediated by bradykinin B_2 receptors, followed by activation of the renal kallikrein–kinin system, is considered as another mechanism for the K^+-induced decrease in blood pressure.

The present study examined whether K^+ loading would induce diuresis and natriuresis, following an increase in renal kallikrein secretion in anesthetized rats. Additionally, the effects of a non-peptide bradykinin B_2 receptor antagonist, 8-[3-[*N*-[(E)-3-(6-acetamidopyridin-3-yl)acryloylglycyl]-*N*-methylamino]-2,6-dichlorobenzyloxy]-2-methylquinoline (FR173657; Aramori et al., 1997), on the K^+-induced diuresis and natriuresis were investigated.

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2. Materials and methods

2.1. Animals and drugs

Male Sprague–Dawley strain rats (specific pathogen-free, 350–450 g; Shizuoka Laboratory Animal Center, Hamamatsu) were used. All rats were given normal rat chow (NMF; Oriental Yeast, Tokyo) and tap water ad libitum immediately after weaning, and were housed at constant humidity ($60 \pm 5\%$) and temperature (25 ± 1°C), and kept on a continuous 12-h light/12-h dark cycle until the time of the experiment.

This study was performed in accordance with the guidelines for animal experiments of Kitasato University School of Medicine.

Pentobarbital sodium (Nembutal) was purchased from Abbott Lab., North Chicago, IL, USA. FR173657 (Fujisawa Pharmaceutical, Osaka, Japan) was suspended at a concentration of 10 mg/ml in 0.1 N HCl.

2.2. Preparation

The rats were initially anesthetized with 40 mg/kg pentobarbital sodium intraperitoneally (Nembutal: Abbott Lab.). Tracheotomy was performed, and a polyethylene canula (PE-100; Clay Adams, Parsippany, NJ, USA) was inserted into the trachea. The urinary bladder was cannulated with a polyethylene cannula (PE-60, Clay Adams) through a small abdominal skin incision. The right femoral vein was cannulated with a polyethylene cannula (PE-10, Clay Adams) for infusion. Body temperature was measured continuously (Model CTM-303 thermometer; Terumo, Tokyo), and was maintained at 37.5 ± 0.5°C, with a desk lamp and heated table. All rats were infused continuously with a 135-mM NaCl solution, containing 0.5% pentobarbital sodium through the femoral vein at a rate of 6.0 ml kg^{-1} h^{-1}, using an infusion pump (Model 235; Atom, Tokyo).

2.3. Experimental procedure

Thirty minutes after the start of infusion was taken as the start of the experiment (time 0 min). Urine was collected every 15 min for 180 min and placed on ice. In the high-K^+-treated group, the NaCl solution was switched to a high-K^+ solution (67.5 mM KCl, 67.5 mM NaCl, containing 5% pentobarbital sodium), 60 min after the start of the experiment, and this infusion was performed for 120 min. In the FR173657-treatment group, the rats were given FR173657 at a dose of 1.0 mg/kg intravenously as a bolus through the dorsal vein of the penis, 45 min after the start of the experiment in the high-K^+-treated group. It has been reported that the oral bioavailability of FR173657 was 44% (Asano et al., 1997), and oral administration of FR173657 at a dose of 3 mg/kg, which corresponded to an intravenous dose of 1.32 mg/kg, significantly inhibited the bradykinin-induced plasma exudation into the pleural cavity by 76% in rats (Majima et al., 1997). Therefore, it is supposed that the dose of FR173657 used in the present study was sufficient for acting on the kidney. Control rats were given 0.1 N HCl.

2.4. Measurement of urine volume and urinary excretion of Na^+, K^+ and Cl^-

Urine volume was determined gravimetrically. The urinary Na^+, K^+ and Cl^- levels were measured with ion-selective electrodes after fivefold dilution with distilled water (Fuji Dri-Chem Slide Na–K–Cl, Fuji Dri-Chem 800V: Fuji Film, Tokyo, Japan).

