

ORIGINAL ARTICLE

An ATP-sensitive potassium channel blocker suppresses sodium-induced hypertension through increased secretion of urinary kallikrein

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It is suggested that an ATP-sensitive potassium channel blocker suppresses sodium-induced hypertension through increased secretion of urinary kallikrein. We reported that glibenclamide, an ATP-sensitive potassium channel blocker, accelerated dose-dependent secretion of renal kallikrein in sliced kidney cortex and *in vivo* in rats. In vehicle-treated normal Brown-Norway-Kitasato (nBN-Ki) rats, the administration of glibenclamide increased urinary kallikrein secretion, but changed neither the systolic blood pressure nor the urinary sodium on low (0.3%) NaCl diets. Although on high (8%) NaCl diets, the systolic blood pressure of the nBN-Ki rats administered glibenclamide was significantly lower ($P < 0.05$). The urinary levels of kallikrein and sodium of the nBN-Ki rats administered glibenclamide were significantly increased ($P < 0.05$, glibenclamide vs. vehicle). A similar result was obtained with a kidney-selective ATP-sensitive potassium blocker, N,N'-dicyclohexyl-4-morpholinecarboxamide (U18177), in SD rats. Mutant kininogen-deficient Brown-Norway Katholiek (muBN-Ka) rats fed high (8%) NaCl diets showed an increase in urinary kallikrein levels, but showed neither hypotensive nor natriuretic actions by glibenclamide. A bradykinin B₂ receptor antagonist, 8-[3-[N-(E)-3-(6-acetamidopyridin-3-yl) acryloylglycyl]-N-methylamino]-2,6-dichlorobenzyloxy]-2-methylquinoline (FR173657), which was administered to SD rats, together with glibenclamide, abolished the hypotensive and natriuretic effects of glibenclamide in high-sodium (8%NaCl) hypertension, despite an accelerated secretion of urinary kallikrein. Therefore, these results indicate that glibenclamide, an ATP-sensitive potassium channel blocker suppressed sodium-induced hypertension through sodium excretion from the kidney resulting from accelerated secretion of urinary kallikrein.

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Keywords: ATP-sensitive potassium channel blocker (glibenclamide); sodium-induced hypertension; renal kallikrein secretion

INTRODUCTION

In the early 1970s, it was reported that patients with essential hypertension excrete less urinary kallikrein than do normal healthy participants.^{1–3} Kallikrein is the enzyme responsible for the generation of kinins, kallidin and bradykinin from the precursor protein, kininogen. Bradykinin is well known to induce vasodilation, increase renal blood flow and excrete water and sodium from the kidney.¹ It was recently revealed that the renal kallikrein–kinin system plays an important role in the localization of the kidney. The components of the tissue kallikrein–kinin system are localized along the distal nephron.^{4,5} After the tubulo-glomerular feedback system completes its role at the distal, connecting tubule cells secrete renal kallikrein, whereas the adjacent collecting tubule cells secrete kininogen.⁴ Bradykinin B₂ receptors are located along the cortical and medullary collecting ducts, which are downstream to the connecting tubules.^{5–8}

Mutant Brown-Norway-Katholiek (muBN-Ka) rats are capable of producing kininogens in the liver, but cannot release them into the bloodstream, because of the point mutation of Ala163 to Thr in the structure of kininogen.⁹ Consequently, muBN-Ka rats excreted a much smaller amount of kinin in the urine than did normal rats of the same strain.¹⁰ In these experiments, muBN-Ka rats were compared with normal Brown-Norway Kitasato (nBN-Ki) rats, which are normal rats of the same original strain. BN-Ka rats fed a low (0.3%) NaCl diet showed neither physiological disorders nor a rise in systolic blood pressure (SBP).¹¹ However, a diet containing 2% NaCl increased SBP to approximately 170 mm Hg in muBN-Ka rats, but did not affect it in nBN-Ki rats.¹⁰ In addition, it was observed that sodium accumulated particularly in cells, such as erythrocytes and in body fluids, such as the cerebrospinal fluid, in muBN-Ka rats.^{10,12} It is important that sodium accumulation in the cells caused a 10-fold and

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a 30-fold increase in vascular sensitivity to angiotensin II and epinephrine, respectively.¹³ Consequently, a dysfunction of the renal kallikrein–kinin system leads to salt sensitivity in rats.

