Salt-Dependent Inhibition of Epithelial Na\(^+\) Channel–Mediated Sodium Reabsorption in the Aldosterone-Sensitive Distal Nephron by Bradykinin

Mykola Mamenko, Oleg Zaika, Peter A. Doris, Oleh Pochynyuk

Abstract—We have documented recently that bradykinin (BK) directly inhibits activity of the epithelial Na\(^+\) channel (ENaC) via the bradykinin B2 receptor (B2R)-G\(_{q/11}\)-phospholipase C pathway. In this study, we took advantage of mice genetically engineered to lack bradykinin receptors (B1R, B2R\(^{-/-}\)) to probe a physiological role of BK cascade in regulation of ENaC in native tissue, aldosterone-sensitive distal nephron. Under normal sodium intake (0.32% Na\(^+\)), ENaC open probability (\(P_o\)) was modestly elevated in B1R, B2R\(^{-/-}\) mice compared with wild-type mice. This difference is augmented during elevated Na\(^+\) intake (2.00% Na\(^+\)) and negated during Na\(^+\) restriction (<0.01% Na\(^+\)). Saturation of systemic mineralocorticoid status with deoxycorticosterone acetate similarly increased ENaC activity in both mouse strains, suggesting that the effect of BK on ENaC is independent of aldosterone. It is accepted that angiotensin-converting enzyme represents the major pathway of BK degradation. Systemic inhibition of angiotensin-converting enzyme with captopril (30 mg/kg of body weight for 7 days) significantly decreases ENaC activity and \(P_o\) in wild-type mice, but this effect is diminished in B1R, B2R\(^{-/-}\) mice. At the cellular level, acute captopril (100 \(\mu\)mol/L) treatment sensitized BK signaling cascade and greatly potentiated the inhibitory effect of 100 nmol/L of BK on ENaC. We concluded that BK cascade has its own specific role in blunting ENaC activity, particularly under conditions of elevated sodium intake. Augmentation of BK signaling in the aldosterone-sensitive distal nephron inhibits ENaC-mediated Na\(^+\) reabsorption, contributing to the natriuretic and antihypertensive effects of angiotensin-converting enzyme inhibition. (Hypertension. 2012;60:1234-1241.)

Key Words: kallikrein-kinin system ▪ collecting duct ▪ connecting tubule ▪ angiotensin-converting enzyme inhibition ▪ natriuresis

Maintenance of constant circulating volume and normal blood pressure is of key importance to all higher organisms. Kidneys play a dominant role in regulation of circulating volume by controlling water and Na\(^+\) excretion. Compromised kidney function is tightly linked to disturbances in extracellular fluid volume and, as a consequence, to altered blood pressure regulation. It is becoming recognized that excessive Na\(^+\) retention by the kidney is predominantly a failure to appropriately suppress tubular Na\(^+\) reabsorption. The aldosterone-sensitive distal nephron (ASDN) is the final site where tubular Na\(^+\) reabsorption is regulated. This segment is formed by the connecting tubule and the cortical collecting duct (CCD). The activity of the epithelial Na\(^+\) channels (ENaC) accounts for electrogeneric Na\(^+\) reabsorption in the ASDN. ENaC-mediated sodium reabsorption in ASDN is stimulated by the renin-angiotensin (Ang)-aldosterone system (RAAS) in response to decreased Na\(^+\) intake and volume contraction. A role of ENaC in regulation of systemic blood pressure is generally recognized. Genetic mutations in humans causing gain-of-function/loss-of-function in ENaC result in monogenic forms of hypertension/hypotension, respectively.

Whereas the RAAS serves to correct states associated with volume contraction, its counterpart, the kallikrein-kinin system (KKS), is activated in response to conditions with volume expansion. KKS lowers blood pressure by promoting vasodilation, natriuresis, and diuresis, generally opposing the hypertensive effects of RAAS. Moreover, activation of KKS reduces generation of reactive oxygen species and plays a protective role against organ damage in the heart and kidney.

