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Early increases in renal kallikrein secretion on administration of potassium or ATP-sensitive potassium channel blockers in rats

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1 This study aimed to examine whether administration of potassium or ATP-sensitive potassium channel (K_{ATP} channel) blockers caused early increases in renal kallikrein (KK) secretion. To clarify this mechanism, the effect on renal KK secretion of a K_{ATP} channel blocker was compared with the effect resulting from use of an osmotic diuretic or volume load. Furthermore, the effect on potassium-induced increases in renal KK secretion by an additional treatment using a K_{ATP} channel blocker was examined. Lastly, the effect of a K_{ATP} channel blocker on renal KK secretion was also examined in superfused slices of kidney cortex.

2 Intravenous infusion of potassium augmented renal KK secretion within 30 min while urine volume increased gradually in both the potassium loading and control groups.

3 Administration of the K_{ATP} channel blocker, 4-morpholinecarboximidine-N-1-adamantyl-N′-cyclohexylhydrochloride (PNU-37883A) or glibenclamide, caused a dose-dependent increase in renal KK secretion.

4 The concentration of KK in urine was higher in the PNU-37883A group as compared to the osmotic-diuretic or volume-load group.

5 PNU-37883A had no additive effect on the potassium-induced increase in renal KK secretion.

6 Renal KK secretion increased in slices of kidney cortex incubated with PNU-37883A within 10 min of superfusion.

7 In conclusion, administration of both potassium and K_{ATP} channel blockers induced early increases in renal KK secretion in the absence of the washout phenomenon. Potassium loading may have increased renal KK secretion through the same mechanism as the K_{ATP} channel blocker.

Keywords: ATP-sensitive potassium channel blocker; glibenclamide; potassium; renal kallikrein secretion; osmotic diuretic; volume load

Abbreviations: b.wt., body wt; K_{ATP} channel, ATP-sensitive potassium channel; KK, kallikrein; PEG200, polyethyleneglycol 200; PNU-37883A, 4-morpholinecarboximidine-N-1-adamantyl-N′-cyclohexylhydrochloride; Saline, physiological saline; U, units

Introduction

Potassium administration has been shown to lower blood pressure in both hypertensive subjects (Svetkey *et al*., 1987; Valdes *et al*., 1991) and hypertensive animal models (Suzuki *et al*., 1981; Fujita & Sato, 1983; Barden *et al*., 1988). Postulated mechanisms for the antihypertensive effect of potassium administration include direct vasodilatation (Emanuel *et al*., 1959), potassium-induced natriuresis (Brandis *et al*., 1972), reduced sympathetic nervous activity (Battarbee *et al*., 1979; Fujita & Ando, 1984), suppression of the renin-angiontensin system (Vander, 1970), and augmentation of urinary secretion of prostaglandins (Barden *et al*., 1985; 1987) and kallikrein (KK). Renal KK, a serine protease, is synthesized in renal tubule cells of the connecting tubules (Figueroa *et al*., 1984) and secreted into the tubular lumen. It has been reported that renal KK preferentially acts on low-molecular weight kininogen and subsequently kinin is released (Hagiwara *et al*., 1994). Enhancement of renal KK secretion is expected to potentiate the renal KK-kinin system thereby preventing the development of hypertension through augmentation of urinary sodium excretion (Majima *et al*., 1993; Majima & Katori, 1995).

An increase in renal KK secretion due to potassium loading has been demonstrated in both human (Murakami *et al*., 1989; Valdes *et al*., 1991) and animal (Vio & Figueroa, 1987; Barden *et al*., 1988) studies. Three studies have proposed aldosterone as a mediator of these effects. In one study, a dietary potassium load caused parallel changes in urinary secretion of KK and aldosterone over 24 h in man (Horwitz *et al*., 1978); in another study, renal KK secretion was increased gradually in rats over the course of 7 days by aldosterone administration *via* osmotic minipumps (Fejes-Toth & Fejes-Toth, 1984); in the third study, 6 days of aldosterone administration increased KK activity in renal homogenates and in the membrane-enriched fractions in rats (Nishimura *et al*., 1980). Additionally, a simultaneous increase in urinary excretion of KK and PGF2α (Fejes-Toth & Fejes-Toth, 1984; Nasjletti *et al*., 1985) or 6-keto-PGF1α (Barden *et al*., 1987) by potassium administration has been reported. Thus, PGs have also been proposed as mediators of the augmentation of renal KK secretion by potassium. In these experiments, the resultant increase in renal KK secretion from potassium administration was examined up to and beyond 24 h, and therefore, aldosterone and PGs were thought to have mediated the above delayed incremental effects of potassium on renal KK secretion. In contrast, an early increase in renal KK secretion, which occurs within 20 min after intravenous infusion of potassium, has been

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reported in rats (Obika, 1989). An increase in plasma aldosterone levels at an intravenous potassium infusion rate of 0.17 mmole min^{-1} took at least 1 h in humans, a rate 68 fold faster than that used in the present study (Himathongkam *et al*., 1975). Therefore, it seems that aldosterone does not participate in this early response. Moreover, an *in vitro* study showed that augmentation of extracellular potassium concentration led to increased KK-like enzyme release in slices of rat kidney (Lauar *et al*., 1982). However, the mechanism of the early augmentation of renal KK secretion by potassium remains unclear.