The increased secretion of urinary kallikrein because of potassium has been reported in animals^{14–16} and in humans with hypertension.¹⁷ Indeed, using the sliced kidney cortex, we have shown that potassium chloride in the perfusate accelerates the secretion of kallikrein in a concentration-dependent manner.¹⁸ Moreover, immediately after intravenous infusion of potassium gluconate solution into anesthetized Sprague–Dawley (SD) rats, urinary kallikrein secretion was accelerated with diuresis and natriuresis.^{19,20} Glibenclamide, an ATP-sensitive potassium channel blocker, is known to release insulin from the β cell in the pancreas and is used for treatment of diabetes mellitus. It was reported that glibenclamide inhibited the transporter of Na^+ and K^+ in the ascending thick limb of Henle's loop by blocking K^+ recycling across the apical membrane.²¹ In addition, inhibition of K^+ secretion in the collecting tubules occurred by decreasing the activity of apical K^+ channels preventing kaliuresis.²¹ A kidney-selective ATP-sensitive potassium channel blocker, U18177 and its 1-adamantyl analog (U37883A) were developed as diuretics and as more selective blockers of the kidney ATP-sensitive channel.^{22,23} Glibenclamide and U37883A also dose-dependently accelerated the secretion of kallikrein from the kidney.^{18–20} A bradykinin B_2 receptor antagonist, FR173657, decreased the secretion of bradykinin and natriuresis.¹⁵ Therefore, diuresis and natriuresis induced by potassium or an ATP-sensitive potassium channel blocker may be mediated by the same mechanism of activation of the renal kallikrein–kinin system.

This study examined whether the sodium-induced rise of blood pressure is suppressed by the administration of either glibenclamide or U18177, through accelerated release of urinary kallikrein, and whether this effect is removed by FR173657.

METHODS

Mutant kininogen-deficient BN-Ka (muBN-Ka) rats and normal BN-Ki (nBN-Ki) rats, and male SD strain rats (specific pathogen-free, 6 weeks old from the Shizuoka Laboratory Animal Center in Hamamatsu) were used. All rats were given normal rat chow containing 0.3% sodium (NMF, Oriental Yeast Co., Tokyo, Japan). They were housed at constant humidity ($60 \pm 5\%$) and temperature ($25 \pm 1^\circ\text{C}$), 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: glibenclamide (Sigma Chemical Co., St Louis, MO, USA), U18177 (a kidney-selective ATP-sensitive potassium blocker, Sigma-Aldrich Inc.) and FR173657 (a bradykinin B_2 receptor antagonist, kindly provided by Fujisawa Pharmaceutical Co. Ltd., Osaka, Japan). We have reported earlier that glibenclamide accelerated dose-dependent secretion of renal kallikrein, *in vivo*, in rats and glibenclamide of 30 mg/kg also acted more strongly.¹⁹ Therefore, we administered glibenclamide of 60 mg/kg/day (30 mg/kg, two times a day).

Induction of hypertension

Salt-induced hypertension: muBN-Ka, nBN-Ki and SD rats were fed a diet to which was added 8% NaCl and were provided distilled water during experimental periods.

Measurement of systolic blood pressure

Systolic blood pressure of unanesthetized rats was determined with tail-cuff plethysmography (model BP-98A, Softron, Tokyo, Japan), as reported previously.¹⁰

Blood collection

Blood was collected from the carotid artery of each strain from rats under light ether anesthesia, at day 8 of the experimental periods, as reported previously.¹⁰

For the determination of the sodium concentration of erythrocytes, blood was collected directly into tubes containing ice-chilled isosmotic lithium chloride solutions.

Collection of urine and measurement of urinary creatinine and sodium

Twenty-four-hour urine samples from individual rats during the experimental periods were collected in metabolic cages. Urine volume was recorded at the end of the 24-h period. Urinary creatinine was measured by a kinetic method using Jaffe's reaction.²⁴ Urinary sodium was determined by using ion-selective electrodes (Fuji Dri-Chem Slide Na-K-Cl, Fuji Dri-Chem 800V; Fuji Film Co. Ltd., Tokyo, Japan).

Measurement of urinary active kallikrein

Activity of the active kallikrein in urine collected in metabolic cages over 24 h was measured using a peptidyl fluorogenic substrate selective for the tissue kallikrein, Pro-Phe-Arg-methyl-coumarinylamide (Peptide Institute, Minoh, Osaka, Japan).¹¹ After a 10-fold dilution of the original urine with 0.2 mol l^{-1} of the Tris-HCl buffer (pH 7.8), 10 μl of the diluted urine was incubated with 1 ml of a $5 \times 10^{-5} \text{ mol l}^{-1}$ of substrate solution in 0.05 mol l^{-1} of the Tris-HCl buffer containing 0.1 mol l^{-1} of NaCl and 0.01 mol l^{-1} of CaCl_2 (pH 8.0). One arbitrary unit (AU) was defined as 1×10^{-10} mol of the Tris-HCl buffer containing 0.1 mol l^{-1} of NaCl and 0.01 mol l^{-1} of CaCl_2 (pH 8.0). One AU was defined as 1×10^{-10} mol of 7-amino-4-methylcoumarin for 10 min per microliter of urine at 37°C .¹⁰ The activity was termed as that which divided the active kallikrein by creatinine (Cr).