The physiological actions of the KKS result from production of local hormone peptides kinins, such as bradykinin (BK), from a precursor kininogen mainly by the action of the serine protease kallikrein. BK interacts with G-protein-coupled B1 and B2 receptors (B1R and B2R). The biological effects of BK are mediated mainly through the B2R, which are constitutively expressed in smooth muscles, neurons, vascular endothelium, and kidney epithelial cells. Cumulative experimental evidence strongly supports a critical role of KKS in the regulation of systemic blood pressure. Disruption of any

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of the KKS components, including kininogen,\textsuperscript{17} kallikrein,\textsuperscript{18} and B2R,\textsuperscript{19,20} produces hypertension when sodium intake is elevated. Consistently, low urinary kallikrein levels are found in individuals with essential hypertension.\textsuperscript{21} A polymorphism in human B2R gene (+9,+9) is linked to increased cardiovascular risk and higher systolic blood pressure.\textsuperscript{22}

KKS and RAAS are interrelated at the level of the Ang-converting enzyme (ACE). ACE inhibition is proved to be potent in the treatment of hypertension, congestive heart failure, and diabetic nephropathy.\textsuperscript{23–25} However, the beneficial actions of ACE inhibition extend beyond blocking Ang II production and inhibition of RAAS. ACE is much more potent in cleaving kinins than in producing Ang II from Ang I.\textsuperscript{9,23} ACE inhibition alters the balance between the KKS and the RAAS in favor of the former, which further contributes to lowering of blood pressure. Inhibition of B2R consistently and significantly attenuates the hypertensive effect of ACE inhibition in both normotensive and hypertensive individuals.\textsuperscript{26}

In the kidney, KKS is thought to be involved in regulation of water and electrolyte handling.\textsuperscript{13,27} In perfused rat kidneys, inhibition of B2 receptors decreased urinary Na\textsuperscript{+} excretion without altering glomerular filtration rate or renal blood flow.\textsuperscript{28} Renal KKS is localized to the distal portion of the renal nephron. Specifically, kallikrein immunoreactivity is detected almost exclusively in the connecting tubule.\textsuperscript{29} Kininogen and B2R expressions are located in connecting tubule and CCD.\textsuperscript{29} This spatial pattern coincides with ENaC localization and argues that the renal KKS is specifically designed to influence tubule function.\textsuperscript{29}

In the current study, we probed physiological aspects of BK regulation of ENaC activity in freshly isolated murine ASDNs. We determined that genetic deletion of both BK receptors (B1R and B2R) results in increased ENaC activity under conditions of normal and elevated salt intake, and this effect is independent of aldosterone status. We proposed that inability to properly suppress ENaC activity during dietary sodium excess contributes to the salt-sensitive hypertension observed in mice with deleted BK receptors.\textsuperscript{19,20} Furthermore, ACE blockade with captopril greatly augments the BK signal to ENaC in murine ASDNs promoting renal sodium excretion. Genetic deletion of BK receptors attenuates the effect of ACE inhibition on sodium handling in the ASDN.

Methods

Reagents and Animals

All chemicals and materials were from Sigma (St Louis, MO), VWR (Radnor, PA), and Tocris (Ellisville, MO) and were at least of reagent grade. Animal use and welfare adhered to the National Institutes of Health Guide for the Care and Use of Laboratory Animals following a protocol reviewed and approved by the Animal Care and Use Committee of the University of Texas Health Science Center at Houston. For experiments, male C57BL/6d mice (Charles River Laboratories, Wilmington, MA) 6 to 8 weeks old were used. B1R, B2R\textsuperscript{−/−} mice (inbred into C57BL/6d background) were originally purchased from Jackson Laboratory (Bar Harbor, ME; strain 012371) and maintained in the animal facility of University of Texas Health Science Center at Houston. To examine the effects of salt intake, animals were provided diets containing nominally free (<0.01\% Na\textsuperscript{+}, TD.90228), regular (0.32\% Na\textsuperscript{+}, TD.7912), and high (2.00\% Na\textsuperscript{+}, TD.92034) sodium for 1 week. All diets were purchased from Harlan Teklad (Madison, WI). Animals had free access to tap water. In some experiments, captopril (30 mg/kg of body weight) was added to drinking water for 7 days.