It is known that membrane permeability to potassium through potassium channels mainly determines the membrane potential in excitable cells. In the case of renal tubular cells, which are non-excitable cells, an increase in membrane potential caused by a high concentration of extracellular potassium was reported in an experiment which used a single perfused proximal tubule of *Necturus* kidney (Kubota *et al*., 1983). Renal potassium channels in distal nephrons not only secreted potassium but also maintained the membrane potential of the tubular cells (Giebisch, 1995). Stanton reported that perfusion of the inner medullary collecting tubules produced membrane depolarizations in tubular cells in the presence of high potassium concentrations (Stanton, 1989). Furthermore, perfusion with barium chloride, a blocker of potassium channels, also produced the same effect (Stanton, 1989). Supposedly, an early stimulatory effect on renal KK secretion of potassium was mediated through the same pathway as that of the renal potassium channel blocker. This study set out to examine whether administration of potassium or potassium channel blockers caused early increases in renal KK secretion in anaesthetized rats. In order to clarify this mechanism, the effect of a K_{ATP} channel blocker on renal KK secretion was compared to effects resulting from an osmotic diuretic and volume load. Furthermore, the effect of an additional treatment consisting of a potassium channel blocker in combination with a potassium-induced increase in renal KK secretion was examined. Lastly, the effect of a potassium channel blocker on renal KK secretion was investigated in superfused slices of kidney cortex.

Since K_{ATP} channels have been reported to be localized on the membranes of the distal tubules and connecting tubules (Boim *et al*., 1995) where KK-secreting cells are present, increases of renal KK secretion brought about by PNU-37883A and glibenclamide, both of which are blockers of K_{ATP} channels (Guillemare *et al*., 1994), were tested in the present experiment.

Methods

Materials

Male Sprague-Dawley rats (specific pathogen-free, 8-weeks-old from the Shizuoka Laboratory Animal Centre in Hamamatsu) were used. All rats were given normal rat chow and tap water *ad libitum*, were housed at constant humidity ($60\pm5\%$) and temperature ($25\pm1^{\circ}C$), and were kept on a continuous 12 h light–dark cycle. This study was performed in accordance with the Kitasato University School of Medicine guidelines for animal experiments.

The following drugs were used: potassium gluconate (Wako Pure Chemical Industries, Ltd., Osaka, Japan), gluconic acid (Wako Pure Chemical Industries), glibenclamide (Sigma Chemical Co., St. Louis, MO, U.S.A.), polyethyleneglycol 200 (PEG200, Wako Pure Chemical Industries), dimethylacetamide (DMA, Wako Pure Chemical Industries), Triton X-100 (Wako Pure Chemical Industries), and 4-morpholinecarboximidine-N-1-adamantyl-N′-cyclohexylhydrochloride (PNU-37883A, Pharmacia & Upjohn, Co., Kalamazoo, Michigan, U.S.A.). Either PNU-37883A or glibenclamide was dissolved in saline or DMA.

General procedures

Rats were initially anaesthetized with 50 mg kg^{-1} pentobarbital sodium (Nembutal: Abbott Lab., North Chicago, IL, U.S.A.) intraperitoneally. Part of the trachea was separated from the surrounding tissue and a polyethylene cannula (PE-100: Clay Adams, Parsippany, NJ, U.S.A.) was placed under the separated trachea. A tracheostomy was then performed. The urinary bladder was cannulated through a small abdominal skin incision using a polyethylene cannula (PE-50: Clay Adams). The left femoral vein was cannulated with a polyethylene cannula (PE-10: Clay Adams) for infusion. Body temperature was measured continuously with a thermometer (Model CTM-303: Terumo, Tokyo, Japan) and maintained at $37.5\pm0.5^{\circ}C$ with a desk lamp and heated table. All rats were infused continuously using an infusion pump (Model 235: Atom, Tokyo) to deliver physiological saline (saline) containing 0.4% pentobarbital sodium through the femoral vein at a rate of 6 ml kg^{-1} h^{-1}. After these conditions were maintained for 15 min, collections of urine were performed every 15 min and placed on ice.

Either 30 mM potassium gluconate dissolved in saline or in mM potassium 75, sodium 75, and chloride solution 150 was used for the potassium loading experiments, and either sodium gluconate dissolved in saline at a concentration of 30 mM or saline was prepared for the control animals. Pentobarbital sodium was added to infused solutions to give a final concentration of 0.4%. Forty-five min after the start of infusion, saline was replaced with either a solution of potassium gluconate ($n=6$) or sodium gluconate ($n=5$), and another infusion was performed for 30 min. In the experiment using potassium chloride, the saline was replaced with the potassium solution ($n=5$) or saline ($n=5$) was continued 90 min after the start of infusion. The infusion was then performed for a further 75 min.

For the study using K_{ATP} channel blockers, either 300 μl of PNU-37883A at doses of 1 mg kg^{-1} ($n=4$), 3 mg kg^{-1} ($n=4$) or 10 mg kg^{-1} ($n=6$), or 100 μl of glibenclamide at doses of 3 mg kg^{-1} ($n=3$), 10 mg kg^{-1} ($n=3$) or 30 mg kg^{-1} ($n=5$), was given as a bolus i.v. injection 45 min after the start of infusion. The infusion was performed for 75 min after the injection of PNU-37883A at a dose of 10 mg kg^{-1}. After the administration of lower doses of PNU-37883A and glibenclamide, the infusion was continued for either 30 min or 15 min. Either saline ($n=5$) or DMA ($n=5$) was given as a vehicle for PNU-37883A and glibenclamide.

In the experiment in which concentrations of KK in urine were examined, either 300 μl of PNU-37883A at a dose of 10 mg kg^{-1} ($n=6$) or 300 μl of PEG200 at a dose of 1.4 μmole kg^{-1} ($n=6$) was injected intravenously and the infusion rate was increased to 60 ml kg^{-1} h^{-1} (volume load, $n=5$) 45 min after the start of saline infusion. The infusion was then performed for 30 min.

