Bradykinin Stimulates Renal Na^+ and K^+ Excretion by Inhibiting the K^+ Channel (Kir4.1) in the Distal Convoluted Tubule

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Abstract—Stimulation of BK2R (bradykinin [BK] B2 receptor) has been shown to increase renal Na^+ excretion. The aim of the present study is to explore the role of BK2R in regulating Kir4.1 and NCC (NaCl cotransporter) in the distal convoluted tubule (DCT). Immunohistochemical studies demonstrated that BK2R was highly expressed in both apical and lateral membrane of Kir4.1-positive tubules, such as DCT. Patch-clamp experiments demonstrated that BK inhibited the basolateral 40-pS K^+ channel (a Kir4.1/5.1 heterotetramer) in the DCT, and this effect was blocked by BK2R antagonist but not by BK1R (BK B1 receptor) antagonist. Whole-cell recordings also demonstrated that BK decreased the basolateral K^+ conductance of the DCT and depolarized the membrane. Renal clearance experiments showed that BK increased urinary Na^+ and K^+ excretion. However, the BK-induced natriuretic effect was completely abolished in KS-Kir4.1 KO (kidney-specific conditional Kir4.1 knockout) mice, suggesting that Kir4.1 activity is required for BK-induced natriuresis. The continuous infusion of BK with osmotic pump for 3 days decreased the basolateral K^+ conductance and the negativity of the DCT membrane. Western blot showed that infusion of BK decreased the expression of total NCC and phosphorylated NCC. Renal clearance experiments demonstrated that thiazide-induced natriuresis was blunted in the mice receiving BK infusion, suggesting that BK inhibited NCC function. Consequently, mice receiving BK infusion for 3 days were hypokalemic. We conclude that stimulation of BK2R inhibits NCC activity, increases urinary K^+ excretion, and causes mice hypokalemia and that Kir4.1 is required for BK2R-mediated stimulation of urinary Na^+ and K^+ excretion. **(*Hypertension*. 2018;72:361-369. DOI: 10.1161/HYPERTENSIONAHA.118.11070.)** ● **Online Data Supplement**

Key Words: bradykinin B2, receptor ■ diuretics ■ hypertension ■ ion transport ■ sodium-chloride symporter

Increasing dietary K^+ intake or infusion of high potassium solution has been shown to augment urinary excretion of kallikrein—a serine protease, which cleaves kinogen and produces kinins, such as bradykinin (BK) in the kidney.[1] Also, these interventions increase BK2R (BK B2 receptor) density and mRNA levels in the kidney.[2–4] Previous studies reported that the overexpression of BK2R increased renal blood flow, glomerular filtration rate, and urine flow in the BK2R transgenic mice.[1,5] Conversely, the inhibition of BK2R or renal kallikrein has been shown to decrease urinary volume and Na^+ excretion (E_{Na}) in rats.[6,7] BK2R is expressed in the proximal tubules, thick ascending limb, and aldosterone-sensitive distal nephron (ASDN), including distal convoluted tubule (DCT) and collecting duct.[8,9] However, the role of BK2R in the regulation of membrane transport in the DCT is not understood.

The DCT is responsible for the absorption of 5% filtered Na^+ load by a thiazide-sensitive NCC (NaCl cotransporter). It is well established that high K^+ (HK) intake inhibits NCC expression and activity thereby contributing to HK intake–induced natriuresis and antihypertensive effect. We have previously demonstrated that HK intake–induced inhibition of NCC requires the presence of inwardly rectifying K^+ channel (Kir4.1) because the deletion of Kir4.1 in the DCT abolished the effect of HK intake on NCC expression.[10] Kir4.1 is expressed in the basolateral membrane of the late thick ascending limb, DCT and cortical collecting duct.[11–14] Kir4.1 interacts with Kir5.1 to form a 40-pS basolateral K^+ channel.[13,15] Moreover, Kir4.1/Kir5.1 heterotetramer is the only type of K^+ channel expressed in the basolateral membrane of the DCT.[15] Furthermore, we demonstrated that HK intake–induced inhibition of the basolateral Kir4.1/Kir5.1 K^+ channels is essential for the inhibition of NCC in the DCT.[10] Also, because HK intake stimulated BK production and BK2R expression,[3] it raises the possibility that BK and BK2R may

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play a role in the regulation of Kir4.1 and NCC in the DCT during increased dietary K^+ intake. Thus, the aim of the present study is to test whether the stimulation of BK2R regulates Kir4.1 and NCC in the DCT.

Methods

The authors declare that all supporting data and detailed methods, including animal preparation, electrophysiology, Western blot, and renal clearance method, are available within the article (and its online-only Data Supplement).

Animals

C57BL/6 mice (either sex, 12 weeks old) and KS-Kir4.1 KO (kidney-specific conditional Kir4.1 knockout) mice were used in the present study. C57BL/6 mice were purchased from the Second Hospital of Harbin Medical University or Jackson Laboratory (Bar Harbor, ME). KS-Kir4.1 KO mice were bred in New York Medical College for the experiments, and the procedure for generating KS-Kir4.1 KO mice is described in the online-only Data Supplement. The mice were fed with normal K^+ diet (1% KCl) or HK diet (5%) for 7 days as indicated and had free access to water. The preparation of DCT for the patch-clamp experiments has been described in detail in the online-only Data Supplement.

Electrophysiology

A Narishige electrode puller (Narishige, Japan) was used to manufacture the patch-clamp pipettes from borosilicate glass (1.7 mm OD). The resistance of the pipette was 5 (for single-channel recording) or 2 mol/LΩ (for whole-cell recording) when it was filled with solution containing (in mmol/L) 140 KCl, 1.8 $MgCl_2$, and 10 HEPES (titrated with KOH to pH 7.4). The details for the single-channel and whole-cell recordings are described in the online-only Data Supplement.