Tissue Isolation

The procedure for isolation of the ASDNs suitable for electrophysiology and Ca\textsuperscript{2+}-imaging has been described previously.\textsuperscript{31–33} Briefly, mice were euthanized by CO\textsubscript{2} administration followed by cervical dislocation, and the kidneys were removed immediately. Kidneys were cut into thin sections (<1 mm), with sections placed into ice-cold physiological saline solution buffered with HEPES (pH 7.4). The ASDN was identified as merging of connecting tubule into CCD and was mechanically isolated from cortical sections of kidney sections by microdissection using watchmaker forceps under a stereomicroscope. Isolated ASDN was attached to a 5×5-mm coverglass coated with poly-L-lysine. A coverglass containing ASDN was placed in a perfusion chamber mounted on an inverted Nikon Eclipse Ti microscope and perfused with room temperature HEPES buffered (pH 7.4) saline solution. ASDNs were split opened with 2 sharpened micropipettes, controlled with different micromanipulators, to gain access to the apical membrane. The tubes were used within 1 to 2 hours of isolation.

Electrophysiology

ENaC activity in principal cells was determined in cell-attached patches on the apical membrane made under voltage-clamp conditions (−V\textsubscript{m}=−60 mV) using standard procedures\textsuperscript{32–34} (see online-only Data Supplement for more details).

Intracellular Calcium Measurements

Intracellular calcium levels were measured in cells of the split-opened ASDNs using Fura-2 fluorescence ratiometric imaging, as described previously.\textsuperscript{35,36} Split-opened ASDNs were loaded with Fura-2 by incubation with 2 μmol/L of Fura-2/acetoxymethyl ester in a bath solution for 40 minutes at room temperature. Subsequently, the ASDNs were washed and incubated for an additional 10 to 15 minutes before experimentation. The ASDNs were then placed in an open-top imaging study chamber (Warner RC-10) with a bottom coverslip viewing window and the chamber attached to the microscope stage of an InCa Imaging Workstation (Intracellular Imaging, Inc.). Cells were imaged with a 20× Nikon Super Fluor objective and regions of interest drawn for individual cells. The Fura-2 fluorescence intensity ratio was determined by excitation (an average for \(≈300\text{ ms}\)) at 340 nm and 380 nm and calculating the ratio of the emission intensities at 511 nm in favor of the former, which further contributes to lowering of blood pressure. Inhibition of B2R consistently and significantly attenuates the hypotensive effect of ACE inhibition in both normotensive and hypertensive individuals.\textsuperscript{22}

Data Analysis

All summarized data are reported as mean±SEM. Data from before and after treatment within the same experiment were compared using the paired \(t\) test. Data from different experiments were compared with Student (2-tailed) \(t\) test or 1-way ANOVA as appropriate. \(P≤0.05\) was considered significant.

Results

Genetic Deletion of B1 and B2 Receptors Disrupts BK Regulation of ENaC

Our recent report\textsuperscript{39} demonstrated that BK acutely regulates ENaC \(P\) in the murine ASDN by stimulation of B2R. Genetic deletion of B2R can lead to compensatory upregulation of B1R expression.\textsuperscript{39,40} To adequately probe the physiological
relevance of BK regulation of ENaC, we used mice lacking both BK receptors (B1R, B2R<sup>−/−</sup>). BK fails to affect ENaC activity in freshly isolated split-opened ASDNs as was assessed with patch clamp electrophysiology (Figure 1A). ENaC <i>P</i><sub>o</sub> was 0.47±0.09, 0.47±0.10, and 0.48±0.09 (n=7; N=5 mice) in the control, on application of 500 nmol/L of BK, and after washout, respectively (Figure 1B). In contrast, the same experimental sequence greatly diminishes ENaC open probability in ASDN cells from wild-type (WT) animals, as exemplified by the representative patch clamp experiment in Figure 1C. The magnitude of inhibition was 73±8% (n=6; N=4 mice; Figure 1D), which is consistent with our previous report. Thirty

**Ablation of BK Signaling Results in Salt-Sensitive Augmentation of ENaC Activity**

We next carefully determined the consequences of the disruption of BK cascade on ENaC activity in mice kept on regular Na<sup>+</sup> intake (0.32% Na<sup>+</sup>). Figure 2A shows representative current traces of ENaC activity in WT and B1R, B2R<sup>−/−</sup> mice. As is clear, ENaC activity is modestly but significantly increased in mutant mice (Figure 2B). This elevation was attributed to a greater ENaC <i>P</i><sub>o</sub> but not to changes in functional ENaC levels (fN). We concluded that dysfunctional BK signaling results in ENaC hyperactivity attributed to augmentation of ENaC gating.