During examination of the additional treatment using PNU-37883A and potassium loading, the saline was replaced with (mM) potassium 75, sodium 75, and chloride solution 150 45 min after the start of infusion, then 15 min later either 300 μl of PNU-37883A at a dose of 3 mg kg^{-1}

($n=4$) or saline ($n=3$) was injected intravenously and infusion was performed for another 15 min.

Procedure using sliced kidney cortices

An abdominal incision was made and the abdominal artery was cannulated with a polyethylene cannula in rats under ether anaesthesia. Both kidneys were removed after saline perfusion, and kidney cortices were sliced to a thickness of two millimeters. The sliced kidney cortex was superfused with Tyrode solution (mM: NaCl 136.9, KCl 2.7, $MgCl_2 \cdot 6H_2O$ 0.5, $CaCl_2$ 1.8, $NaHz_2PO_2 \cdot 2H_2O$ 0.4, $NaHCO_3$ 11.9, Glucose 5.6) at a rate of 8.4 ml h^{-1}. After a 50 min superfusion using Tyrode solution, superfusion was either replaced with 300 μM PNU-37883A dissolved in Tyrode solution ($n=7$) or continued ($n=4$). The superfusion was performed for a further 40 min. Next, 2% Triton X-100 dissolved in distilled water was superfused for 20 min. The perfusate was collected every 10 min. Renal KK-containing cells are located only in certain lesions of tubular cells (Figueroa *et al*., 1984). Therefore, we used data from samples of sliced kidney cortices in which the amount of renal KK released into the perfusate was 30 times greater than the level before superfusion with Triton X-100 solution (data not shown). This may reflect the presence of large amounts of KK-containing cells on the surface of sliced kidney cortices.

Measurement of urine volume and urinary excretion of sodium, potassium and chloride

Urine volume was measured by weight and expressed in terms of volume by specific gravity as 1. To measure urinary excretion of sodium, potassium, and chloride a portion of the urine was diluted 10 fold with distilled water. The measurement was performed with ion-selective electrodes (Fuji Dri-Chem Slide Na-K-Cl, Fuji Dri-Chem 800V: Fuji Film Co., Ltd., Tokyo, Japan). Urine volume and excreted electrolytes were expressed in μl 15 min^{-1} 100 g body weight (b.wt.)$^{-1}$ or μmole 15 min^{-1} 100 g b.wt.$^{-1}$.

Measurement of KK activity in urine and perfusate

KK activity in the collected urine and perfusate was measured using a peptidyl fluorogenic substrate for glandular KK, Pro-Phe-Arg-methyl-coumarinylamide (MCA, Peptide Institute, Minoh, Osaka, Japan) (Majima *et al*., 1993). A portion of the urine was diluted 10 fold with 0.2 M Tris-HCl buffer (pH 7.8) and 10 μl of the diluted urine was mixed with either 10 μl of soy bean trypsin inhibitor (SBTI: Worthington Biochem., Corp., Halls Mill Road, NJ, U.S.A.), an inhibitor of glandular KK, or aprotinin (Wako Pure Chemical Industries), an inhibitor of both glandular and plasma KK, to a final concentration of 0.5 μg μl^{-1}. MCA diluted with 0.05 M Tris-HCl buffer containing 0.1 M $CaCl_2$ (pH 8.0) was added to either the mixed urine solution or 500 μl of the perfusate to attain a final concentration of 0.05 mM. The reaction mixture was incubated at 37°C for either 10 min or 30 min. After the incubation, 2.0 ml of 17% acetic acid was added to the incubation mixture to stop the reaction, and the fluorescence intensity of the incubation mixture was measured by fluorescence spectrophotometer (M850: Hitachi, Ltd., Tokyo, Japan, excitation; 380 nm, emission; 460 nm). One unit was defined as either the amount of renal KK that released 1 μmole of 7-amino-4-methylcoumarin (Peptide Institute, Minoh, Osaka, Japan) for 10 min per 1 μl of urine at 37°C or for 30 min per 500 μl of perfusate at 37°C. Urinary KK activity was expressed as units (U).

Data analysis

Values are expressed as mean ± s.e.mean. Statistical analysis of comparative values was performed using the unpaired *t*-test. Significance levels of the dose-response increases in urinary KK excretion by PNU-37883A and glibenclamide, and the differences in the concentrations of KK in urine by PNU-37883A, PEG200 and volume load were estimated by one-factor ANOVA followed by a Scheffé's F multiple comparison. Those differences with a probability of 5% or less were considered to be significant ($P<0.05$).

Results

Effect of sodium and potassium gluconate on urinary KK excretion

Figure 1 shows that the increase in urinary KK excretion during the 30 min infusion of potassium gluconate solution (0.22 ± 0.05 mU 30 min^{-1} 100 g b.wt.$^{-1}$) was larger than that of sodium gluconate solution (0.03 ± 0.04 mU 30 min^{-1} 100 g b.wt.$^{-1}$).