Immnoblotting and Immunohistochemistry

The details for immunoblotting are described in the online-only Data Supplement. For immunohistochemistry, renal slices were fixed in Bouin fixative and processed as described.[9,16] The localization of BK2R and Kir4.1 was assessed by double immunolabeling in the same tissue sections. Briefly, the first antibody immunostaining (Kir4.1; 1:1200; Alomone Laboratories, Israel) was developed with diaminobenzidine–hydrogen peroxide to give a brown color, whereas BK2R immunostaining was developed with Vector SG substrate to give a blue color (1:1000; Santa Cruz). The sections were observed and photographed on a Nikon Eclipse 600 microscope with a Nikon DS-Ri1 digital camera.

Material and Statistical Analysis

All chemicals, including hydrochlorothiazide, phorbol 12-myristate-13 acetate, BK, HOE140, and Lys-(des-Arg^9, Leu^8)-BK were purchased from Sigma Chemicals (St. Louis, MO). Polyclone antibodies for NCC, pNCC (phosphor-NCC at Thr^{53}), and NKCC2 (type II Na-K-Cl symporter) were purchased from EMD Millipore, Phosphosolutions, and Abcam, respectively. Antibody for epithelial Na channel (ENaC)-α was obtained from Sigma Chemicals. Data were analyzed using Student *t* test for comparisons between 2 groups or 1-way ANOVA for comparisons among >2 groups. *P* values <0.05 were considered statistically significant. Data are presented as the mean±SEM.

Results

We first used immunohistochemistry to examine whether BK2R was expressed in the DCT using Kir4.1 as a marker. Figure 1A shows an image of double staining with low magnification, and it demonstrates that BK2R is expressed in Kir4.1-positive tubules. Two areas of the image (indicated by a square) in Figure 1B are enlarged and demonstrate a detailed view of BK2R staining (Figure 1C and 1D). It is apparent that BK2R is expressed in the DCT as evidenced by strong basolateral Kir4.1 staining and convoluted tubule appearance. Moreover, BK2R staining is visible not only in the apical membrane but also in the lateral membrane of the DCT (indicated by a red arrow).

Because BK2R is expressed in both apical and lateral membranes of the DCT, we next used the patch-clamp technique to examine whether BK regulated the basolateral 40-pS K^+ channel (a Kir4.1/Kir5.1 heterotetramer) in the DCT. Figure 2A is a channel recording showing the effect of BK on the basolateral 40-pS K^+ channel activity in the DCT. Adding 1-μM BK decreased K^+ channel activity and reduced NP_o (a product of channel number and open probability) of the 40-pS K^+ channels to 1.45±0.3, although this effect was not significant (Figure 2B). However, 10-μM BK significantly inhibited the basolateral 40-pS K^+ channel. Results

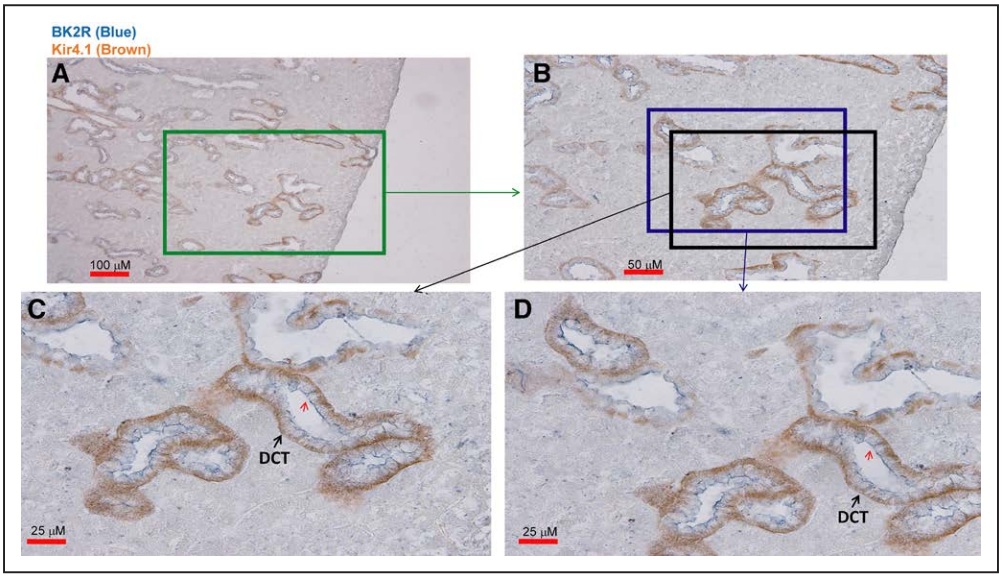

Figure 1. BK2R (bradykinin B2 receptor) is expressed in Kir4.1-positive distal tubules. A double staining image shows the expression of Kir4.1 (brown) and BK2R (blue) with low magnification (**A**). Areas marked by 2 squares (**B**) are enlarged, demonstrating detailed view of BK2R staining in (**C** and **D**), respectively. The distal convoluted tubules (DCTs) are indicated by arrows. A red arrow indicates BK2R staining in the lateral membrane of the DCT.

