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Title

Effect of *In-vivo* administration of nafamostat on the onset of renal hyperkalemia and association of urine kallikrein in rats.

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Running title

In vivo effect of nafamostat on renal hyperkalemia and kallikrein.

Summary

Hyperkalemia is known as a major adverse effect of nafamostat, a serine protease inhibitor, and its mechanism is thought as inhibition of an amiloride-sensitive Na^+ channel. Kallikrein is a serine protease and can activate the amiloride-sensitive Na^+ channel. The study examined whether urine potassium excretion and kallikrein activity decrease in a nafamostat-induced hyperkalemia model. First, rats were administered nafamostat (1.2 mg/kg) or 5% glucose by a continuous intravenous infusion (c.i.) under general anesthesia, and urine and blood samples were collected every 15 minutes from 30 minutes before and by 90 minutes after drug administration. Next, nafamostat (0.13, 0.4, 1.2 mg/kg c.i.) or 5% glucose was co-administered by a low-dose amiloride (3.33 μg/kg i.v. followed by 12 μg/kg c.i.) in the same time course. Changes in serum Δ potassium concentrations, a difference from the baseline, and those in urine potassium excretion and kallikrein activity, and their means of the last three points were evaluated. Statistical analyses were conducted by a repeated measure ANOVA($\alpha = 0.05$, one-tail test). In results, increasing and decreasing trends were observed regarding with the changes inΔpotassium and potassium excretion, and their corresponding means of the last three points, respectively, in the nafamostat group compared with control group. In the next experiment, changes in Δ the potassium and those in potassium excretion, and their corresponding means of the last three points significantly increased and decreased, respectively, in the nafamostat group compared with the control group. A significant decrease in urine kallikrein activity was shown by the maximum

dose of nafamostat regarding with the means of the last three points compared to the control. In conclusions, nafamostat induced renal hyperkalemia *in vivo* via inhibition of the amiloride-sensitive Na^+ channel, which was associated with inhibition of urine kallikrein activity.

Key words

Tissue kallikrein, Nafamostat, Amiloride, Renal hyperkalemia, Rat.

Introduction

In the present study, we investigated whether nafamostat administration induced hyperkalemia and reduced urinary potassium excretion and urinary kallikrein activity in rats. Nafamostat is a serine protease inhibitor and its target is the proteolytic enzymes including thrombin, activated coagulation factors (XIIa, Xa, VIIa), kallikrein, plasmin, and complements [1)]. It is clinically used for the treatment of acute pancreatitis, disseminated intravascular coagulation and prevention for coagulation of the circulating blood during the extracorporeal circulation in the patients with bleeding or bleeding tendency (hemodialysis and plasmapheresis). Hyperkalemia is known as a major and serious adverse effect caused by nafamostat in several cases [2-7)]. Renal and extrarenal potassium imbalance have been reported as mechanisms for the nafamostat-induced hyperkalemia, i.e., a decrease in urine potassium excretion by inhibition of an amiloride-sensitive Na+ currents in the apical membrane of isolated renal

cortical collecting duct cells in rabbits [8] and suppression of potassium influx in the erythrocytes by inhibition of a Na^+-K^+ ATPase in healthy men [9].

The authors have studied administration of a high potassium solution to rats increases urine kallikrein excretion [10] and a high potassium has a direct stimulatory effect on kallikrein secretion in renal cortical slices and dissected renal connecting tubules [11, 12]. We have also studied administration of furosemide, a loop diuretic and used for the treatment of hyperkalemia, to rats increases urine kallikrein excretion accompanying an increase in urine potassium excretion [13]. Kallikrein is known as a serine protease and there are two types of kallikrein existed in the plasma and tissues. Renal kallikrein is the tissue one and is synthesized and secreted by the connecting tubule cells of the distal nephron. Functions of tissue kallikrein are thought to release kinins from kininogens, which increases renal blood flow and natriuresis by activation of the B2 receptors [14], and to activate the amiloride-sensitive Na^+ channel by promoting a cleavage of that channel [15].

Therefore, the authors thought the following hypothesis: nafamostat induces hyperkalemia by decreasing urine potassium excretion via inhibition of the amiloride-sensitive Na^+ channel which is accelerated by inhibition of renal kallikrein. The aims of the present study are to investigate the *in vivo* effect of nafamostat on the onset of renal hyperkalemia in the absence and presence of amiloride, and whether the nafamostat-induced hyperkalemia is associated with a decrease in urine kallikrein activity in rats.