Effect of potassium chloride on urinary KK excretion and urine volume, and urinary excretion of sodium, potassium and chloride

In order to clarify whether other potassium solutions increase urinary KK excretion and whether urinary KK excretion was increased by potassium loading as a result of the washout phenomenon, the effect of potassium chloride on urinary KK excretion, urine volume and urinary excretion of electrolytes were examined. Urinary KK excretion tended to increase immediately after infusion of the 75 mM potassium solution and significantly during the 30 min infusion of potassium (0.51 ± 0.03 mU 15 min^{-1}

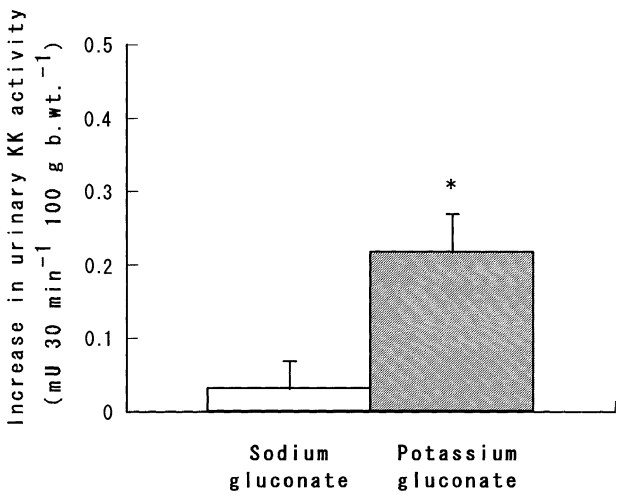

Figure 1 Increases in urinary KK excretion during the 30 min infusion of sodium and potassium gluconate. Values are expressed as mean ± s.e.mean; $n=5$ for the sodium gluconate group and $n=6$ for the potassium gluconate group. After a 45 min intravenous infusion of saline, either sodium gluconate (30 mM) or potassium gluconate (30 mM) was substituted and infused for a further 30 min. *Significantly different from the sodium gluconate value, $P<0.05$.

100 g b.wt.$^{-1}$) in comparison with saline (0.41 ± 0.02 mU 15 min^{-1} 100 g b.wt.$^{-1}$). The increase in urinary KK excretion was maintained during the potassium infusion (Figure 2a). Urine volume and urinary excretion of sodium, potassium and chloride increased gradually during both the infusion of potassium and saline (Figure 2b–e).

Effects of PNU-37883A on urinary KK excretion and urine volume, and urinary excretion of sodium, potassium and chloride

Figure 3 shows changes in urinary KK excretion (a), urine volume (b), urinary excretion of sodium (c), potassium (d),