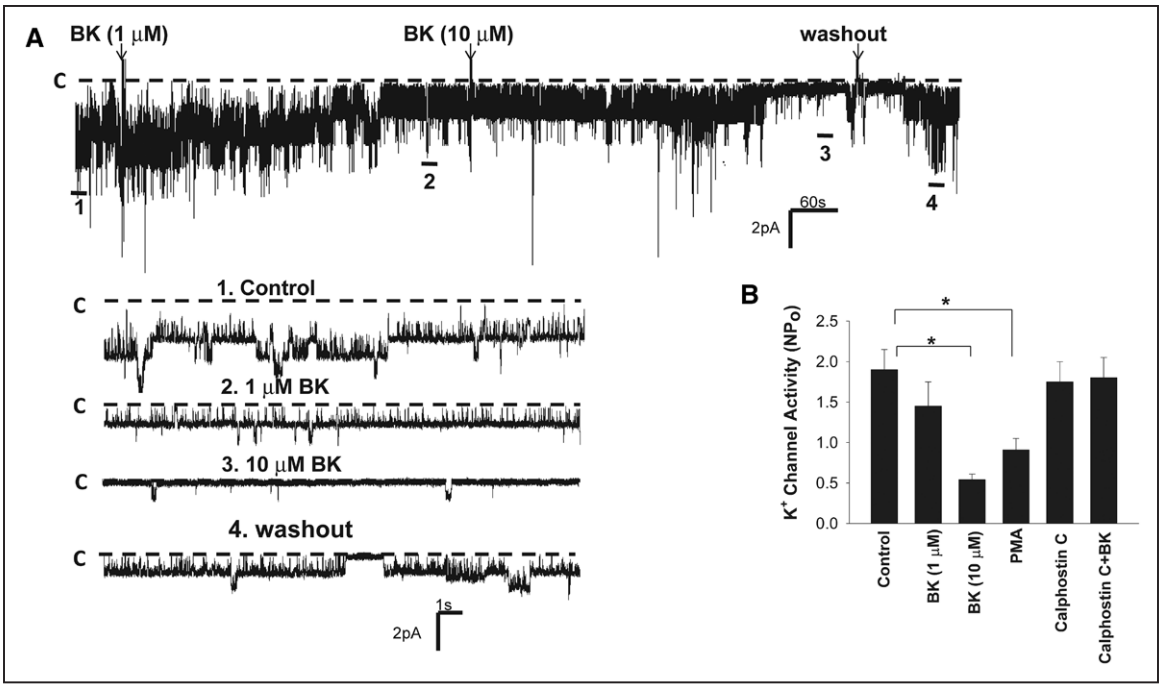

Figure 2. Bradykinin (BK) inhibits the basolateral 40-pS K^+ channels in the distal convoluted tubule (DCT). **A**, A single-channel recording shows the effects of 1 and 10 μM BK on the basolateral K^+ channels in the DCT. The top trace shows the time course of the experiments, and 4 parts of the record indicated by numbers are extended to demonstrate the fast time resolution. The holding potential was 0 mV, and the channel closed level is indicated by a dotted line and C. The experiments were performed in cell-attached patches with 140 mmol/L K^+ in the pipette and 140 mmol/L Na^+/5 mmol/L K^+ in the bath solution. **B**, A bar graph summarizes the results of experiments in which the effects of BK, PMA (10 μM), calphostin C (1 μM), and BK+calphostin C on the basolateral 40-pS K^+ channel were examined (n=6). * indicates a significant significance.

from 6 experiments are summarized in Figure 2B demonstrating that BK (10 μM) significantly decreased the channel activity defined by NP_o from 1.9±0.25 to 0.54±0.07. The effect of BK on the basolateral K^+ channels was reversible because the washout was able to partially restore the channel activity. Moreover, the inhibitory effect of BK on the basolateral K^+ channel was mediated by PKC (protein kinase C) pathway because calphostin C (1 μM) abolished the inhibitory effect of BK (10 μM) on the basolateral K^+ channels in the DCT (NP_o=1.8±0.25, n=6; Figure 2B). The notion that PKC mediated the effect of BK on the basolateral K^+ channels was also supported by the observation that the stimulation of PKC with phorbol 12-myristate-13 acetate (10 μM) also inhibited the basolateral K^+ channel activity and reduced NP_o to 0.91±0.14 (n=6).

We have also used the whole-cell recording to measure the Ba^{2+}-sensitive K^+ currents under control conditions and in the presence of 10 μM BK in the early portion of the DCT. Because Kir4.1/Kir5.1 heterotetramer is the only type of K^+ channel in the early portion of the DCT, the whole-cell K^+ currents represent the activity of whole population of Kir4.1/Kir5.1 in the DCT. Figure 3A is a whole-cell recording showing the Ba^{2+}-sensitive K^+ currents measured from −60 mV to 60 mV at a 20-mV step, and Figure 3B is a recording demonstrating the whole-cell K^+ currents measured with the RAMP protocol from −100 mV to 100 mV. It is apparent that BK decreased the Ba^{2+}-sensitive whole-cell K^+ currents. Figure 3C is a bar graph summarizing the results of 6 experiments in which Ba^{2+}-sensitive K^+ currents were measured at −60 mV using step protocol (as shown in Figure 3A), demonstrating that BK treatment decreased the K^+ currents from 1280±60 to 860±50 pA (n=6). Because the basolateral Kir4.1/Kir5.1 channels participate in generating the negative membrane potential, BK-induced inhibition of the basolateral K^+ channels is expected to decrease the negativity of the DCT membrane (depolarization). This speculation is confirmed by measuring K^+-current (I_K) reversal potential—an index of the membrane potential—with perforated whole-cell recording. From the inspection of Figure 3D, it is apparent that BK treatment shifted the I_K reversal potential of the DCT to a positive range (depolarization). Results summarized in Figure 3C show that I_K reversal potential was −66±3 mV under control conditions, and it was −55±3 mV after acute BK treatment. Thus, BK decreases the basolateral Kir4.1 activity and depolarizes the DCT membrane. Moreover, the inhibitory effect of BK on the basolateral K^+ conductance was enhanced in the DCT of the mice on a HK diet for 7 days. Figure S1A in the online-only Data Supplement is a whole-cell recording showing the Ba^{2+}-sensitive K^+ currents measured from −60 to 60 mV at a 20-mV step, and Figure S1B is a recording demonstrating the whole-cell K^+ currents measured with RAMP protocol from −100 mV to 100 mV. Like under control conditions, BK inhibited the basolateral Kir4.1/Kir5.1 in the DCT of the mice on HK diet. Figure S1C is a bar graph summarizing the results of 7 experiments showing that BK treatment decreased the K^+ currents from 690±60 to 330±40 pA. Thus, HK intake enhanced the inhibitory effect of BK on the basolateral K^+ channels because BK-induced inhibition of basolateral K^+ conductance was significantly larger in the mice on HK diet (51±2%) than in the mice on normal K^+ diet (32±2%; Figure S1D).