Measurement of erythrocyte sodium concentration

Erythrocyte from the carotid artery was washed three times with an isotonic lithium chloride solution. The pellets of the obtained erythrocyte were lysated with distilled water, and the resulting sample was diluted with 0.5 N of HNO_3 . Intracellular sodium concentrations of erythrocyte (RBC[Na]i) were determined with plasma optical emission spectrometer (SPS-7700, Seiko Instruments Inc., Chiba, Japan). The results were expressed as mmol l^{-1} red blood cell (RBC).

Measurement of intracellular sodium concentrations of vascular tissues

Measurement of intracellular sodium concentrations of vascular tissues (vascular [Na]i) was performed using abdominal aorta. The abdominal aorta was isolated and the connective tissues around the aorta were carefully removed. The vascular tissues were homogenized with distilled water (1 ml for 100 mg of wet tissue of aorta) with polytrone. The sodium concentrations in the supernatant of the homogenates were determined with the above-mentioned plasma optical emission spectrometer. The results were expressed as mmol kg^{-1} of wet tissue.

Measurement of blood glucose

Blood glucose was measured by enzymatic colorimetry using D-glucose oxidase (Medisefu-Mini, Terumo corp., Tokyo, Japan). This method used arterial blood of about 1.2 μl at any time till the end of the experiment.

Statistical analysis

Values are expressed as mean \pm s.e.m. One-way ANOVA or Student's *t*-test or *post hoc* Dunnett's test was used to evaluate the statistical significance of the difference. Differences with a probability of 5% or less were considered to be significant.

RESULTS

Normal BN-Ki rats, which continued a diet containing 0.3% of NaCl for 5 days, did not show a rise of SBP on day 5 ($112 \pm 3 \text{ mm Hg}$, $n=6$). Administration of glibenclamide (30 mg kg^{-1} , p.o., two times a day) significantly increased the secretion of urinary kallikrein to $8.5 \pm 1.0 \text{ AU mg}^{-1} \text{ Cr}$ compared with $3.9 \pm 0.5 \text{ AU mg}^{-1} \text{ Cr}$ in

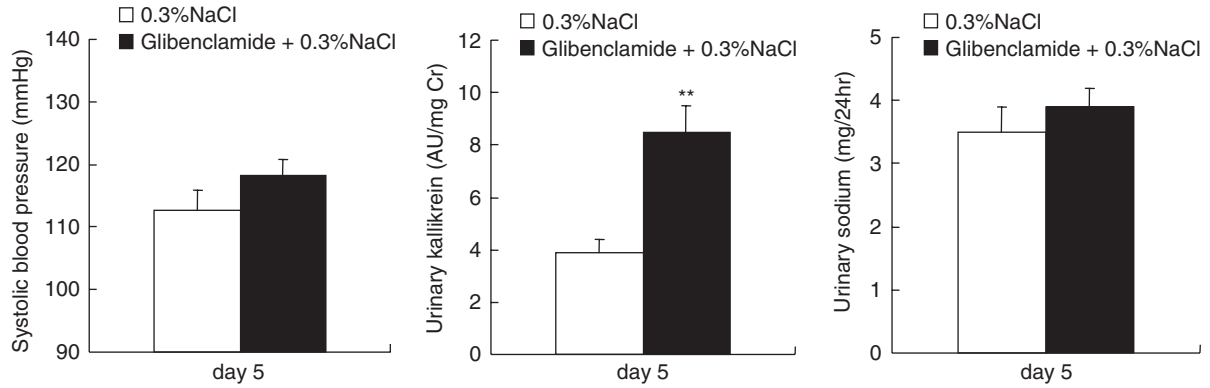


Figure 1 Systolic blood pressure (SBP), urinary kallikrein and urinary sodium in normal Brown Norway (nBN-Ki) rats fed 0.3% NaCl diet. Open columns are a vehicle group. Glibenclamide (60 mg kg^{-1}) was administered to rats throughout the experiment period. Values are mean \pm s.e.m., $n=6$. ** $P<0.01$ vs. value in the vehicle control group.