Materials and Methods

Animals

Nine to eleven-week-old male Wistar-Imamichi rats weighing 270 g to 350 g were used (Japan SLC Inc. Shizuoka, Japan). They were delivered to the laboratory animal center one to three weeks before the experiment date to acclimatize. The animals were housed under constant humidity and temperature and a 12-h light/dark cycle, and maintained on a certified diet and tap water ad libitum. The study was approved by the Research Center for Laboratory Animals, Dokkyo Medical University (approval number 1120).

Reagents

Sevoflurane and pentobarbital (Somnopentyl®) were purchased from Mylan（Tokyo, Japan）and Kyoritsu Seiyaku (Tokyo, Japan), respectively. Nafamostat and amiloride were purchased from FUJIFILM Wako Pure Chemical Corp. (Osaka, Japan). Both reagents were dissolved in 5% glucose. Soybean trypsin inhibitor and aprotinin were purchased from Warthington Biochem Corp. (Lakewood, NJ, USA) and FUJIFILM Wako Pure Chemical Corp. (Osaka, Tokyo), respectively. They were dissolved in saline. A fluorogenic synthetic substrate of urine kallikrein, L-Pro-Phe-Arg 4-Methyl-Coumaryl-7-Amide, and its reference, 7-amino-4-methylcoumarin, were purchased from Peptide Institute, Inc. (Osaka, Japan). They were dissolved in dimethyl sulfoxide.

Surgical procedure

Rats were anesthetized by an inhalation anesthetic, sevoflurane, where induction and maintenance were conducted at concentrations of 4.0-5.0 % with room air. In order to administer the drugs by an intravenous injection (i.v.) or continuous intravenous infusion (c.i.), a catheter was inserted into the femoral vein (Intramedic polyethylene tubing PE No. 50, Nippon Becton Dickinson and Company, Tokyo, Japan). For urine and blood sampling, the bladder was cannulated by a polyethylene tube (TOP Corp., Tokyo, Japan) after a small abdominal incision, and jugular vein was cannulated by a polyethylene tube (PE No. 50), respectively. After the cannulation rats were placed on a heated table at 37.5°C. The catheter into the femoral vein was connected to the infusion pump (Terumo Corp., Tokyo, Japan) to administer a 5% glucose solution at a rate of 6 mL/kg/h (c.i.). After urine was excreted through the bladder tube, urine was started to collect, and subsequently blood was taken. Urine and blood were collected every 15 min from 30 minutes before as a baseline (-15, 0 min), and for 90 min after the start of drug administration (15, 30, 45, 60, 75, 90 min), i.e., two and six samples were taken, respectively. After urine and blood sampling was completed, an additional blood (about 2.5 mL) was drawn in case of need. Then, euthanasia was conducted by administration of an excess amount of pentobarbital sodium (200 mg/kg, i.v.)

Study design

The study consisted of two separate protocols. In the first protocol, nafamostat (1.2 mg/kg/h) or 5% glucose was administered (c.i.) in the experimental and control groups, respectively. During the baseline in the experimental group 5% glucose was administered. A dose of nafamostat was determined on the bases of the clinical maximum dose (clinical doses, 0.06~0.20 mg/kg/h, c.i. for 24h) and two times more than the clinical dose (10 mg/2h c.i. once to twice per day), for which are indicated disseminated intravascular coagulation and acute pancreatitis, respectively. The animal dose was calculated by conversion of the clinical dose to animal dose based on body surface area, where the clinical dose in mg/kg was divided by 0.162, a standard conversion factor of rats, to obtain animal dose in mg/kg [16)]. In the second protocol, nafamostat and a low-dose amiloride or a low-dose amiloride was administered in the experimental and control groups, respectively. During the baseline in both groups 5% glucose was administered (c.i.). Then, amiloride was first administered (3.33 μg/kg, i.v.) taking 1 min in both groups, and subsequently nafamostat (0.13, 0.4, 1.2 mg/kg/h, c.i.) and amiloride (12 μg/kg/h, c.i.) or amiloride (12 μg/kg/h, c.i.) alone were administered in the experimental and control groups, respectively. The doses of nafamostat and amiloride were determined on the bases of the clinical and subclinical doses and those of the minimum effective dose in rats [17)], respectively.

Measurement of potassium concentrations in serum and urine

The urine sample was placed in 1.5 mL Hyper Microtube (WATSON Corp., Tokyo, Japan) and stored at 4℃ until the end of the experiment (1-6 hours). The blood sample was placed on MICROTAINER® (Nippon Becton Dickinson and Company, Tokyo, Japan) and stored at room temperature during the same time as urine. After completion of the experiment, centrifugation for urine and blood samples was performed at 1200 rcf for 20 minutes under 4℃ conditions and the serum or urine supernatant was collected for measurement. A portion of the urine sample was diluted 16 folds with distilled water.