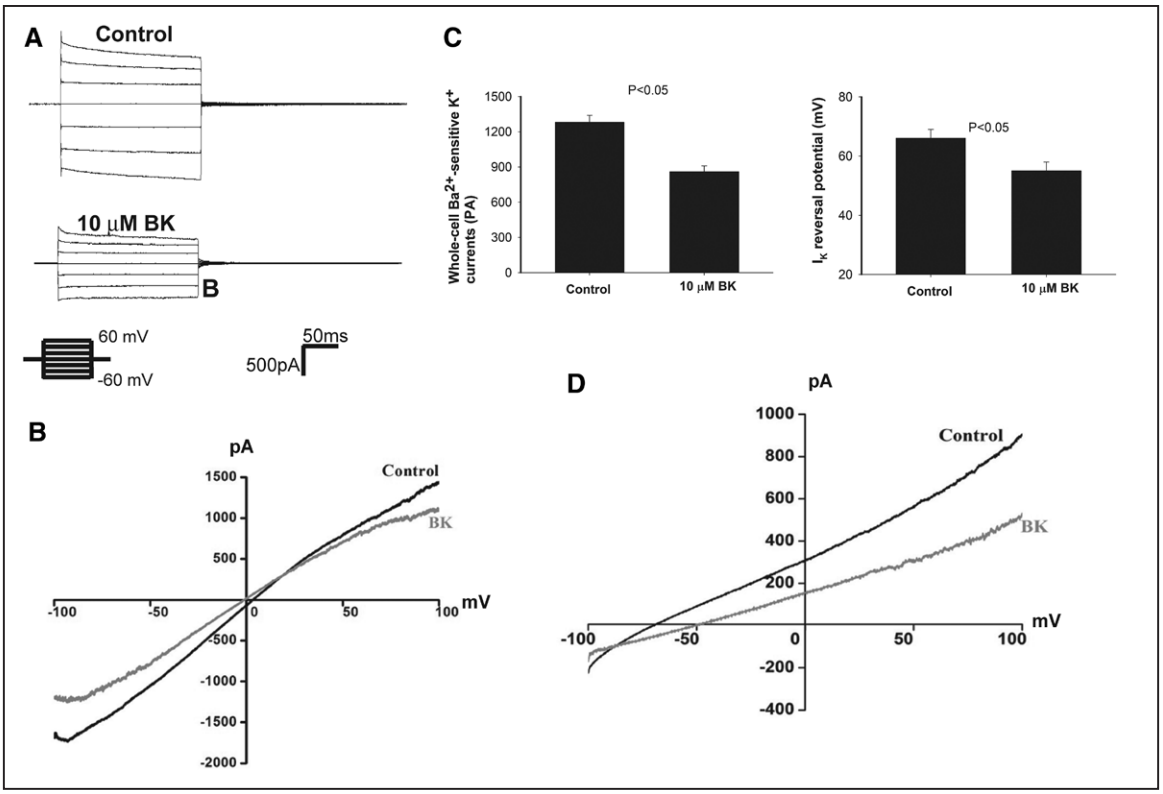

Figure 3. Bradykinin (BK) decreases the basolateral K^+ conductance of the distal convoluted tubule (DCT) and depolarizes the membrane. A whole-cell recording shows Ba^{2+}-sensitive K^+ currents in the DCT treated with 10 μM BK, and the K^+ currents were measured with a step protocol from −60 to 60 mV at a 20-smV step (**A**) or with RAMP protocol from −100 to 100 mV (**B**). Symmetrical 140 mmol/L KCl solution in the bath and pipette was used for the measurement. Results of 6 experiments are summarized in a bar graph (**C**). A whole-cell recording shows the effect of 10 μM BK on K^+-current (I_K) reversal potential of the DCT (**D**). The bath solution contains (in mmol/L) 140 NaCl and 5 KCl, whereas the pipette solution has 140 KCl. Results of 5 experiments are summarized in a bar graph (**C**, **left**).

To further examine whether the inhibitory effect of BK on the basolateral K^+ channels was mediated by BK2R, we examined the effect of BK on the basolateral K^+ channels in the presence of HOE140—a specific BK2R antagonist.[7] Figure 4A is a channel recording showing the effect of BK on the basolateral 40-pS K^+ channel activity in the DCT treated with HOE140 (1 μM). Results from 6 similar experiments are summarized in Figure 4B showing that HOE140 abolished the inhibitory effect of BK on the basolateral K^+ channel activity in the DCT (NP_o=1.7±0.14), although BK2R antagonist, per se, had no significant effect on the channel activity (NP_o=1.7±0.14). In contrast, BK was still able to inhibit the basolateral K^+ channel activity in the presence of 1 μM Lys-(des-Arg^9, Leu^8)-BK (BK1R [BK B1 receptor] antagonist; Figure 4C) and decreased NP_o to 0.8±0.1 (n=6; Figure 4B). Thus, the results suggest that BK-induced inhibition of basolateral K^+ channels in the DCT is mediated by BK2R and PKC-dependent pathway.

Previous studies have demonstrated that the basolateral K^+ channel activity in the DCT plays a key role in regulating NCC activity such that a stimulation of the basolateral Kir4.1 activity increases, whereas an inhibition of the basolateral K^+ channel activity decreases NCC activity.[17] Because BK inhibits the basolateral Kir4.1 activity in the DCT and depolarizes the membrane, it is conceivable that BK might increase E_{Na} by inhibiting NCC. This hypothesis was tested with renal clearance experiments to examine the effect of BK on renal E_{Na} by 1-time infusion of BK (0.5 ng/kg body weight [BW] within 60 seconds). For the clearance study, the mice were intravenously perfused with isotonic saline for 4 hours (0.3 mL per 1 hour), and urine collections started 1 hour after saline infusion. Results from 4 experiments are summarized in Figure 5A demonstrating that acute BK infusion increased E_{Na} from 0.76±0.06 to 1.48±0.12 μEq/min per 100 g BW. Also, Figure S2 shows the time course of BK infusion–induced changes in urine volume. It is apparent that BK infusion–induced urine volume changes appear in the second (60 minutes after injection) and third collections (90 minutes after injection). The BK infusion–induced stimulation of E_{Na} was, at least in part, because of the inhibition of NCC because BK failed to increase E_{Na} in KS-Kir4.1 KO in which NCC activity was inhibited.[10,17] Results summarized in Figure 5B show that the deletion of Kir4.1 not only increased the basal level of renal E_{Na} (1.56±0.12 μEq/min per 100 g BW) but also abolished the effect of BK infusion on E_{Na} (1.58±0.12 μEq /min per 100 g BW). We have also examined the effect of BK infusion on renal K^+ excretion (E_K) with renal clearance methods. Figure 5C is a line graph demonstrating the results of each experiment, and Figure 5D summarizes the results of 4 experiments demonstrating that BK infusion increased E_K from 0.61±0.04 to 0.9±0.05 μEq/min per 100 g BW. The deletion of Kir4.1 not only caused K^+ wasting (1.06±0.05 μEq / min per 100 g BW) but also abolished the effect of BK on