We further probed whether disruption of BK signaling affects adaptation of ENaC activity to changes in salt intake. Indeed, increased Na<sup>+</sup> intake (2% Na<sup>+</sup>) exacerbates the difference in ENaC <i>P</i><sub>o</sub> between B1R, B2R<sup>−/−</sup> and WT mice (Figure 3A). In contrast, we found no significant difference in ENaC activity when animals were placed on a sodium-deficient diet (<0.01% Na<sup>+</sup>). Of note, we did not detect significant differences in functional ENaC levels in WT and B1R, B2R<sup>−/−</sup> mice under these conditions. We concluded that BK signaling is critical to suppress ENaC activity when sodium intake is elevated.

The blunted modulation of ENaC activity by salt intake in B1R, B2R<sup>−/−</sup> mice may arise from altered sensitivity to aldosterone. To test this, we placed animals on high-salt intake to suppress endogenous aldosterone secretion and exogenously saturated mineralocorticoid signaling with deoxycorticosterone acetate injections. As summarized in Figure 3B, deoxycorticosterone acetate induces comparable increases in ENaC activity in both strains. This suggests that BK cascade does not overlap with aldosterone regulation of ENaC.

**Figure 1.** Bradykinin inhibition of epithelial Na<sup>+</sup> channel (ENaC) is absent in B1R, B2R<sup>−/−</sup> mice. A, A representative continuous current trace from a cell-attached patch containing single ENaC in the control condition, under application of 500 nmol/L of bradykinin (BK), and after washout with regular bath solution. The patch was formed on the apical plasma membrane of a principal cell within a split-opened area of aldosterone-sensitive distal nephron (ASDN) isolated from B1R, B2R<sup>−/−</sup> mice. The patch was held at a test potential of <i>V</i><sub>h</sub>−<i>V</i><sub>p</sub>=−60 mV. Areas control (1) and on BK treatment (2) are shown below at an expanded time scale. Inward Li<sup>+</sup> currents are downward. **Dashed lines** indicate the respective current state with C denoting the closed state. B, Summary graph of ENaC <i>P</i><sub>o</sub> changes in response to BK and after washout from paired patch clamp experiments similar to that shown in part A. C, A representative continuous current trace from a cell-attached patch monitoring ENaC activity in ASDN isolated from wild-type (WT) mice in the control condition, under addition of 500 nmol/L of BK, and after washout of BK with regular bath solution. D, Summary graph of relative ENaC activity after treatment with 500 nmol/L of BK for WT and B1R, B2R<sup>−/−</sup> mice, respectively. ENaC activity was normalized to the corresponding values before treatment. Here and below, numbers of experiments for each data set are shown. **#Significant change vs WT.**
Newly generated kinins are known to be rapidly degraded via ACE-dependent cleavage. Because ACE activity is present in the ASDN,41,42 we investigated whether ACE interferes with BK regulation of ENaC. Systemic inhibition of ACE with captopril (30 mg/kg of body weight for 7 days) significantly decreases total ENaC activity and $P_0$ in WT mice kept on regular salt regimen (Figure 4). Importantly, genetic deletion of BK receptors diminishes ACE-dependent ENaC inhibition. Snapshot measurements of urinary sodium and creatinine concentrations in the bladder (Figure S1 in the online-only Data Supplement) support the conception that natriuretic effect of captopril is blunted in B1R, B2R−/− mice. More careful analysis is required to definitively prove this. Overall, our results suggest that tonic ACE activity limits BK inhibition of ENaC-mediated sodium reabsorption.

Figure 2. Genetic deletion of bradykinin (BK) receptors augments basal epithelial Na$^+$ channel (ENaC) activity. A, Representative current traces of ENaC activity in aldosterone-sensitive distal nephron (ASDN) from wild-type (WT) (top) and B1R, B2R−/− mice (bottom). All other conditions are identical to those described in Figure 1A. B, Summary graphs comparing total ENaC activity ($f_NP_0$, left), ENaC open probability ($P_0$, middle), and functional ENaC expression ($f_N$, right) for split-opened ASDNs from WT and B1R, B2R−/− mice. #Significant increase vs WT.