Potassium concentrations in the serum sample and urine one were measured by Fuji Dri-Chem System (Fuji Dri-Chem 3500V, Fujifilm Corp., Tokyo, Japan) using the ion-selective electrode (Fuji Dri-Chem Slide Na-K-Cl, Fujifilm Corp., Tokyo, Japan). Values for the serum potassium concentrations were expressed as Δserum potassium concentrations, a difference from just before the start of drug administration. A reason for this is that the serum potassium concentrations are maintained in the narrow range *in vivo*, i.e., lower and upper normal limits are 3.6 and 5.0 mEq/L, respectively [18)], it makes the changes more apparent to compare the Δ data than actual data. Values for the urine were expressed as mEq per mg of urine creatinine to correct for a difference in the glomerular filtration volume. Data was evaluated as the changes in Δserum potassium concentrations and urine potassium excretion, and their corresponding mean of the last 3 points (60, 75, 90 min). A reason for choosing the last 3 points is that these data could reflect the maximum effect of the treatment.

Measurement of creatinine concentrations in urine

A portion of the urine sample was diluted two-fold with distilled water. The QuantiChrom™ Creatinine Assay Kit (BioAssay Systems, Hayward, CA, USA) was used to measure the creatinine concentrations (mg/dL). The diluted urine sample (2.5 µL) was mixed with reagent A (25 µL), reagent B (25 µL) and distilled water (50 µL) in a clear bottom plate. The plate was gently tapped and mixed. The optical density was measured immediately and 5 min later at 510 nm. The difference in the optical density was calculated, quantified by a reference calibration curve.

Measurement of kallikrein activity in urine

Enzymatic activity of urine kallikrein was determined using the fluorogenic synthetic substrate according to our previous study [11)]. A portion of the urine sample was diluted 10-fold with 0.2M Tris-HCl buffer (pH 7.8). The diluted urine sample (10 µL) was mixed with 0.05 mM synthetic substrate solution (0.05 M Tris-HCl buffer containing 0.1 M NaCl and 0.01 M $CaCl_2$, pH 8.0) , in the presence of soybean trypsin inhibitor (10 µL, 1 mg/mL) or that of aprotinin (10 µL, 1 mg/mL). The mixture was incubated at 37°C for 20 min. Soybean trypsin inhibitor and aprotinin inhibits a kallikrein-like activity in plasma or that in plasma and urine, respectively. Thus, a difference between the values of these incubation mixtures indicates urine kallikrein activity. The fluorescence intensity was measured at 380

nm as excitation and 460 nm as emission by fluorescence spectrophotometer (Thermo Scientific Varioskan Flash®, Thermo Fisher Scientific Corp., Vantaa, Finland). Kallikrein activity was quantified by a calibration curve of the reference. Values were expressed as μmoles per mg of urine creatinine to correct for a difference in the glomerular filtration volume. Data was evaluated as the changes in urine kallikrein activity and its corresponding mean of the last 3 points.

Statistical analysis

Data was expressed as mean ± standard error (SE). A repeated measure two-way ANOVA was conducted for the comparison of the changes in Δpotassium concentrations and those in urine potassium excretion and kallikrein activity by a single dose of nafamostat or 5% glucose in the absence or presence of amiloride. Unpaired *t* test was conducted for the comparison of the means of the last three points regarding with Δpotassium concentrations and urine potassium excretion in the study where a single dose of nafamostat or 5% glucose was examined in the absence of amiloride. A repeated measure one-way ANOVA was conducted for the comparison of the means of the last three points regarding with Δpotassium concentrations and urine potassium excretion and kallikrein activity in the study where multiple doses of nafamostat or 5% glucose were examined in the presence of amiloride. A multiple comparison test (Dunnett test, one-tail test) was applied for the comparison of the changes in Δ potassium concentrations and those in urine potassium excretion and kallikrein activity, and their

corresponding means of the last three points in the study where multiple doses of nafamostat or 5% glucose were examined in the presence of amiloride. A significance level was set at 0.05. IBM SPSS Statistics, version 26 (IBM, Armonk, NY, USA) was used for statistical analyses.

Results

Effect of nafamostat or 5% glucose on Δserum potassium concentrations and urine potassium excretion in the absence of co-administration of amiloride.