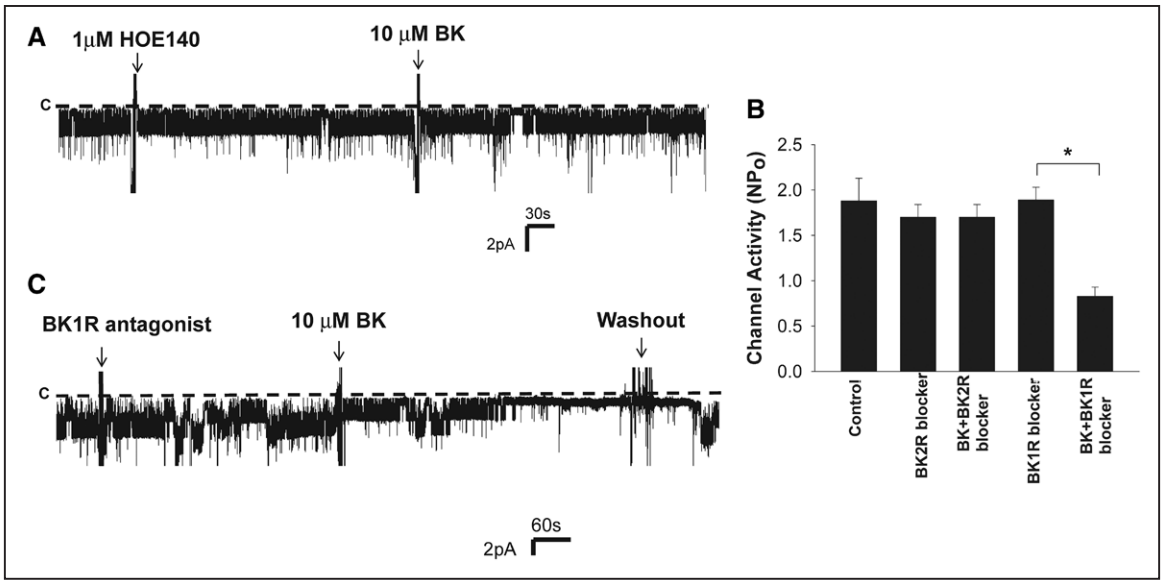

Figure 4. BK2R (bradykinin B2 receptor) mediates bradykinin (BK)-induced inhibition of the basolateral 40-pS K^+ channels in the distal convoluted tubule (DCT). A single-channel recording demonstrates the effect of 10 μM BK on the basolateral K^+ channels in the DCT in the presence of 1 μM HOE (BK2R antagonist; **A**) or in the presence of 1 μM Lys-(des-Arg^9, Leu^8)-BK (BK1R [BK B1 receptor] antagonist; **C**). The holding potential was 0 mV, and the channel closed level is indicated by a dotted line and C. **B**, Bar graph summarizes the results of experiments in which the effects of HOE140 (1 μM), BK (10 μM)+HOE140, BK1R antagonist (1 μM), and BK+BK1R antagonist on the basolateral 40-pS K channel were examined (n=6). * indicates a significant significance.

renal E_K (1.03±0.05 μEq /min per 100 g BW). Thus, BK infusion–induced stimulation of both E_{Na} and E_K depends on the presence of Kir4.1.

To further test whether BK infusion–induced stimulation of renal E_{Na} was because of the inhibition of Kir4.1 and NCC in the DCT, we examined the basolateral K^+ channel activity in the DCT and NCC activity in the mice receiving BK infusion through an osmotic pump (4 μg/min per kg BW). Figure 6A is a recording showing Ba^{2+}-sensitive K^+ currents of DCT cells clamped from −60 mV to 60 mV at a 20-mV step from untreated control and BK-treated mice. As summarized in Figure S3A, the mean whole-cell K^+ current at −60 mV was −1250±60 pA (n=6) in the control mice, and it was −520±30 pA (n=6) in BK-treated mice. Because basolateral K^+ channels participate in generating the membrane potential of DCT cells, an inhibition of the basolateral K^+ channels should depolarize DCT membrane. Thus, we used the whole-cell recording to measure the I_K reversal potential in the control (vehicle) and BK-treated mice. Figure 6B is a perforated whole-cell recording showing current/voltage curve of the DCT with 140 mmol/L K^+ in the pipette (intracellular solution) and 140 mmol/L Na^+/5 mmol/L K^+ in the bath from the control and BK-treated mice. The results from 7 experiments are summarized in Figure S3B showing that BK treatment depolarized DCT membrane and decreased I_K reversal potential of the DCT from −65±4 (control) to −42±5 mV (BK infusion). Thus, patch-clamp experiments confirm that the BK inhibited the basolateral K^+ channel activity in the DCT and depolarized the membrane. We have also used the renal clearance method to examine the effect of hydrochlorothiazide on E_{Na} in the control mice and in the mice receiving BK infusion for 3 days. Figure 6C summarizes the results of 4 experiments showing that hydrochlorothiazide-induced natriuretic effect (from 1.96±0.23 to 3.20±0.13 μEq/min per 100 g BW) in BK-treated mice was significantly smaller than that in the untreated group (from 0.84±0.13 to 2.68±0.16 μEq/min per 100 g BW). Moreover, the fact that basal level of E_{Na} was higher in BK-treated mice (1.96±0.23 μEq/min per 100 g BW) than that of the control animals (0.84±0.13 μEq/min per 100 g BW) also indicates that BK inhibits Na^+ transporters, such as NCC. The notion that BK infusion inhibited NCC activity was also supported by the Western blot analysis. Figure 6D is a Western blot showing the effect of BK infusion (3 days) on the expression of pNCC and total NCC. The normalized band intensity for pNCC and total NCC (n=6) is summarized in Figure 6E. BK infusion for 3 days significantly decreased the abundance of pNCC (36±4% of the control) and total NCC (55±5% of the control). Thus, Western blot data are consistent with the results of the renal clearance study, suggesting that BK infusion inhibits the NCC activity in the DCT. The effect of BK infusion on NCC was specific because BK infusion for 3 days had no effect on the expression of NKCC2 and full-length ENaC-α subunit (Figure S4). However, from the inspection of Figure 4B, it is apparent that BK treatment decreased the expression of cleaved ENaC-α subunit (65±5% of the control), suggesting that BK treatment inhibits both ENaC and NCC. Because BK-induced inhibition of NCC should increase the flow-stimulated K^+ secretion by increasing Na^+ and volume delivery to the late portion of ASDN, the mice receiving BK infusion for 3 days were hypokalemic (Figure 6F). This strongly suggests the role of BK2R in regulating Na^+ and K^+ transport in ASDN.