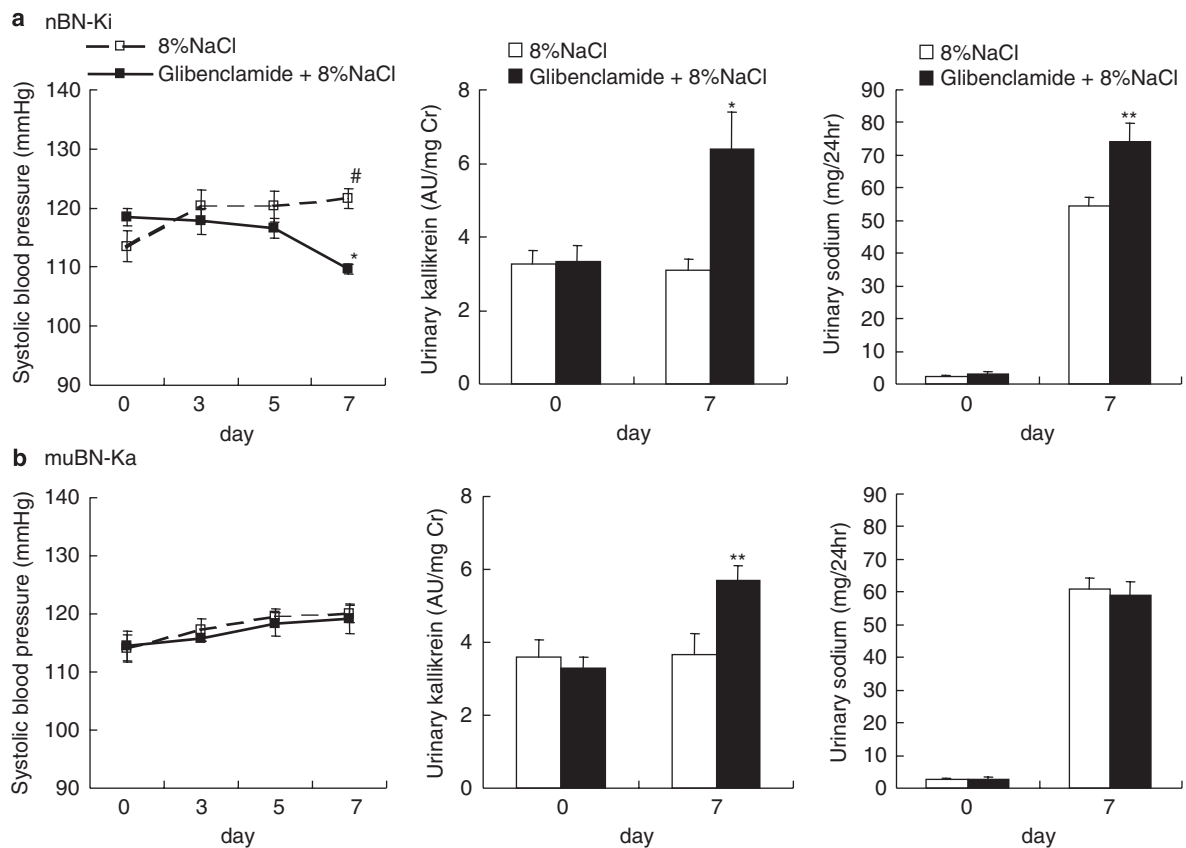


Figure 2 Line and columns show results of systolic blood pressure (SBP), urinary kallikrein and urinary sodium in normal Brown Norway-Kitasato (nBN-Ki) rats (a) and mutant Brown Norway Katholiek (muBN-Ka) rats (b) fed an 8% NaCl diet. Straight line and open columns represent the vehicle control group. Broken line and closed columns represent the glibenclamide-treated group (60 mg kg^{-1}). Blood pressure was measured on days 0, 3, 5 and 7. Urinary kallikrein and urinary sodium were measured on days 0 and 7. Values are mean \pm s.e.m., $n=6$. # $P<0.05$ vs. SBP on day 0 in the vehicle control group. * $P<0.05$, ** $P<0.01$ vs. value in the vehicle control group.

vehicle-treated rats (Figure 1). Neither SBP nor urinary sodium was changed by glibenclamide. In our earlier report, the SBP of normal BN-Ki rats that were fed a high (8%) NaCl diet, increased from $124 \pm 4 \text{ mmHg}$ on day 0, $146 \pm 1 \text{ mmHg}$ on day 7 and to $163 \pm 3 \text{ mmHg}$ on day 14.¹⁰ In this experiment, during 7 days, the SBP of normal BN-Ki rats increased from 113.5 ± 2.6 to $122 \pm 2 \text{ mmHg}$ under an 8% NaCl diet, whereas the SBP of rats

that were administered glibenclamide fell to $110 \pm 1 \text{ mmHg}$ on day 7 (Figure 2a). Urinary kallikrein on day 7 increased to $6.4 \pm 1.0 \text{ AU mg}^{-1} \text{ Cr}$, compared with $3.4 \pm 0.3 \text{ AU mg}^{-1} \text{ Cr}$ in vehicle-treated rats. Urinary sodium excretion increased in vehicle-treated rats from $2.4 \pm 0.4 \text{ mg/24h}$ on day 0 and $54.3 \pm 2.9 \text{ mg/24h}$ on day 7, but increased to $74.0 \pm 7.3 \text{ mg/24h}$ on day 7 in rats administered with glibenclamide.