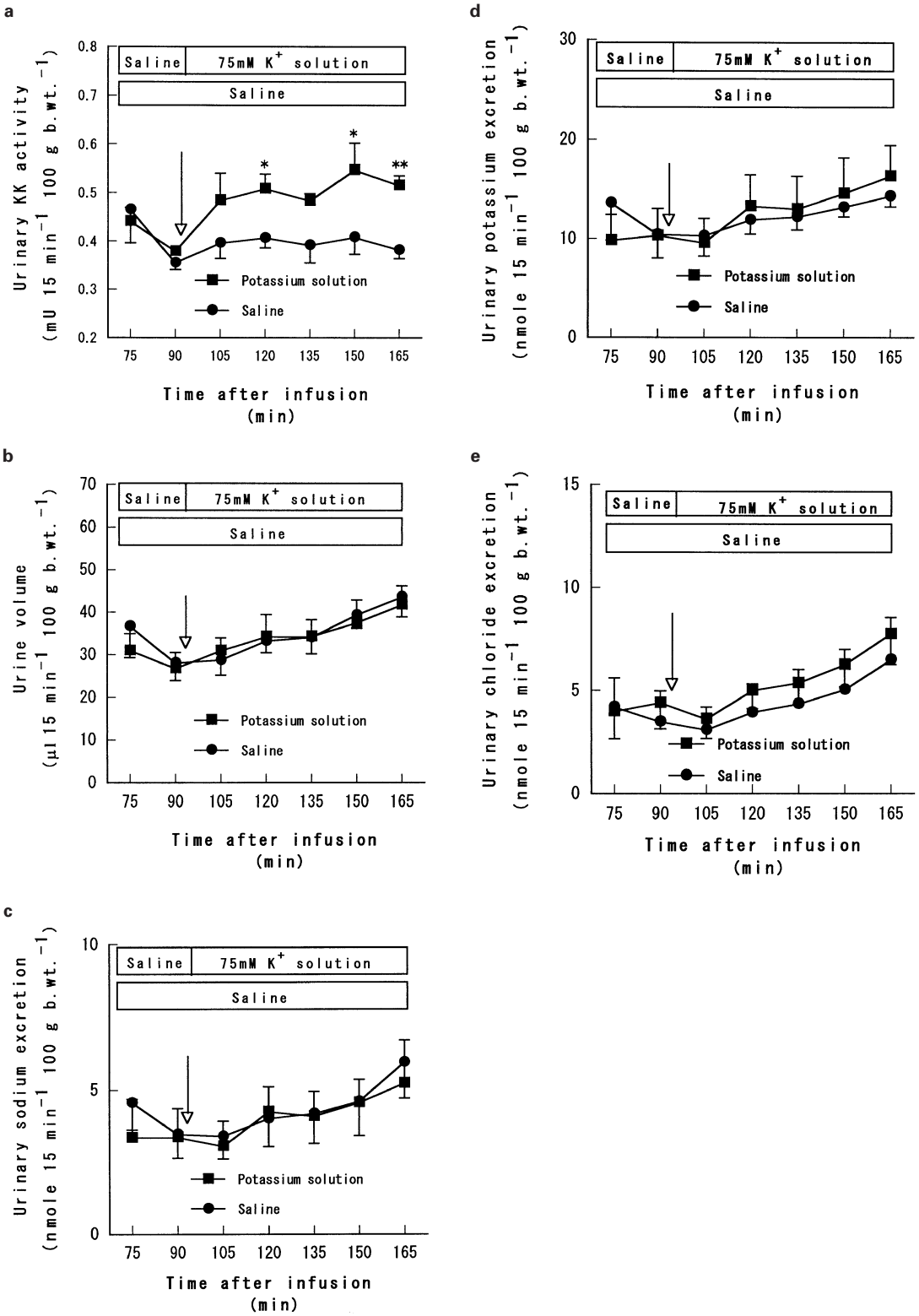

Figure 2 Changes with time in urinary KK excretion (a); urine volume (b); urinary excretion of sodium (c); potassium (d); and chloride (e) after infusion of (mM) K^+ 75, Na^+ 75 and Cl^- 150 solution or saline. Values are expressed as mean $\pm$ s.e.mean; $n=5$ for each group. After intravenous infusion of saline for 90 min, either (mM) K^+ 75, Na^+ 75 and Cl^- 150 solution was substituted (indicated by the arrow in the figure) or saline was continued. The infusion was performed for a further 75 min. *,**Significantly different from corresponding control value, $P<0.05$, $P<0.01$.

and chloride (e) after the administration of PNU-37883A at a dose of 10 mg kg^{-1}. Urinary KK excretion started to increase immediately after the administration of PNU-37883A (0.24 ± 0.03 mU 15 min^{-1} 100 g b.wt.$^{-1}$), peaked 30 min later (0.26 ± 0.02 mU 15 min^{-1} 100 g b.wt.$^{-1}$), and then decreased to its original level within the next 30 min (0.17 ± 0.04 mU 15 min^{-1} 100 g b.wt.$^{-1}$). In the control group, the urinary activity of KK corresponding to the maximum level resulting from PNU-37883A administration was 0.15 ± 0.01 mU 15 min^{-1} 100 g b.wt.$^{-1}$. Urine volume

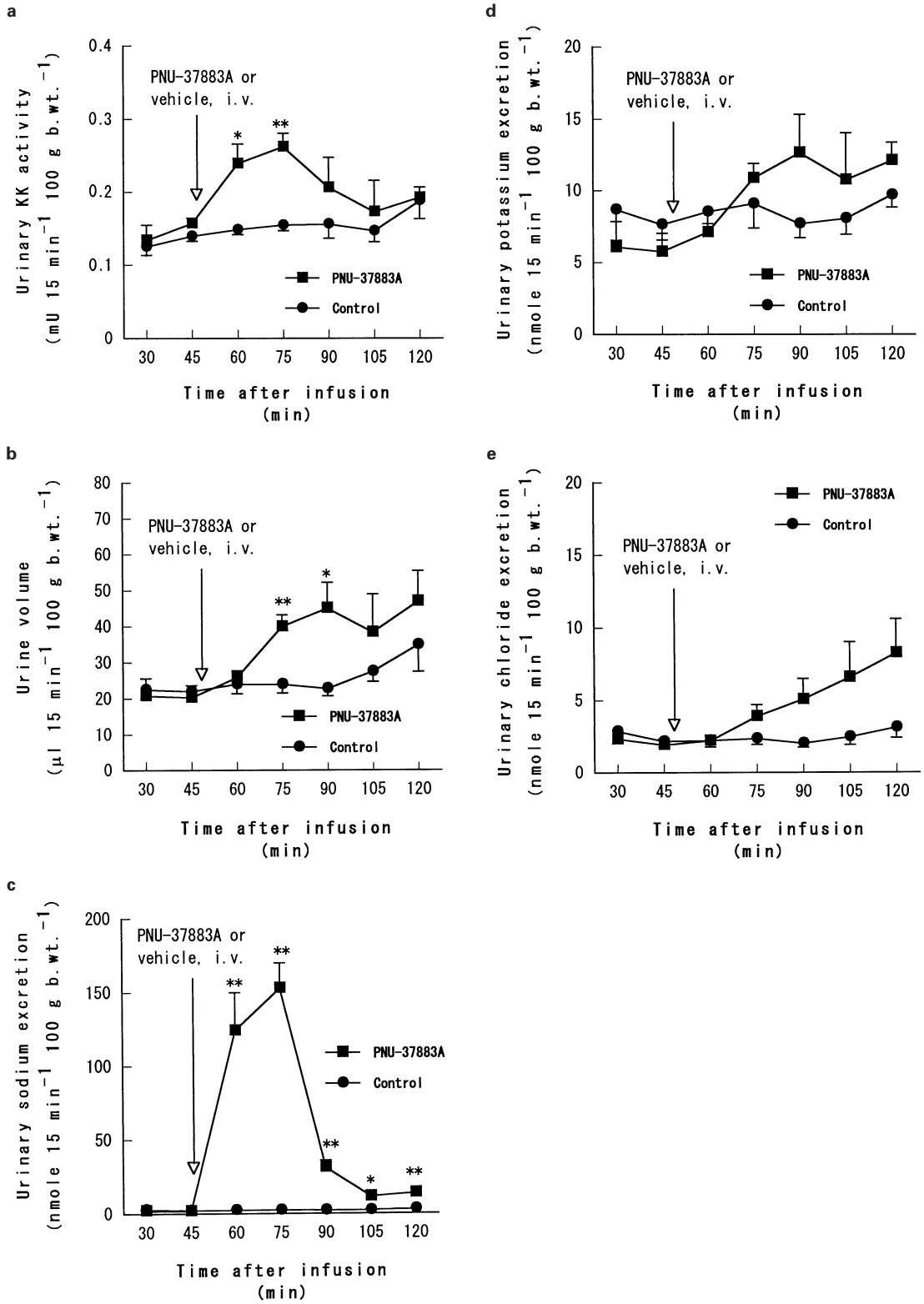

Figure 3 Changes with time in urinary KK excretion (a); urine volume (b); urinary excretion of sodium (c); potassium (d); and chloride (e) after the administration of either PNU-37883A or vehicle. Values are expressed as mean $\pm$ s.e.mean; $n = 6$ for PNU-37883A and $n = 5$ for vehicle. Either PNU-37883A (10 mg kg^{-1}) or vehicle was administered 45 min after the start of infusion. *,**Significantly different from corresponding vehicle value, $P < 0.05$, $P < 0.01$.