Discussion

In the present study, we demonstrate that BK inhibits the basolateral 40-pS K^+ channel activity in the DCT. Moreover, the observation that PKC inhibitor abolished whereas PKC stimulator mimicked the effect of BK on the K^+ channels strongly suggests that the effect of BK on the basolateral K^+ channels

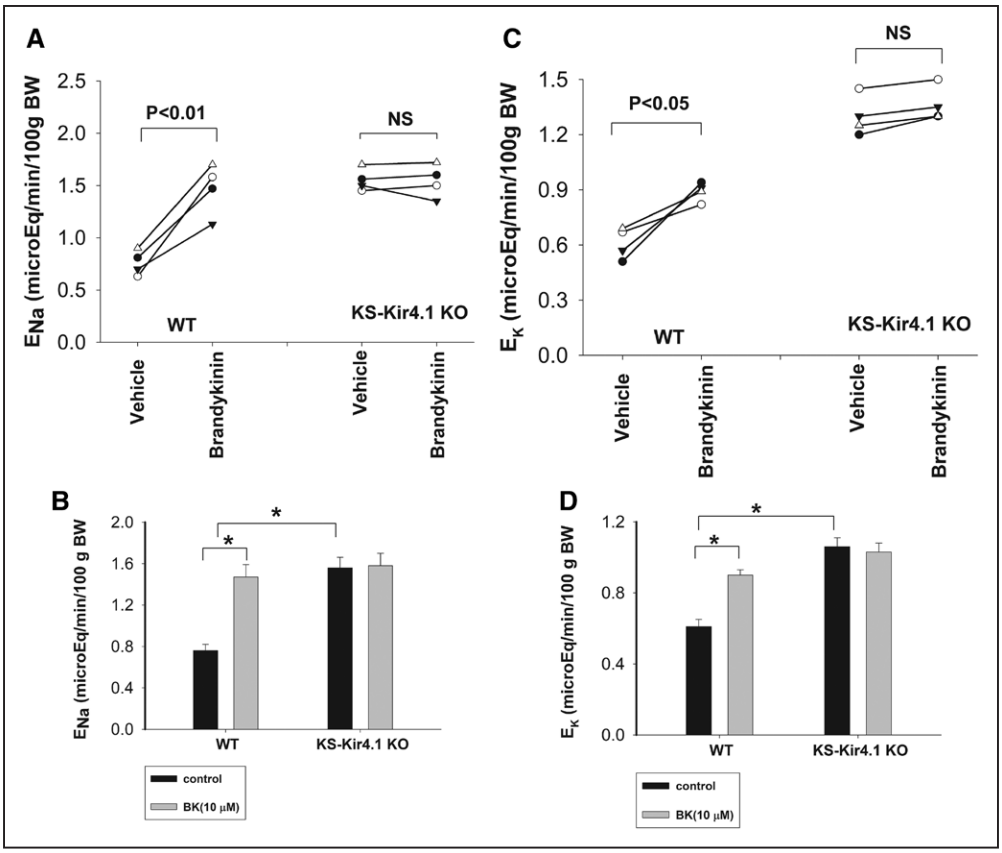

Figure 5. Bradykinin (BK) infusion stimulates renal Na^+ and K^+ excretion (E_{Na} and E_K). **A**, A line graph shows the results of each experiment in which urinary E_{Na} was measured before and after BK infusion in WT (wild type) mice and KS-Kir4.1 KO (kidney-specific conditional Kir4.1 knockout) mice. **B**, The mean value and statistical information are shown in a bar graph. The basal level of E_{Na} of KS-Kir4.1 KO mice is significantly different in comparison with WT mice. **C**, A line graph shows the results of each experiment in which urinary E_K was measured before and after BK infusion in WT mice and KS-Kir4.1 KO mice. **D**, The mean value of E_K and statistical information are shown in a bar graph. The basal level of E_K of KS-Kir4.1 KO mice is significantly different in comparison with WT mice. NS indicates nonsignificant. * indicates a significant significance.

in the DCT was mediated by PKC. Moreover, 2 lines of evidence suggest that BK2R is responsible for the effect of BK on the basolateral 40-pS K^+ channels: (1) immunostaining shows positive BK2R staining in the DCT; and (2) HOE140 (a specific BK2R antagonist) but not BK1R antagonist abolished the effect of BK on the basolateral 40-pS K^+ channels in the DCT. Thus, BK2R plays a role in tonic regulation of the basolateral 40-pS K^+ channels in the DCT.

The basolateral 40-pS K^+ channel in the DCT is composed of Kir4.1 (encoded by *Kcnj10*) and Kir5.1 (encoded by *Kcnj16*). Moreover, Kir4.1 is a pore-forming component for the Kir4.1/Kir5.1 heterotetramer because the deletion of *Kcnj10* completely eliminates the basolateral K^+ conductance in the DCT.[13,17,18] Consequently, the Ks-Kir4.1 KO mice recapitulated the Gitelman syndrome, including modest Na^+ wasting, high aldosterone, hypokalemia, and metabolic alkalosis.[17] Because this 40-pS K^+ channel is the only type of K^+ channel expressed in the basolateral membrane of the DCT,[17] it is conceivable that BK2R-mediated inhibition of the 40-pS K^+ channel activity should have a significant effect on the basolateral K^+ conductance and the membrane potentials. Indeed, we observed that BK treatment not only decreased the basolateral K^+ conductance but also depolarized the DCT membrane. This suggests that the BK–BK2R pathway is involved in tonic regulation of the membrane potential in the DCT.