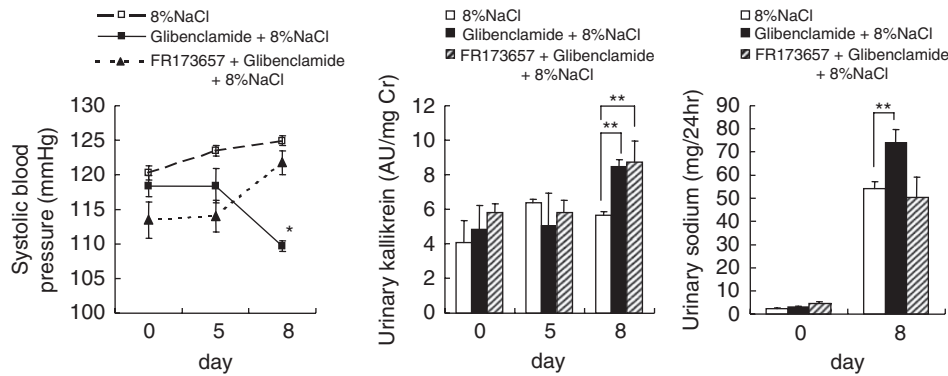


Figure 3 Systolic blood pressure (SBP), urinary kallikrein and urinary sodium in Sprague–Dawley strain (SD) rats fed an 8% NaCl diet. A bradykinin B₂ receptor antagonist (FR173657) was given to rats at a dose of 100 mg/kg. Blood pressure, urinary kallikrein and urinary sodium were measured on days 0, 5 and 8. Values are mean \pm s.e.m., $n=6$. * $P<0.05$, ** $P<0.01$ vs. value on same days in the vehicle control group.

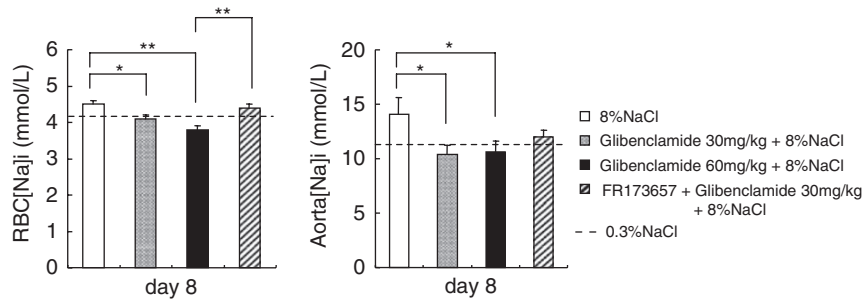


Figure 4 Columns show results of intracellular sodium concentration of erythrocyte (RBC[Na]i) and aorta in SD rats fed an 8% NaCl diet on day 8. Broken line shows the mean intracellular sodium concentration in SD rats fed a 0.3% NaCl diet on day 8. Values are mean \pm s.e.m., $n=6-10$. * $P<0.05$, ** $P<0.01$ vs. value in the vehicle-treated group.

Kininogen-deficient BN-Ka rats that were fed an 8% NaCl diet, also raised SBP (120 ± 2 mm Hg) and increased urinary sodium (61 ± 3 mg/24 h) on day 7 (Figure 2b). Glibenclamide did not inhibit the rise of SBP and the increase in urinary sodium excretion on day 7, although urinary kallikrein increased significantly to 5.7 ± 0.4 AU/mg Cr.

In SD strain rats that were fed an 8%NaCl diet, suppression of SBP rise by glibenclamide was ceased by the administration of a bradykinin B₂ antagonist, FR173657 (100 mg kg⁻¹; p.o., two times a day) (Figure 3). The administration of glibenclamide significantly decreased SBP from 118 ± 2 mm Hg on day 0 to 110 ± 1 mm Hg on day 8. The administration of FR173657 did not decrease SBP. Nevertheless, urinary kallikrein was increased by glibenclamide, from 4.9 ± 1.4 AU/mg Cr on day 0, to 8.5 ± 0.4 AU/mg Cr on day 8 and from 5.8 ± 0.5 AU/mg Cr on day 0, to 8.7 ± 1.2 AU/mg Cr on day 8. However, the increase in urinary sodium was only found in the solo administration of glibenclamide. These results indicated that the effects of glibenclamide on the blood pressure and urinary sodium were abolished by FR173657.