increased 15 to 30 min after the injection of PNU-37883A (40.3 ± 3.3 vs 24.0 ± 2.4 (control) μl 15 min^{-1} 100 g b.wt.$^{-1}$) and peaked 30 min later (45.1 ± 7.2 vs 22.8 ± 2.0 (control) μl 15 min^{-1} 100 g b.wt.$^{-1}$). Urinary sodium excretion markedly increased immediately after the administration of PNU-37883A; whereas, changes in the urinary excretion of potassium and chloride after the administration of PNU-37883A were not significantly different from control.

Dose-dependent increases of urinary KK excretion due to PNU-37883A and glibenclamide

Several doses of PNU-37883A and glibenclamide were examined to determine whether urinary KK excretion increase dose dependently. The potency of the increase in urinary KK excretion by PNU-37883A was compared with that of glibenclamide. Figure 4 shows the dose dependent increases in urinary KK excretion following administration of PNU-37883A and glibenclamide. The increase in urinary KK excretion with PNU-37883A was 4.9 and 7.9 fold relative to the control value at doses of 3 mg kg^{-1} and 10 mg kg^{-1} respectively. Concerning glibenclamide, urinary KK excretion was increased by 2.8 and 4.8 fold at the same doses.

Differences in KK urine concentrations after administration of PNU-37883A and PEG200 and after volume load

In order to ensure that the PNU-377883A-induced increase in renal KK secretion was not due to the washout phenomenon, we compared the effect of PNU-37883A on concentrations of KK in urine to that of PEG200 and volume load. As shown in Figure 5, the concentrations of KK in urine during the 30 min infusion was higher in the PNU-37883A group (0.65 ± 0.10 mU μl^{-1}) than that in the PEG200 ($2.5 \pm 0.4 \times 10^{-4}$ U μl^{-1}) or volume load (0.29 ± 0.07 mU μl^{-1}) group.

Effect of additional PNU-37883A treatment on the potassium-induced increase in urinary KK excretion

PNU-37883A was administered additionally during the potassium infusion to examine whether the stimulatory effect on urinary KK excretion by PNU-37883A and potassium loading appeared additively, synergistically or without change. The increase in urinary KK excretion due to treatment with either vehicle or PNU-37883A was expressed as a value relative to the pre-treatment value (Figure 6). Urinary KK excretion significantly increased (68%) after treatment with PNU-37883A at a concentration of 3 mg kg^{-1} during saline infusion. The increase caused by PNU-37883A was significantly larger than that of the control. The potassium infusion increased urinary KK excretion by 50% in both the control and PNU-37883A groups (data not shown). The additional PNU-37883A administration increased urinary KK excretion by only 19%. This increase was not significantly different from control.

Effect of superfusion with PNU-37883A on renal KK secretion in sliced kidney cortices

In order to investigate whether PNU-37883A has a direct stimulatory effect on renal KK secretion, an experiment using sliced kidney cortices was performed. As shown in Figure 7, renal KK secretion peaked 10 min after superfusion with PNU-37883A (0.46 ± 0.07 mU 10 min^{-1}) and subsequently decreased to the pre-superfusion level during the following 30 min. In the control group, the activity of

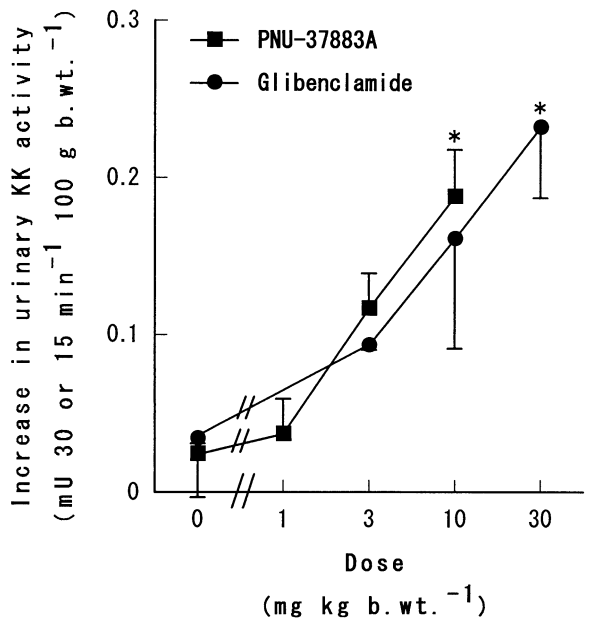

Figure 4 Increases in urinary KK excretion after administration of either PNU-37883A or vehicle and either administration of glibenclamide or vehicle 30 min and 15 min after either the administration. Values are expressed as mean$\pm$s.e.mean; $n=5$ for each vehicle, $n=4$ for PNU-37883A at a dose of 1 mg kg^{-1} or 3 mg kg^{-1}, $n=6$ for PNU-37883A at a dose of 10 mg kg^{-1}, $n=3$ for glibenclamide at a dose of 3 mg kg^{-1} or 10 mg kg^{-1}, $n=5$ for glibenclamide at a dose of 30 mg kg^{-1}. PNU-37883A, glibenclamide or vehicle was administered 45 min after the start of infusion.