Not only inhibiting the basolateral K^+ channels in the DCT, BK infusion also increased urine E_{Na}. Three lines of evidence suggest that BK-induced natriuresis was at least, in part, mediated by inhibiting NCC. First, renal clearance study showed that BK-induced natriuresis was absent in KS-Kir4.1 KO mice in which NCC function was inhibited.[17] Second, BK infusion decreased the expression of both total NCC and pNCC in the kidney. Third, hydrochlorothiazide-induced natriuretic effect—an indication of NCC function—was smaller in the mice treated with BK than untreated control animals, suggesting that BK suppressed NCC. A large body of evidence has demonstrated that the stimulation of BK–BK2R increases renal E_{Na} by mechanisms of increasing renal blood flow or inhibiting Na^+ transport in distal nephron.[6,19,20] Our present study has further suggested that BK-induced inhibition of NCC should contribute to kallicrein–kinin system–induced natriuresis. Thus, the results of the present study have suggested that BK–BK2R should play a role in the regulation of DCT function by inhibiting both Kir4.1 and NCC.

BK2R is also highly expressed in the vascular structure, and BK is a powerful vasodilator,[1]; it is conceivable that BK infusion may increase glomerular filtration rate thereby

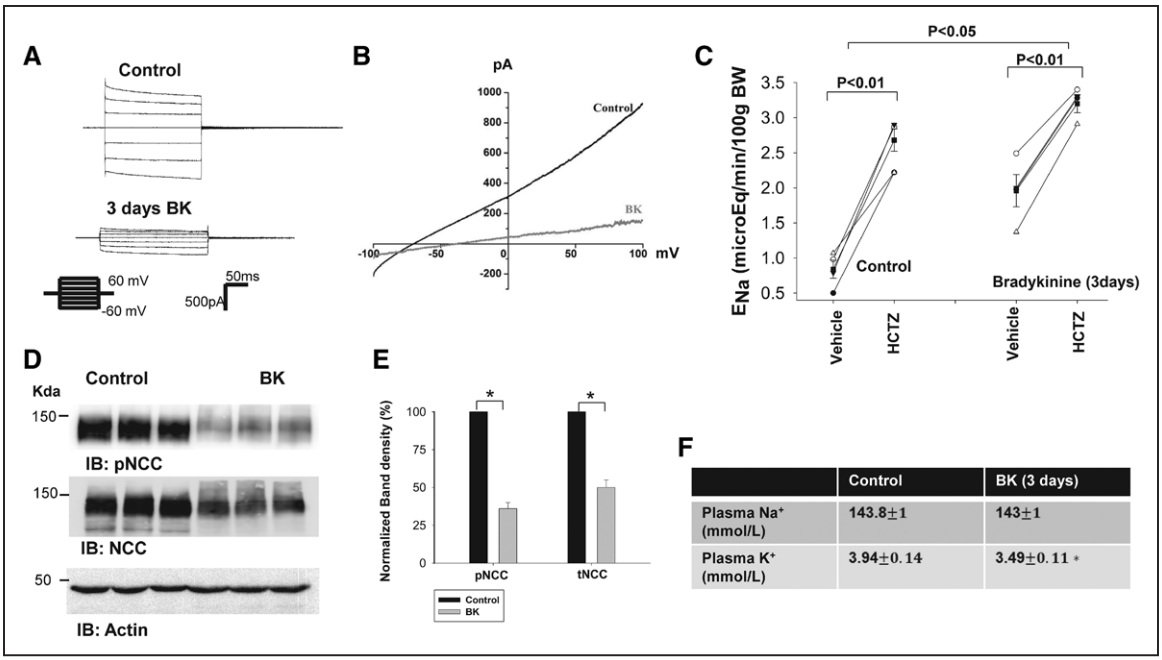

Figure 6. Bradykinin (BK) infusion inhibits Kir4.1 and NCC (NaCl cotransporter). **A**, A whole-cell recording shows Ba^{2+}-sensitive K^+ currents in the distal convoluted tubule (DCT) of mice treated with vehicle (control) or BK infusion for 3 d. K^+ currents were measured with a step protocol from –60 to 60 mV at a 20-mV step. **B**, A whole-cell recording shows K^+-current (I_K) reversal potential of the DCT in mice treated with vehicle (control) or BK infusion for 3 d. **C**, A line graph shows the results of experiments in which urinary sodium excretion (E_{Na}) was measured before and after a single dose of hydrochlorothiazide (HCTZ; 25 mg/kg body weight) in control and BK-treated mice. BK was delivered for 3 d through an osmatic pump. **D**, A Western blot shows the expression of phosphor-NCC at Thr^{53} (pNCC) and total NCC (tNCC) in control and BK-treated mice. **E**, A bar graph summarizing the normalized band density of pNCC and tNCC from tissues obtained in the control and BK-treated mice (n=6). **F**, A table shows the plasm Na^+ and K^+ concentrations in the control and BK-treated mice (n=7). *Significant difference between 2 groups.

increasing E_{Na}. However, BK-induced hemodynamic changes may have a minor role in stimulating E_{Na} in our experiment settings. This speculation is supported by 2 pieces of the observations. First, the largest increase in urinary volume after BK infusion appeared in the second (60 minutes after injection) and third collection (90 minutes after injection) rather than the first collection (30 minutes after injection; Figure S2). Second, the effect of BK on urinary E_{Na} and urine volume was absent in Ks-Kir4.1 KO mice. These findings suggest that BK-induced hemodynamic change in glomerular filtration rate may not play a major role in increasing urinary E_{Na}.