When intracellular sodium concentrations were determined, SD strain rats that were fed an 8% NaCl diet increased in RBC[Na]i compared with SD strain rats that were fed a 0.3% NaCl diet (Figure 4, left panel, 0.3% NaCl: 4.1 ± 0.4 mmol l⁻¹, 8% NaCl: 4.5 ± 0.4 mmol l⁻¹). In addition, sodium concentrations of vascular smooth muscles in the aorta showed similar results (Figure 4, right panel, 0.3% NaCl: 11.4 ± 0.7 mmol l⁻¹, 8% NaCl: 14.1 ± 1.5 mmol l⁻¹). Meanwhile, glibenclamide dose-dependently reduced RBC[Na]i, which were elevated by an 8% high-sodium diet

(Figure 4, left panel, 30 mg day⁻¹: 4.1 ± 0.1 mmol l⁻¹, 60 mg day⁻¹: 3.8 ± 0.1 mmol l⁻¹). The effect of 60 mg kg⁻¹ of glibenclamide was removed by the simultaneous administration of FR173657 (Figure 4, left panel). The same result was obtained in the aorta. Glibenclamide reduced the intracellular sodium concentration of the vascular smooth muscle, which was elevated by an 8% high-sodium diet (Figure 4, right panel, 30 mg day⁻¹: 10.4 ± 0.8 mmol l⁻¹, 60 mg day⁻¹: 10.6 ± 1.0 mmol l⁻¹). The effect of 30 mg kg⁻¹ of glibenclamide was also removed by the simultaneous administration of FR173657 (Figure 4, right panel).

A kidney-selective ATP-sensitive potassium blocker, U18177, also significantly suppressed the rise of SBP in the SD strain rats that were fed an 8% NaCl diet (122 ± 2 mm Hg on day 4) in comparison with vehicle rats (112 ± 1 mm Hg) (Figure 5). Moreover, urinary sodium excretion (84.5 ± 7.3 mg/24 h on day 5) was increased through the markedly accelerated secretion of urinary kallikrein (7.1 ± 0.3 AU/mg Cr on day 4).

No rat died during the experiments, in spite of the use of a sufficient dose of glibenclamide. The blood glucose levels by the end of the experiment (day 8) were reduced slightly, but dose dependently, with glibenclamide (0.3% NaCl: 177 ± 13 mg per 100 ml⁻¹, 8%NaCl: 181 ± 6 mg per 100 ml⁻¹, glibenclamide 30 mg day⁻¹: 169 ± 4 mg per 100 ml⁻¹, glibenclamide 60 mg day⁻¹: 160 ± 8 mg per 100 ml⁻¹).

DISCUSSION

The importance of dietary sodium chloride in the regulation of blood pressure has received much attention over the past few years, but this area of research and knowledge is still being debated.²⁵ We have

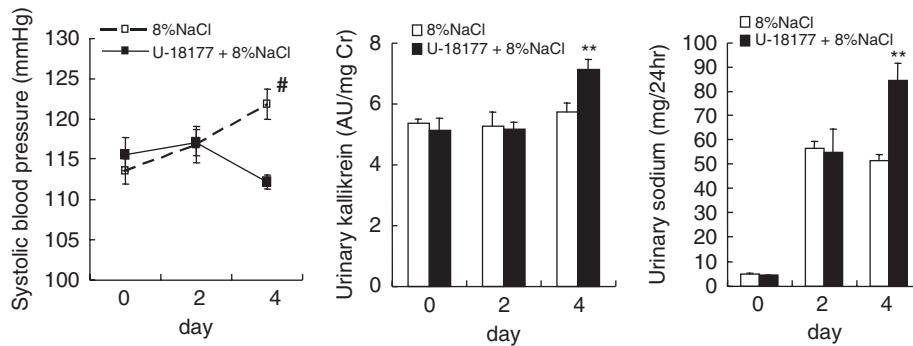


Figure 5 Systolic blood pressure (SBP), urinary kallikrein and urinary sodium in Sprague–Dawley strain (SD) rats fed an 8% NaCl diet. A kidney-selective ATP-sensitive potassium blocker (U-18177) was given to rats (60 mg kg^{-1}). Blood pressure, urinary kallikrein and urinary sodium were measured on days 0, 2 and 4. Values are mean \pm s.e.m., $n=6$. [#] $P < 0.05$ vs. SBP on day 0 in the vehicle control group. ^{**} $P < 0.01$ vs. value on same days in the vehicle control group.

reported earlier that high sodium loading, which included more than a 4% NaCl diet, raised the SBP of normal dosage-dependent BN-Ki rats, whereas in contrast, mutant kininogen-deficient BN-Ka rats were sensitive to sodium, so that a loading of 2% NaCl diet raised SBP. In brief, we infer that the SBP is increased by the relation between the dysfunction of the renal kallikrein–kinin system and sodium loading.