Figure 3. Effect of bradykinin (BK) on epithelial Na$^+$ channel (ENaC) is independent of aldosterone status. A, Summary graph of ENaC $P_0$ for wild-type (WT) and B1R, B2R−/− mice maintained on sodium-deficient (<0.01% Na$^+$) and high-sodium (2.00% Na$^+$) diet for 1 week before the experimentations. #Significant increase vs WT. B, Summary graph comparing total ENaC activity for WT and B1R, B2R−/− mice kept on high-sodium intake and subcutaneously injected with deoxycorticosterone acetate (DOCA) for 3 consecutive days (2.4 mg per injection per animal). #Significant increase vs WT 2% Na$^+$; ##significant increase vs B1R, B2R−/− 2% Na$^+$.
nmol/L of BK significantly decreased ENaC $P_o$ in a reversible manner in control conditions (Figure 5A). The magnitude of this effect (inhibition by 43±10%) is considerably smaller than observed with 500 nmol/L of BK (Figure 1D). Acute captopril application had no measurable effect on ENaC (Figure 5B). However, as is clear from the representative current trace (Figure 5B) and the summary graph (Figure 5C), BK had a significantly stronger action on ENaC when ACE was blocked (83±5%). The comparison of BK inhibition of ENaC $P_o$ is shown in Figure 5D.

Activation of BK receptors is known to increase intracellular Ca$^{2+}$ concentration via phospholipase C-IP3–dependent mechanism. To assess the functional status of the receptors, we used Ca$^{2+}$-sensitive dye Fura-2 to directly monitor changes in [Ca$^{2+}$]i in individual cells within a split-opened ASDN in response to activation of BK signaling. As demonstrated on the average time course of changes in [Ca$^{2+}$]i (Figure 6), 100 nmol/L of BK elicits a remarkably greater Ca$^{2+}$ response after pretreatment with captopril. In contrast, we did not observe any significant changes in the magnitude of the [Ca$^{2+}$]i transient elicited by subsequent BK application (data not shown).

Overall, our results strongly suggest that ACE blockade augments BK signaling in the ASDN leading to a greater ENaC inhibition likely contributing to the increased renal sodium excretion by captopril.

### Discussion

The initial evidence that BK regulates sodium handling in the distal nephron was provided by Tomita et al. Although it was suggested that BK inhibits electroneutral Na$^+$ and Cl$^-$ transport, this does not exclude an effect of BK on ENaC, because changes in ENaC activity can occur in the absence of changes in transepithelial membrane potential in perfused CCDs. Indeed, we reported direct inhibitory action of nanomolar concentrations of BK on

![Figure 4](image-url)

**Figure 4.** Systemic inhibition of angiotensin-converting enzyme decreases epithelial Na$^+$ channel (ENaC) activity in wild-type (WT) but not in B1R, B2R−/− mice. Summary graph of total ENaC activity (left) and open probability (right) for WT and B1R, B2R−/− mice in the control condition and after treatment with captopril for 7 days. Captopril (30 mg/kg of body weight) was given with drinking water. #Significant decrease vs WT; ##significant increase vs WT+captopril.

![Figure 5](image-url)

**Figure 5.** Acute angiotensin-converting enzyme inhibition potentiates bradykinin (BK) inhibition of epithelial Na$^+$ channel (ENaC). A, A representative continuous current trace from a cell-attached patch containing single ENaC in the control condition, under application of 100 nmol/L of BK, and after washout with regular bath solution. All other conditions are identical to those described in Figure 1A. The patch was formed on the apical plasma membrane of a principal cell within a split-opened area of aldosterone-sensitive distal nephron isolated from wild-type mice. B, A representative continuous current trace from a cell-attached patch monitoring ENaC activity in the control condition, under application of 100 pmol/L of BK, and washout with regular bath solution. Drug application times are shown with respective bars on the top. C, Summary graph of ENaC $P_o$ changes in response to captopril and after BK+captopril from paired patch clamp experiments similar to that shown in part B. D, Summary graph of relative ENaC activity after treatment with 100 nmol/L in the absence and presence of pretreatment with captopril. ENaC activity was normalized to the corresponding values before treatment. #Significant change vs 100 nmol/L of BK.
responses by captopril. The average time course of [Ca2+]i reported observation that inhibition of B2 receptors with HOE-sive sodium retention. Our results resonate with previously cortex than those in the medulla. 46 Furthermore, urinary BK 10- to 100-nmol/L range, and these values are higher in the BK. Interstitial fluid BK levels in the rat kidney are in the when BK receptors are deleted.19,20 Because BK receptors are also expressed in vasculature,16 future studies are necessary to carefully determine the extent of this contribution.