Also, BK2R is highly expressed in the collecting duct and cortical collecting duct, and previous elegant study by Zaika et al[20] has shown that BK inhibits ENaC in the cortical collecting duct. Our present study also shows that BK treatment decreased the expression of cleaved ENaC-α isoform. Thus, it is conceivable that BK-mediated inhibition of ENaC should contribute to the BK infusion–induced increase in urinary E_{Na}. Moreover, BK-induced increase in urinary volume delivery to the collecting duct should stimulate flow-dependent E_K thereby enhancing urinary E_K. However, the observation that BK infusion did not increase urinary E_{Na} in Kir4.1 KO mice, despite of the upregulation of ENaC activity in the collecting duct,[21] suggests that BK-induced inhibition of ENaC activity may be blunted in Kir4.1 KO mice. One possibility is that the volume depletion in Ks-Kir4.1 KO mice may suppress the inhibitory effect of BK on ENaC. Further experiments are needed to explore this possibility. However, BK-induced inhibition of NCC and ENaC should have a synergistic effect on overall renal E_{Na} under physiological conditions.

Two lines of evidence suggest the possibility that Kir4.1 activity is essential for the effect of BK on NCC, although we could not completely rule out the direct effect of BK on NCC. First, BK-induced natriuresis was largely abolished in KS-Kir4.1 KO mice. Second, BK-induced inhibition of NCC was closely correlated with BK-induced depolarization of DCT membrane. A large body of evidence has suggested the membrane potential in the DCT plays an important role in the regulation of NCC activity such that an increase in the membrane negativity (hyperpolarization) stimulates, whereas a decrease in membrane negativity (depolarization) inhibits NCC activity.[10,17,22,23] Our previous studies have demonstrated that the membrane potential is linked to NCC expression through the modulation of Cl^--sensitive WNK (with-no-lysine kinase), which is suppressed by high intracellular Cl^- levels and stimulated by decreased intracellular Cl^- levels.[22,24,25] Thus, the BK-induced inhibition of the basolateral Kir4.1 is expected to increase the intracellular Cl^- levels thereby inhibiting WNK. Because WNK activity determines the activity of both SPAK (Ste20 proline- and alanine-rich kinase) and OSR (oxidation-sensitive responsive kinase), the inhibition of WNK should suppress SPAK and OSR activity thereby inhibiting NCC.[26–30] Thus, it is conceivable that the BK-induced inhibition of the basolateral Kir4.1 in the DCT is involved for BK-induced decrease in NCC expression and activity.

Recent developments in the field of renal E_K have indicated that NCC activity in the DCT plays an important role in the

regulation of E_K and K^+ homeostasis.[22,31,32] For instance, an increase in dietary K^+ intake suppresses the NCC activity thereby increasing Na^+ and volume delivery to the late ASDN, whereas a decrease in dietary K^+ intake stimulates the NCC activity thereby decreasing Na^+ and volume delivery to the late ASDN.[33] Genetic and clinical studies have also shown that abnormal NCC activity is associated with hyperkalemia or hypokalemia. For instance, hyperkalemia in patients with pseudohypoaldosteronism type II is the result of high NCC activity,[28,34,35] whereas hypokalemia and K^+ wasting are 2 main phenotypes in patients with Gitelman syndrome in which genetic mutations cause the inhibition of NCC.[36] Indeed, our present study has also demonstrated that the application of BK-induced inhibition of NCC was associated with increasing urinary E_K. Consequently, the mice treated with BK were hypokalemic. This finding suggests the role of BK in the stimulation of E_K in ASDN and in maintaining K^+ homeostasis. In this regard, increased dietary K^+ intake has been reported to augment urinary kallikrein excretion and to stimulate BK2R expression,[3,4] suggesting that BK may play an important role in stimulating E_K during high dietary K^+ intake. This notion is also suggested by our finding that BK-induced inhibition of the basolateral K^+ channel activity was enhanced in the mice on HK diet. Our previous study has shown that increasing dietary K^+ intake inhibited the basolateral K^+ channel activity and that HK-induced inhibition of Kir4.1 was essential for the effect of HK intake on NCC.[10] Because BK2R regulates basolateral Kir4.1 in the DCT, it is possible that the stimulation of BK–BK2R pathway is an important mechanism by which HK intake stimulates renal E_K.

Perspectives

The main finding of the present study is to demonstrate that the stimulation of BK–BK2R inhibited the basolateral Kir4.1 in the DCT and increased renal Na^+ and E_K by inhibiting NCC. Although we and others have demonstrated that BK–BK2R pathway plays a role in increasing urinary E_{Na},[6,7,19] animals deficient in BK2R are normotensive under control conditions.[37–39] This suggests that BK–BK2R pathway may not be essential for maintaining overall Na^+ homeostasis and controlling normal blood pressure under physiological conditions. However, the finding that BK stimulates urinary E_K and causes hypokalemia suggests the possible role of BK2R in maintaining K^+ homeostasis. In this regard, the previous study has also shown that the mice with overexpression of BK2R increased urinary E_K.[5] Thus, it is possible that BK2R is involved in stimulating renal E_K through suppressing Kir4.1 and NCC in the DCT. Our previous experiments have also demonstrated that AT2R (type II angiotensin II receptor) plays a role in regulating urinary E_K.[40] Thus, both AT2R and BK2R may play a role in regulating urinary E_K during increased dietary K^+ intake.

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Disclosures

None.

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Novelty and Significance

What Is New?

- Bradykinin (BK) inhibits the basolateral Kir4.1 in the distal convoluted tubule by activating BK2R (BK B2 receptor) and depolarizes distal convoluted tubule membrane.
- BK application increases renal Na^+ excretion and inhibits NCC (NaCl cotransporter) activity. This natriuretic effect of BK depends on the presence of Kir4.1 in the distal convoluted tubule.
- BK stimulates renal K^+ excretion and causes mice hypokalemic.

What Is Relevant?

- NCC plays a key role in regulating renal K^+ excretion and maintaining K^+ homeostasis, whereas Kir.4.1 plays a key role in controlling NCC activity. The finding that BK2R is involved in the regulation of Kir4.1 and NCC is relevant for understanding an integrated mechanism of regulating K^+ homeostasis.
- The finding that BK inhibits NCC is highly relevant for understanding the mechanism of BK2R-induced natriuretic effect.

Summary

BK2R plays a role in inhibiting NCC activity by suppressing the basolateral Kir4.1 activity in the distal convoluted tubule, and BK2R plays a role in stimulating renal K^+ excretion and K^+ homeostasis.