2.5. Measurement of urinary kallikrein excretion

Kallikrein activity in the collected urine was measured using a peptidyl fluorogenic substrate selective for glandular kallikrein, Pro–Phe–Arg–methyl-coumarinylamide (Pro–Phe–Arg–MCA; Peptide Institute, Minoh, Osaka, Japan). MCA, diluted with 0.05 M Tris–HCl buffer, containing 0.1 M NaCl and 0.01 M $CaCl_2$ (pH 8.0), was added to 5 μl of urine diluted fivefold with distilled water to a final concentration of 0.05 mM. Kallikrein activity was calculated as the difference between amidase activity in the presence of soy bean trypsin inhibitor (SBTI, 0.5 μg/μl of diluted urine; Worthington Biochem., Halls Mill Road, NJ, USA) and that in the presence of aprotinin (0.5 μg/μl; Wako). This determination method is assumed to exclude the contamination by proteases, other than kallikrein in the urine (Fujita et al., 1999). One arbitrary unit was defined as the amount of urinary kallikrein that released 10^{-10} M of 7-amino-4-methyl-coumarin (Peptide Institute, Minoh, Osaka, Japan) for 15 min/μl urine at 37°C.

2.6. Statistical analyses

Values are expressed as means ± S.E.M. The significance of differences between means was analyzed by repeated-measure analysis of variance (ANOVA). A P value less than 0.05 was considered significant.

3. Results

3.1. Changes in urine volume and urinary excretion of Na^+, K^+, Cl^- and kallikrein in K^+-infused rats

Changes in urine volume and urinary excretion of Na^+, K^+, Cl^- and kallikrein after infusion with the NaCl solution and high-K^+ solution are shown in Fig. 1. Changes in these parameters were assessed in every 60-min infusion. Urine volume increased significantly 60 min after infusion with the high-K^+ solution (1830 ± 230 μl kg^{-1} h^{-1},