ENaC in native distal nephrons and cultured principal cells with patch clamp electrophysiology.30 In the study by Tomita et al,41 nanomolar BK concentrations inhibited NaCl transport only from the basolateral side in the perfused rat CCD. In our experiments, we applied BK apically, although back-leak to the basolateral side is also generally recognized for split-opened ASDN preparations. In addition, differences in species and preconditioning might potentially cause the discrepancies about the apical versus basolateral BK actions on ENaC and sodium transport, respectively.

Kidney is capable of producing a significant amount of BK. Interstitial fluid BK levels in the rat kidney are in the 10- to 100-nmol/L range, and these values are higher in the cortex than those in the medulla.46 Furthermore, urinary BK and kallikrein levels are elevated during high-salt diet.53 This suggests that physiologically relevant concentrations of BK in the kidney are sufficient to suppress ENaC activity. Indeed, in the current study, we directly demonstrate that 100 nmol/L of BK inhibits ENaC activity in the distal nephron (Figure 5) and that disruption of BK signaling augments ENaC activity. This becomes particularly apparent during volume-expanded conditions, such as elevated Na+ intake. Thus, genetic deletion of BK receptors recapitulates the state of gain-of-function mutations in ENaC causing hypertension in humans.49-51 This strongly suggests that BK signaling plays an important physiological role by decreasing ENaC activity in the ASDN during euvoletic and volume-expanded states to avoid excessive sodium retention. Our results resonate with previously reported observation that inhibition of B2 receptors with HOE-140 (icatibant) in perfused kidney increases tubular Na+ reabsorption in rats fed a normal but not a sodium-deficient diet.28 Therefore, it is reasonable to propose that enhanced ENaC activity contributes to the salt-sensitive hypertension observed when BK receptors are deleted.19,20 Because BK receptors are also expressed in vasculature,46 future studies are necessary to carefully determine the extent of this contribution.

We also found that BK regulation of ENaC does not overlap with salt-dependent regulation of ENaC by mineralocorticoids. Despite the long-standing dogma that aldosterone is the major determinant of ENaC activity, we increasingly appreciate that regulation of sodium handling in the distal nephron is much more complex and requires integrated inputs from several sources. Recent evidence identifies endothelin-150,51 and purinergic33,52 signaling cascades as important inhibitors of ENaC during elevated salt intake. In contrast, we56 and others57 pointed to a direct effect of Ang II in stimulation of ENaC that may occur during volume restriction. It is clear that the ENaC-mediated sodium handling in the ASDN is a critical component of sodium homeostasis. Gain-of-function mutations of the channel cause hypertension and volume expansion (Liddle syndrome).54 Loss-of-function mutations, in contrast, lead to salt wasting and low BP (pseudohypoaldosteronism type I).54 Water and electrolyte transport in the tubule cannot be further compensated downstream of the ASDN, and sodium reabsorption in this segment is not under negative feedback control, as occurs in proximal tubular segments. Thus, it is tempting to speculate that multiple signaling inputs are designed to fine-tune functional properties of epithelial cells in the ASDN protecting the kidney from unopposed modulation of transport rates. At the same time, these regulatory inputs are not redundant because disruption of either of them results in mild-volume imbalance, as reported in mice lacking purinergic P2Y2 receptor,52 or with disrupted ATP release in the distal nephron (Cx30−/− mice)55 and mice lacking endothelin receptor type-B.57 In the current study, we provide strong evidence about a functional role of BK signaling in the ASDN with its disruption leading to the salt-sensitive ENaC activation.