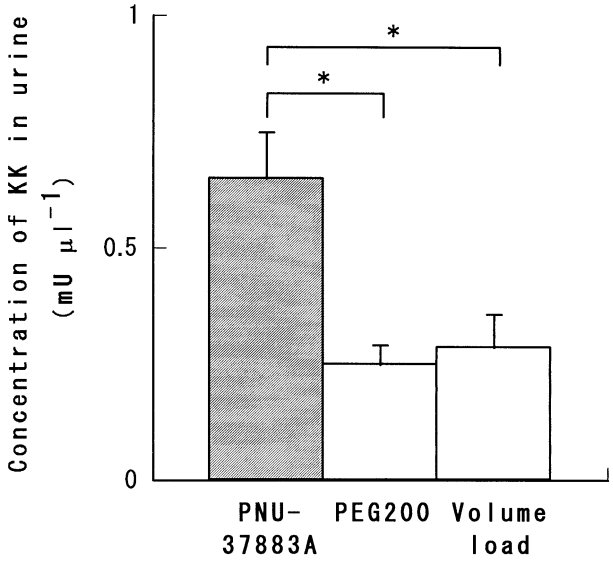

Figure 5 Differences in urine KK concentrations 30 min after the administration of PNU-37883A, PEG200 and volume load with saline. Values are expressed as mean$\pm$s.e.mean; $n=6$ for PNU-37883A and PEG200, $n=5$ for volume load with saline. Either PNU-37883A (10 mg kg^{-1}) or PEG200 (1.4 μmole kg^{-1}) was administered 45 min after the start of infusion. In volume–load group, after intravenous infusion of saline at a rate of 6 ml kg^{-1} h^{-1} for 45 min, the infusion rate was increased to 60 ml kg^{-1} h^{-1}. *Significantly different between indicated values, $P<0.05$.

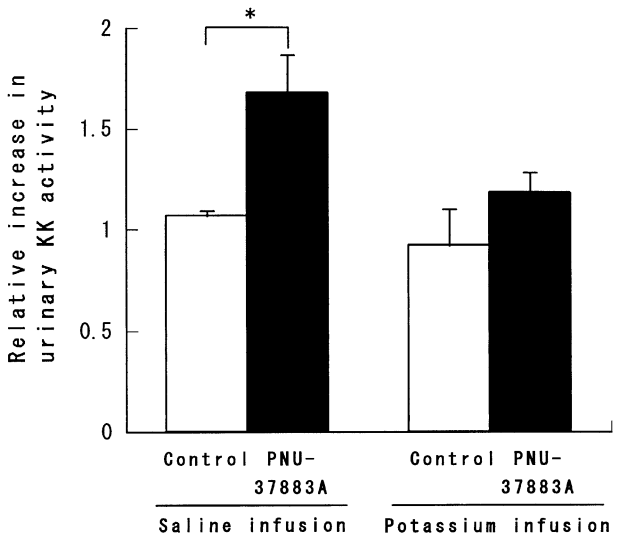

Figure 6 Relative increase in urinary KK excretion following intravenous injection of vehicle or PNU-37883A after infusion of potassium solution and saline. Values are expressed as mean ± s.e.mean; $n=3$ or $n=5$ for vehicle and $n=4$ for PNU-37883A during the infusion of potassium solution or saline. After 45 min intravenous infusion of saline, either (mM) K^+ 75, Na^+ 75 and Cl^- 150 solution was substituted or saline infusion was continued. Fifteen minutes later, either vehicle or PNU-37883A (3 mg kg^{-1}) was administered intravenously. *Significantly different from correspond-

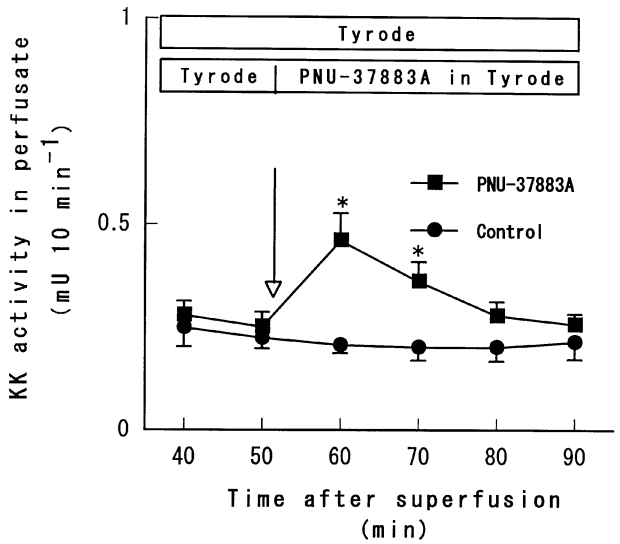

Figure 7 Changes with time in renal KK secretion after superfusion using either PNU-37883A solution or Tyrode solution. Values are expressed as mean ± s.e.mean; $n=7$ for PNU-37883A and $n=4$ for control. After a 50 min superfusion using Tyrode solution, superfusion was either replaced with 300 μM PNU-37883A (indicated by the arrow in the figure) or continued. Forty minutes later, 2% Triton X-100 solution was substituted and was superfused for 20 min. *Significantly different from corresponding control value, $P<0.05$.

renal KK corresponding to the maximum level resulting from PNU-37883A superfusion was 0.21 ± 0.02 mU 10 min^{-1}.