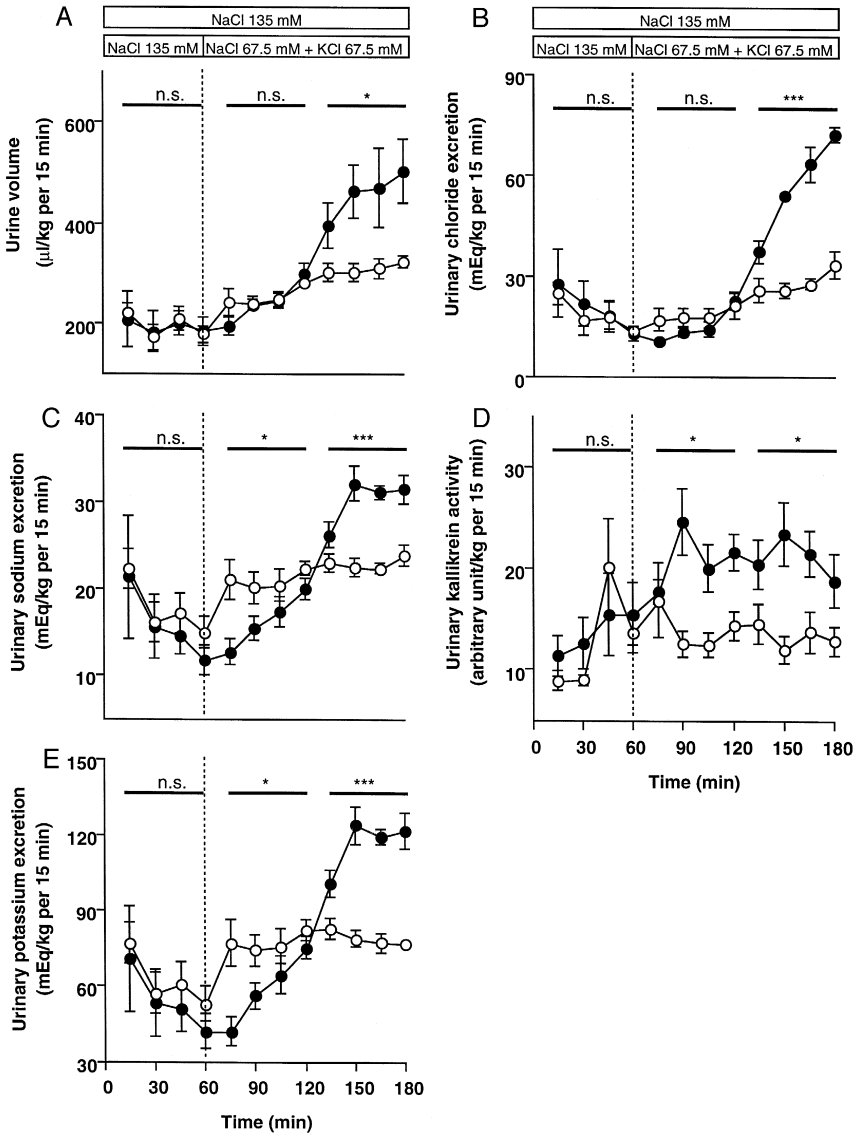

Fig. 1. Time course of the changes in urine volume (A), and urinary excretion of Na^+ (B), K^+ (C), Cl^- (D), and kallikrein (E) during intravenous infusion of Na^+ or K^+. Na^+ solution or high-K^+ solution was infused at a rate of 6.0 ml kg^{-1} h^{-1}. Values are means ± S.E.M. from five rats. Values for each 60-min period in the K^+-infused group (closed circles) were compared with those for the Na^+-infused group (open circles) (n.s. not significant, $^*P < 0.05$, $^{***}P < 0.001$).

$n = 5$) compared with the NaCl solution (1240 ± 60 μl kg^{-1} h^{-1}, $n = 5$, $P < 0.05$; Fig. 1A). Urinary Na^+ excretion during the first 60-min infusion with the high-K^+ solution (65.4 ± 3.8 meq kg^{-1} h^{-1}, $n = 5$) was less than that with the corresponding NaCl solution (83.7 ± 5.7 mEq kg^{-1} h^{-1}, $n = 5$, $P < 0.05$). Urinary Na^+ excretion, however, increased significantly during the second 60-min infusion with the high-K^+ solution (121 ± 4 meq kg^{-1} h^{-1}, $n = 5$) compared with the NaCl infusion (91.5 ± 3.2 meq kg^{-1} h^{-1}, $n = 5$, $P < 0.001$; Fig. 1B). The change in urinary Cl^- excretion was similar to the change in urine volume in both groups (Fig. 1D). Compared with that in the Na^+-treated group, the urinary K^+ excretion in the K^+-treated group was lower during the first 60-min infusion and higher during the second-60 min infusion (Fig. 1C).

As shown in Fig. 1E, urinary kallikrein excretion during the first and second 60-min infusion with the high-K^+ solution (83.8 ± 6.2 and 84.0 ± 8.7 arbitrary unit kg^{-1} h^{-1}, $n = 5$) was higher than that with the NaCl solution (56.2 ± 6.0 and 53.0 ± 5.9 arbitrary unit kg^{-1} h^{-1}, $n = 5$, $P < 0.05$), respectively.