A weak increase in Δpotassium concentrations was shown by administration of nafamostat compared to the control in the change with time and mean of the last 3 points, but the differences were not significant (Fig 1A. and 1B.). Regarding with the urine potassium excretion, a weak decrease was shown by administration of nafamostat compared to the control in the change and mean of the last 3 points, but the differences were not significant (Fig 2A. and 2B.).

Effect of nafamostat or 5% glucose on Δserum potassium concentrations and urine potassium excretion in the presence of co-administration of amiloride.

Nafamostat was administered at three doses in the presence of co-administration of amiloride. An increase in Δserum potassium concentrations was shown by administration of a low-dose amiloride

compared with the baseline for the change with time and mean of the last 3 points (Fig 3A. and 3B.). The increase was significantly augmented by administration of nafamostat at a dose of 0.4 mg/kg/h compared to the control both for the change with time and mean of the last 3 points (Fig 3A. and 3B.). Attenuation of the increasing effects on Δserum potassium concentrations were shown by administration of nafamostat at 1.2 mg/kg/h (Fig 3A. and 3B.). Regarding with the urine potassium excretion a decrease was shown by administration of amiloride compared with the baseline for the change with time and mean of the last 3 points (Fig 4A. and 4B.). The decrease was significantly augmented by administration of nafamostat at a dose of 1.2 mg/kg/h compared to the control for the change with time, and they were significantly decreased by nafamostat at a dose of 0.13 and 1.2 mg/kg/h for the mean of the last 3 points (Fig 4A. and 4B.).

Effect of nafamostat or 5% glucose on urine kallikrein activity in the presence of co-administration of amiloride.

An increase in Δserum potassium concentrations was shown by administration of a low-dose amiloride compared with the baseline for the change with time and mean of the last 3 points (Fig 5A. and 5B.). The effect of nafamostat on the urine kallikrein activity was not significantly different from the control for the changes with time (Fig 5A.). A further decrease was significantly shown by administration of nafamostat at 1.2 mg/kg/h compared to the control regarding with the mean of the

last 3 points (Fig 5B.).

Discussion

This study is the first to evaluate the *in vivo* effect of nafamostat on development of hyperkalemia and examine the association of urine kallikrein activity with its development. Doses and route for administration of nafamostat were set as same as the clinical form in this study. The results show that nafamostat had a weak effect on both the increase in serum potassium and decrease in urine potassium excretion when it was administered solely. Whereas, nafamostat, at lower doses, showed significant changes of the increase in potassium concentrations in the changes with time and mean of the last 3 points (0.4mg/kg/h, $P = 0.036$, $P = 0.038$) in the presence of co-administration of amiloride. Similarly, significant decreases in urine potassium excretion (1.2 mg/kg/h, $P = 0.025$, $P = 0.0003$) were shown by nafamostat in the presence of co-administration of amiloride. Urine kallikrein activity was decreased by the maximum dose of nafamostat in the mean of the last 3 points, with statistical significance (1.2 mg/kg/h, $P = 0.046$), though it was not significant in the changes with time ($P = 0.074$).

The study had several limitations. First, there was a large variation of the serum potassium concentrations and urine potassium excretion in the nafamostat and control groups in the study where nafamostat was administered alone (Fig 1A. and 1B., Fig 2A. and 2B.), and that of the serum potassium

concentrations in the nafamostat and control groups in the study where nafamostat was co-administered with amiloride (Fig 3A. and 3B.). Major reasons for this were a small number of the animals in the former study, and an effect of hemolysis on the serum potassium concentrations which was accompanied with the blood collection and effect of respiratory suppression due to inhalation anesthesia on the serum potassium concentrations as the study did not use a ventilator to the animals. Second, administration of amiloride, of which dose were shown to be minimal in the previous study, increased the serum potassium concentrations and decreased the urine potassium excretion in the present study (Fig 3A. and 3B., Fig 4A. and 4B.). Thus, the augmentive effect of nafamostat might be limited so that the dose-dependent effects were not shown by several doses of nafamostat regarding with the serum potassium concentrations (1.2 mg/kg/h, Fig 3B.), urine potassium excretion (0.4 mg/kg/h, Fig 4B.) and urine kallikrein activity (0.4 mg/kg/h, Fig 5B.).