Initially, we confirmed that the administration of glibenclamide accelerated the secretion of urinary kallikrein and thereby changed SBP and urinary sodium. The accelerated secretion of urinary kallikrein was not tied to the increase of sodium excretion or to the suppression of SBP rise as long as the rats were put on a normal 0.3% of NaCl diet (Figure 1). On the other hand, it showed the rise of SBP and increase in urinary sodium excretion with the secretion of urinary kallikrein, when the same rats were put on loading of a high 8% NaCl diet (Figure 2a). Increased kallikrein secretion might stimulate sodium excretion through kinin formation, which was judged from the effect of a B_2 antagonist on urinary sodium excretion treated with an ATP-sensitive potassium blocker.

Further, we confirmed whether the natriuretic actions and the suppression of SBP rise appeared by the administration of glibenclamide in the presence of the dysfunction of the renal kallikrein–kinin system. In mutant kininogen-deficient BN-Ka rats, glibenclamide had no effect on the suppression of SBP rise and the increase in urinary sodium excretion (Figure 2b). Similarly, when FR173657 was administered simultaneously with glibenclamide, the suppression of SBP rise and the increase in urinary sodium excretion did not occur, although urinary kallikrein increased (Figure 3). These results showed that the natriuretic actions and the suppression of SBP rise did not appear unless the kallikrein–kinin system functioned normally under high sodium intake, even if glibenclamide increased the secretion of kallikrein. In other words, the natriuretic actions and thereby the suppression of the SBP rise occurred by the action of kallikrein, and its secretion increased by glibenclamide.

The measurement of intracellular sodium concentrations not only in RBC but also in vascular smooth muscles revealed that the high sodium intake resulted in the accumulation of sodium in the cells. Glibenclamide administration really canceled the accumulation of sodium possibly through increased secretion of kallikrein (Figure 4). This was confirmed by a bradykinin B_2 antagonist administration, the receptor signaling of which was reported to increase renal sodium excretion (Figure 4). However, there was no significant difference in the intracellular concentrations of vascular smooth muscles between glibenclamide administration and bradykinin B_2 antagonist adminis-

tration. RBC[Na]_i has been used for many studies of hypertension, because RBC is a specimen that can easily be obtained and has the lowest quantity of Na^+/K^+ ATPase activity in per tissue weight.²⁶ Meanwhile, in vascular smooth muscles, isolation of the abdominal aorta needs a close technique. Furthermore, the reduction of $\text{Na}^+/\text{Ca}^{2+}$ exchanger activity, the increase of Na^+ influx and Na^+/K^+ ATPase activity have been reported in experimental hypertension with a high salt diet.^{27–29} We think that these different results were obtained with RBC and vascular smooth muscles because of the influence of various factors. It is plausible that increased accumulation of sodium in smooth muscles may become sensitive to the evaluation of vasoconstricting substances through increased mobilization of calcium from the intracellular store sites.

In this experiment, we evaluated natriuretic actions and the suppression of SBP rise using another ATP-sensitive potassium blocker, U18177 (Figure 5). Nevertheless, the period of administration with U18177 was brief as opposed to glibenclamide, natriuretic actions and the suppression of the SBP rise were observed. We could speculate that this effect of U18177 appeared more rapidly than that of glibenclamide, because U18177 was an ATP-sensitive potassium channel blocker of a selective kidney.

Collectively, it was suggested that glibenclamide, an ATP-sensitive potassium channel blocker, suppressed sodium-induced hypertension through the increased secretion of urinary kallikrein. We reviewed the kind of results that would be obtained, whether a similar experiment was carried out on Dahl salt-sensitive rats. There are no experimental reports that used glibenclamide in Dahl salt-sensitive rats. Instead, it was known that urinary kallikrein decreased in Dahl salt-sensitive rats.³⁰ Therefore, glibenclamide would suppress sodium-induced hypertension through the increased secretion of urinary kallikrein in Dahl salt-sensitive rats. We suggested that kallikrein played a large role as a mechanism of hypertension by a natriuretic fine adjustment in the kidney.