3.2. Effects of FR173657 on the K^+-induced increases in urine volume and urinary excretion of Na^+

Changes in urine volume and urinary Na^+ excretion during each 60-min infusion are shown in Fig. 2. Increases

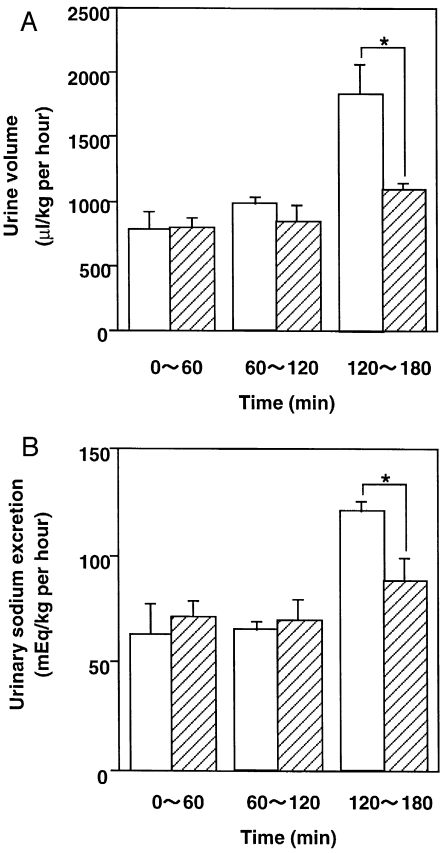

Fig. 2. Changes in urine volume (A) and urinary excretion of Na^+ (B) during intravenous infusion of K^+ in rats treated with FR173657 (hatched columns; $n = 4$) or vehicle (open columns; $n = 5$) 45 min after the start of the experiment. Values are means ± S.E.M. Values for each 60-min period in the FR173657-treated group were compared with those in the vehicle-treated group ($^* P < 0.05$).

in both urine volume and urinary Na^+ excretion in the second 60-min infusion with the high-K^+ solution were significantly inhibited in the FR173657-treated group by 41.0 and 26.7%, respectively.

4. Discussion

In the present study, the increase in renal kallikrein secretion appeared earlier than the increase in urine volume or urinary Na^+ excretion after intravenous infusion of the high-K^+ solution (Fig. 1). We had shown earlier that a high-K^+ solution increased renal kallikrein secretion in urine within 30 min after infusion, and in the perfusate, within 10 min after superfusion of sliced kidney cortex (Fujita et al., 1999, Hayashi et al., 1999). Therefore, the early increase in renal kallikrein secretion caused by K^+ in the present study probably occurred through a direct effect on the connecting tubules, where renal kallikrein-containing cells are localized (Figueroa et al., 1984). A prolonged increase in renal kallikrein activity in urine might be caused by an increase in plasma aldosterone, which is reported to increase renal kallikrein activity in renal homogenates and membrane-enriched fractions (Nishimura et al., 1980). Previous experiments with kininogen-deficient Brown Norway–Katholiek rats revealed that the renal kallikrein–kinin system prevents the development of hypertension through augmentation of urinary excretion of Na^+ and water accumulated in the body (Majima and Katori, 1995). In view of the results which showed the inhibition of the K^+-induced increases in diuresis and natriuresis by FR173657 (Fig. 2), we suggest that the K^+-induced diuresis and natriuresis are associated with augmentation of kinin release from low-molecular weight kininogens, a substrate of renal kallikrein, in urine, followed by augmentation of renal kallikrein secretion. The increase in urinary excretion of Cl^- correlated with that of Na^+ after K^+ infusion that probably resulted from the increase in kinin activity, as blockade of the bradykinin B_2 receptor was shown to decrease urinary Cl^- excretion in rats (Mukai et al., 1996). It is reported that K^+ homeostasis, following an acute K^+ load, is largely regulated by extrarenal tissues (Margaret and Defronzo, 1981). Our preliminary study showed that the plasma concentration of K^+ did not increase immediately, but only 60 min after K^+ infusion (75 mM KCl, 75 mM NaCl). Therefore, the reason why the urinary K^+ excretion did not increase during the first 60-min infusion with the K^+ solution might be translocation of most retained K^+ from the extracellular to the intracellular compartment.

In conclusion, the present study indicated that K^+-induced diuresis and natriuresis are mediated by activation of the renal kallikrein–kinin system. Elucidation of mechanisms for the activation of the renal kallikrein–kinin system by K^+ may be an important step for developing a new anti-hypertensive drug.

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