In the present study, administration of nafamostat alone did not induce renal hyperkalemia, which was contrary to our hypothesis. The result suggests that nafamostat itself may have weak effect on the decrease in urinary potassium excretion. In clinical practice most cases of the nafamostat-induced hyperkalemia have been reported in patients with renal dysfunction [2-7], and a package insert of nafamostat gives attention to the onset of hyperkalemia when co-administering with the potassium-sparing diuretics. Thus, we thought it may be necessary to limit renal function, specifically, urine potassium excretion, to some extent in rats as well. Since nafamostat has been reported to inhibit

amiloride-sensitive Na^+ currents in the renal cortical collecting duct cells in rabbits [8], amiloride was used in combination with naphamostat in the second study.

In the second study the maximum effect on the Δserum potassium concentrations was not shown by nafamostat at a dose of 1.2 mg/kg/h, whereas the maximum effect was shown on the urine potassium excretion by this dose (Fig 3. and 4.). A reason for the difference is possibly due to an apparent decrease in serum potassium concentrations at a dose of 1.2 mg/kg/h, as fibrin clots were formed in the serum sample after centrifugal separation of the blood by this dose of nafamostat. Nafamostat is the inhibitor against coagulating factors (thrombin, XIIa, Xa, VIIa) and fibrinolytic factors (plasmin) [19]. A high dose nafamostat might inhibit the action of plasmin on degradation of fibrin into fibrin degradation products. The presence of fibrin clots might influence measurement of potassium concentrations in the serum.

Regarding with the result of urine kallikrein activity the value gradually increased in the control group after administration of amiloride. We suggest that the increase in the serum potassium concentrations might attribute to the increase in urine kallikrein activity after administration of amiloride. Bases of these suggestions are come from our previous study where administration of high potassium to rats increased the urine kallikrein secretion from renal tubules [10-12]. Furthermore, as the baseline level of the urine kallikrein activity had been increased by administration of amiloride, the inhibitory effect of nafamostat on it might be attenuated.

In conclusions, *in-vivo* administration of nafamostat induced renal hyperkalemia via inhibition of the amiloride-sensitive Na^+ channel, which was associated with inhibition of the urine kallikrein activity. We described inferred mechanisms for the nafamostat-induced hyperkalemia in Fig 6. Further studies are needed to deal with the limitations of the present study, i.e., to conduct under the less dose of inhalation anesthesia to avoid respiratory suppression and less dose of amiloride which has a minimal effect on potassium sparing.

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Conflicts of interest

The authors declare that they have no conflict of interest.

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Figure legends

Fig 1. Time course of Δserum potassium concentrations (A) and the mean of the last 3 points (60, 75, 90 min) of Δserum potassium concentrations after administration of nafamostat (1.2 mg/6 mL/kg/h c.i.) (closed triangles) and 5% glucose (6 mL/h c.i.) (closed circles). The first two blood samples were taken as a baseline, and the next six samples were taken after changing from 5% glucose to nafamostat or continuing 5% glucose (control). Data points are the mean of measurements from 4 rats; error bars indicate the S.E. $P = 0.081$ between the groups, a repeated measures two-way ANOVA (A) and $P = 0.052$ vs 5% glucose group, an unpaired *t*-test (one-side test) (B). Glu: 5% Glucose, Naf 1.2: Nafamostat.

Fig 2. Time course of urine potassium excretion (A) and the mean of the last 3 points (60, 75, 90 min) of urine potassium excretion (B) after administration of nafamostat (1.2 mg/6 mL/kg/h c.i.) (closed triangles) and 5% glucose (6 mL/h c.i.) (closed circles). Urine potassium excretion was corrected by urine creatinine excretion. Data points are the mean of measurements from 4 rats; error bars indicate the S.E. $P = 0.635$ among the groups, a repeated measures two-way ANOVA (A) and $P = 0.467$ vs 5% glucose group, an unpaired *t*-test (one-side test) (B). Glu: 5% Glucose, Naf 1.2: Nafamostat.

Fig 3. Time course of Δserum potassium concentration (A) and the mean of the last 3 points (60, 75,

90 min) of Δserum potassium concentration (B) after administration of nafamostat (0.13, 0.4, 1.2 mg/kg/6 mL/h c.i) co-administered with amiloride (3.33μg/kg i.v. and 1.2 μg/kg/6 mL/h c.i.) (closed triangles, squares and diamonds) or 5% glucose co-administered with amiloride (closed circles). The first two samples of the blood were taken as a baseline, and the next six samples of those were taken after changing from 5% glucose to amiloride and nafamostat, or that to amiloride (control). Data points are the mean of measurements from 13, 12 and 5 rats for nafamostat 0.13, 0.4 mg/kg and 1.2 mg/kg, respectively and 13 rats for 5% glucose; error bars indicate the S.E.. $P = 0.163$ among the groups, a repeated measures two-way ANOVA. $P = 0.036$ vs control group, a Dunnett test following the two-way ANOVA (A). $P = 0.038$ vs control group, a Dunnett test following the one-way ANOVA (B). $^{*}P < 0.05$ vs control group.