It was difficult to explain the mechanisms of glibenclamide on the secretion of renal kallikrein at the cellular level of the connecting tubule. When a high sodium diet was given to rats, the sodium excreted from the glomerulus was markedly increased in spite of increased reabsorptions in the proximal tubules and other segments of the nephrons. It was known that more than 90% of sodium secreted from the glomerulus was reabsorbed by the portions of nephrons situated before the collecting ducts. Kallikrein was secreted mainly from the connecting ducts that were located before the collecting ducts where B_2 receptors were present. The functional relevance of urinary

kallikrein to sodium handling was restricted to the collecting ducts because of the localization of the components of the renal kallikrein systems. We recognized the increase in excretion of sodium under high sodium diets even when the urinary kallikrein secretion was unchanged in normal rats as well as in kininogen-deficient rats (Figure 2). But, as shown in Figures 2a and 3, the urinary kallikrein that was increased by glibenclamide by 50–100% was active in terms of the increased excretion of sodium. The segment of the collecting ducts was the final portion where sodium handling could be adjusted. As shown here, we would like to show the activity of the renal kallikrein–kinin system to enhance sodium excretion. Glibenclamide inhibited the transport of Na^+ and K^+ in the ascending thick limb of Henle's loop by blocking K^+ recycling across the apical membrane.²¹ This inhibition led to the delivery of the large amounts of fluid and Na^+ at the initial and cortical collecting tubule.³¹ This action was similar to a loop diuretic such as furosemide. Moreover, the secretion of K^+ was stimulated with increased luminal pressure at the distal tubule flow dependently. In fact, the maxi K^+ channel, besides the ATP-sensitive potassium channel in the apical membrane, was responsible for the flow-dependent K^+ secretion by coupling with the stretch-activated cation channel in the connecting tubule of the rabbit.³² On the other hand, kaliuresis was not caused by glibenclamide, because its control depended on the initial and cortical collecting tubules.³³ We could speculate that this increased K^+ in the lumen led to the inhibition of the ATP-sensitive potassium channel and depolarization of the cell of the connecting tubules. Therefore, these results suggested that the inhibition of the ATP-sensitive potassium channel by glibenclamide might increase the secretion of renal kallikrein.

It is possible that the secretion of renal kallikrein was explained by the calcium influx. In the experiment using dissected renal-connecting tubule of SD rats, we reported that potassium-induced secretion of renal kallikrein was inhibited by disodium-ethylenediamine-tetraacetate dihydrate (EDTA-2Na) and by NiCl_2 .³⁴ In addition, an L-type calcium channel blocker, verapamil, slightly inhibited the secretion of renal kallikrein. An IP_3 receptor-selective antagonist, xestspingonin C and a ryanodine-sensitive receptor blocker, ruthenium, also yielded the same results. The maxi K^+ channel on the apical membrane in the distal nephron with the epithelial calcium channel (ECaC) was inhibited by verapamil.³² These results showed that calcium influx through ECaC was necessary for the mobilization of the intracellular calcium store for the secretion of renal kallikrein. There was a possibility that the calcium influx was induced by the action through the $\text{Na}^+/\text{Ca}^{2+}$ exchanger on the vasolateral membrane,³⁵ although this was not clarified in this study.

At present, however, we consider that the depolarization that resulted from increased calcium influx may accelerate the secretion of renal kallikrein because of the changes in the transport of electrolyte caused by glibenclamide-induced ATP-sensitive potassium channel blockade. More accurate knowledge of a mechanism by glibenclamide will be obtained by electrochemical studies using a dissected renal-connecting tubule cell.

We considered whether other effects of glibenclamide affected blood pressure and natriuresis. Glibenclamide releases insulin from the β cell in the pancreas. Although insulin is generally known to have a hypoglycemic action, it produces various actions in metabolism. Insulin acted on the distal renal tubule to retain Na^+ and water.³⁶ Insulin also stimulated the cell-membrane Na^+/K^+ ATPase, which raised intracellular Na^+ concentrations in vascular smooth muscles and increased systolic Ca^{2+} levels, which enhanced contractility and increased peripheral resistance.^{37,38} On one hand, the main action of insulin is hypoglycemia. Through its effect on the central nervous

system, sympathetic tension might be enhanced. No hypoglycemia was measured at any time during the experimental period. We could not confirm whether blood pressure was changed by hypoglycemia, because we did not measure blood glucose on any day. On the other hand, insulin was an endothelium-dependent vasodilator, releasing nitric oxide (NO) from the endothelium, which relaxes the vascular smooth muscle.³⁹ Therefore, insulin released by glibenclamide might influence blood pressure and natriuresis partly.

In conclusion, this study pointed out the possibility that glibenclamide, an ATP-sensitive potassium channel blocker, activated the kallikrein–kinin system, caused diuretic and natriuretic action and finally declined systolic blood pressure. Although this study is an animal experiment under an unusually high salt diet, we suggest that it would lead to the development of a critical prevention of hypertension, if molecular mechanisms of the kallikrein–kinin system were elucidated in future.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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