Discussion

KK is secreted into urine as two forms: active KK and inactive prekallikrein. The inactive KK converts to the active form through limited proteolysis in urine. It is reported that the activity of inactive KK, measured as the amidolytic activity, is about one-third that of total KK (Shimamoto *et al.*, 1982; Mohsin *et al.*, 1992). The active KK level in the urine was calculated as the difference between the amidolytic activity in the presence of SBTI and aprotinin in order to exclude nonspecific hydrolysis of the synthetic substrate. Aprotinin, which can inhibit tissue KK, reduced amidolytic activity by more than 80% relative to that in the presence of SBTI (data not shown). Therefore, this method of determination excludes urine contamination from plasma KK and other proteases. Potassium loading over a period of two to three weeks increases active and total KK levels (measured as kininogenase activity) with predominantly increased active KK (82% active KK as a percentage of the total) in the distal convoluted tubules of the mouse nephron (Guder *et al.*, 1987). Therefore, we measured active KK, a major fraction of urinary KK. Possibly, the increase in renal KK secretion seen in the present study resulted from increased secretion of the active KK form, but did not result from increasing conversion of the inactive form into the active form.

As shown in Figures 1 and 2a, potassium-induced increases in renal KK secretion were observed within 30 min after initiation of potassium administration. These results are consistent with those obtained by Obika, in which intravenous infusion of 100 mM potassium chloride solution increased renal KK secretion within 20 min in rats (Obika, 1989).

In the study using PNU-37883A, a transient increase in renal KK secretion was observed within 30 min after administration. A dose-dependent effect on the increase in renal KK secretion was also shown during treatment with PNU-37883A and glibenclamide. As shown in Figure 3, urinary excretion of sodium markedly increased on administration of PNU-37883A. Both our preliminary experiment and Ludens *et al.* (1995) showed that glibenclamide also induces natriuresis. The increase in urinary sodium excretion caused by the administration of either PNU-37883A or glibenclamide may be explained by the findings of previous reports in which PNU-37883A suppresses sodium reabsorption at the apical membrane of the thick ascending limb of Henle's loop by inhibiting the potassium recycling across the K_{ATP} channels (Wang *et al.*, 1995) required for the Na^+-K^+-2Cl^--cotransport system or the cation selective paracellular shunt pathway (Greger, 1985). However, our previous report shows that natriuresis induced by potassium loading is inhibited by simultaneous administration of Hoe 140, a bradykinin B_2 receptor antagonist (Suzuki *et al.*, 1997), which suggests that natriuresis induced by potassium as well as K_{ATP} channel blockers seen in the present study may be related to increased kinin generation resulting from the increase in renal KK secretion.

As shown in Figure 2a,b, renal KK secretion showed a greater increase with potassium loading than with saline loading. Urine volume gradually increased in the potassium loading group, whilst there was no significant difference from the saline loading group. A similar result was observed in Figure 5; when compared with the PEG200 treatment and volume–load groups, concentration of KK in urine was higher in the PNU-37883A treatment group. Therefore, it is suggested that potassium loading and PNU-37883A increased renal KK secretion *via* mechanisms that differ from the washout phenomenon.

The involvement of increased urinary sodium excretion in the augmentation of renal KK secretion by PNU-37883A can possibly be ignored; since our previous report showed that renal KK secretion did not change within 90 min after

intravenous infusion of 300 mM sodium chloride solution (Fujita *et al*., 1999).

Early increases in renal KK secretion induced by PNU-37883A and glibenclamide are similar to those induced by potassium loading. During the study in which PNU-37883A was administered with potassium, no additive effect of PNU-37883A on the potassium-induced increase in renal KK secretion was observed. These findings suggest that the early augmentation of renal KK secretion by potassium is mediated through the same pathway as that of the K_{ATP} channel blocker.

Insulin secretion is increased by a high concentration of extracellular potassium resulting from membrane depolarization of β cells and followed by enhanced calcium influx (Henquin & Lambert, 1974). It has been reported that the closure of K_{ATP} channels also increases insulin secretion through the same mechanism (Henquin & Meissner, 1982). Similarly, the present study demonstrates that administration of a K_{ATP} channel blocker such as PNU-37883A or glibenclamide, leads to an increase in renal KK secretion. As Figure 7 shows, a direct augmentation of renal KK secretion by PNU-37883A administration also occurred within 10 min following the addition of PNU-37883A to sliced kidney cortices. Additionally, a separate experiment reported that this increase was 31 or 49% suppressed after the addition of nifedipine or nickel chloride respectively, both of which are voltage-dependent calcium channel blockers (Hayashi *et al*., 1999). We consider it a possibility that the augmentation of renal KK secretion induced by potassium loading and K_{ATP} channel blockers may result from membrane depolarization of KK secreting cells in the renal connecting tubules, followed by an enhanced calcium influx.

In this study, both potassium loading, known to have antihypertensive effects and K_{ATP} channel blockers augment renal KK secretion. These effects do not seem to be the result of a washout phenomenon, although they probably occurred through the same mechanism. Renal KK secretion is decreased in both hypertensive subjects (Margolius *et al*., 1974; Mersey *et al*., 1979) and in the development of hypertension in hypertensive animal models (Mohsin *et al*., 1992). We previously reported that the renal KK-kinin system blunts the development of hypertension in experimental models through natriuresis (Majima *et al*., 1991; 1995; Ito *et al*., 1999). Therefore, it is suggested that both potassium loading and K_{ATP} channel blockers probably cause antihypertensive effects by increasing renal KK secretion followed by natriuresis. Elucidation of mechanisms responsible for the increase of renal KK secretion by potassium loading may therefore be an important step in the development of new antihypertensive drugs.

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