Ami: Amiloride 3.33 μg/kg i.v. and 1.2 μg/kg/6 mL/h c.i.,

Ami + Naf 0.13: Amiloride 3.33 μg/kg i.v. and 1.2 μg/kg/6 mL/h c.i. + Nafamostat 0.13 mg/kg/h,

Ami + Naf 0.4: Amiloride 3.33 μg/kg i.v. and 1.2 μg/kg/6 mL/h c.i. + Nafamostat 0.4 mg/kg/h,

Ami + Naf 1.2: Amiloride 3.33 μg/kg i.v. and 1.2 μg/kg/6 mL/h c.i. + Nafamostat 1.2 mg/kg/h

Fig 4. Time course of urine potassium excretion (A) and the mean of the last 3 points (60, 75, 90 min) of urine potassium excretion (B) after administration of nafamostat (0.13, 0.4, 1.2 mg/kg/6 mL/h c.i) co-administered with amiloride (3.33μg/kg i.v. and 1.2 μg/kg/6 mL/h c.i.) (closed triangles, squares

and diamonds) or 5% glucose co-administered with amiloride (closed circles). Urine potassium excretion was corrected by urine creatinine excretion. The first two samples of the urine were taken as a baseline, and the next six samples of those were taken after changing from 5% glucose to amiloride and nafamostat, or that to amiloride (control). Data points are the mean of measurements from 12, 11 and 5 rats for nafamostat 0.13, 0.4 mg/kg and 1.2 mg/kg, respectively and 13 rats for 5% glucose; error bars indicate the S.E. $P = 0.091$, among the groups, a repeated measures two-way ANOVA. $P = 0.025$, a Dunnett test following the two-way ANOVA (A). $P = 0.034$, $P = 0.0003$, a Dunnett test following the one-way ANOVA (B). * $P < 0.05$, ** $P < 0.01$ vs control group.

Ami: Amiloride,

Ami + Naf 0.13: Amiloride + Nafamostat 0.13 mg/kg/h,

Ami + Naf 0.4: Amiloride + Nafamostat 0.4 mg/kg/h,

Ami + Naf 1.2: Amiloride + Nafamostat 1.2 mg/kg/h.

Fig 5. Time course of urine kallikrein activity (A) and the mean of the last 3 points (60, 75, 90 min) of urine kallikrein activity (B) after administration of nafamostat (0.13, 0.4, 1.2 mg/kg/6 mL/h c.i) co-administered with amiloride (3.33μg/kg i.v. and 1.2 μg/kg/6 mL/h c.i.) (closed triangles, squares and diamonds) or 5% glucose co-administered with amiloride (closed circles). Urine kallikrein activity was corrected by urine creatinine excretion. The first two samples of the urine were taken as a baseline,

and the next six samples of those were taken after changing from 5% glucose to amiloride and nafamostat, or that to amiloride (control). Data points are the mean of measurements from 12, 12 and 5 rats for nafamostat 0.13, 0.4 mg/kg and 1.2 mg/kg, respectively, and 13 rats for 5% glucose; error bars indicate the S.E. $P = 0.181$, among the groups, a repeated measures two-way ANOVA. $P = 0.074$, a Dunnett test following the two-way ANOVA (A). $P = 0.046$, a Dunnett test following the one-way ANOVA (B). $^{*}P < 0.05$ vs control group.

Ami: Amiloride,

Ami + Naf 0.13: Amiloride + Nafamostat 0.13 mg/kg/h,

Ami + Naf 0.4: Amiloride + Nafamostat 0.4 mg/kg/h,

Ami + Naf 1.2: Amiloride + Nafamostat 1.2 mg/kg/h.

Fig 6. Inferred mechanisms for the nafamostat-induced hyperkalemia.

Hyperkalemia increases kallikrein secretion from the connecting tubular cells into the urine. Urine kallikrein activates ENaC and increases sodium reabsorption. This promotes the urine potassium excretion and attempts to correct hyperkalemia. Nafamostat may inhibit ENaC activity by inhibiting kallikrein. It may decrease the urine potassium excretion by decreasing the sodium reabsorption. ENaC: epithelial Na^+ channel.