We also demonstrate that ACE determines functional status of BK signaling in the ASDN. ACE activity constitutes one of the major pathways responsible for cleavage of kinins. Emerging evidence suggests that sufficient ACE activity is present at the apical plasma membrane of principal cells.41,42 We found that, under regular salt diet, prolonged ACE blockade markedly decreases ENaC activity, and this effect was greatly attenuated by disruption of BK receptors. Importantly, we were also able to correlate changes in ENaC activity with changes in urinary Na+ excretion under these conditions, indicating physiological relevance of this regulation. It should be noted, however, that a part of captopril actions on ENaC could be mediated via decreased Ang II and aldosterone levels. However, animals are not volume depleted under normal sodium intake, and the concentrations of these hormones are low. In addition, Ang II can be formed by enzymes other than ACE, such as chymase.56 In our preliminary observations, we found a mild effect of systemic inhibition of mineralocorticoid receptors with spironolactone on ENaC activity in mice kept on a regular salt diet. However, the effect was associated with a decreased number of functional channels but not PNa, as observed during captopril treatment in this study (Figure 4). We also found that acute ACE blockade with captopril potentiates the inhibitory effect of BK on ENaC. Furthermore, this treatment augments activation of B2 receptors in response to external application of the same concentration of BK (Figure 6). It is possible that close association of ACE with B2R, as was reported for different cell types,57 could decrease the actual concentration of the agonist in the vicinity of the receptors.
attributed to tonic kininase activity of ACE. Alternatively, ACE inhibitors were suggested to act as allosteric enhancers of BK receptor function.65,66 Whereas it is not feasible to precisely determine which scenario actually takes place, our results favor the concept that BK-mediated inhibition of ENaC activity contributes to the natriuretic and antihypertensive effects of ACE inhibition.

Interestingly, the effects of renal KKS on Na+ transport could be more complex and occur independent of BK production. Tissue kallikrein is a serine protease that can proteolytically cleave ENaC at the prostanin site to augment its activity.59,60 It has been proposed that this may activate ENaC during low Na+ and high K+ intakes.63 However, the physiological relevance of this regulation requires further verification because the renal phenotype in animal models with low kallikrein levels18 is similar to that resulting from deletion of B2R.19,20 Furthermore, overexpression of human kallikrein in rats and mice causes hypotension.61,62 On the other hand, similar dual actions on Na+ handling in the kidney were reported for prostaglandins. Despite the fact that renal prostaglandins cause natriuresis and diuresis,63 they also play a critical role in promoting renin secretion.64 It is possible that such opposite effects serve to partially balance each other, thus protecting from extreme disturbances in kidney function.

Perspectives

Detailed understanding of discrete systems controlling sodium homeostasis is fundamental to understanding physiology and treating diseases, such as hypertension. ENaC-mediated Na+ reabsorption in the distal nephron finalizes adjustments of renal sodium excretion to match dietary sodium intake and maintain sodium balance. This is known to be critical for normal blood pressure control. The current study defines a previously underappreciated role of the renal KKS in the regulation of sodium handling and specifically of ENaC activity in the distal nephron by dietary salt intake. Disruption of this regulation leads to overactive ENaC, which is detrimental under sodium-loaded conditions. Functional BK signaling in the distal nephron also appears to be a critical component of the natriuresis promoted by ACE inhibition. It is possible that genetic polymorphism in genes encoding B2R and other functional components of KKS may contribute to different susceptibility of patients to the anti-hypertensive actions of ACE blockade.

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Disclosures

None.

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Salt-dependent inhibition of ENaC-mediated sodium reabsorption in the aldosterone-sensitive distal nephron by bradykinin

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METHODS

Electrophysiology. ENaC activity in principal cells was determined in cell-attached patches on the apical membrane made under voltage-clamp conditions (-V_p = -60 mV) using standard procedures\textsuperscript{1-3}. Current recordings were made in a permanently perfused bath (1.5 mL/min). Drug application times are shown with bars on the top of representative single channel traces. Recording pipettes had resistances of 8-10 megaohms. Typical bath and pipette solutions were (in mM): 150 NaCl, 5 mM KCl, 1 CaCl\textsubscript{2}, 2 MgCl\textsubscript{2}, 5 glucose and 10 HEPES (pH 7.4); and 140 LiCl, 2 MgCl\textsubscript{2} and 10 HEPES (pH 7.4), respectively. For each experimental condition, ASDNs from at least three different mice were assayed. Gap-free single channel current data from gigaohm seals were acquired (and subsequently analyzed) with an Axopatch 200B (Axon Instr.) patch clamp amplifier interfaced via a Digidata 1440 (Axon Instr.) to a PC running the pClamp 10.2 suite of software (Axon Instr.). Currents were low-pass filtered at 100 Hz with an eight-pole Bessel filter (Warner Instr.). Events were inspected visually prior to acceptance. ENaC activity was analyzed over a span of 60-120 sec for each experimental condition after reaching a new steady-state in response to a treatment. Channel activity in individual patches, defined as $NP_o$, was calculated using the following equation: $NP_o = (t_1 + 2t_2 + \ldots + nt_n)$, where $N$ and $P_o$ are the number of ENaC in a patch and the mean open probability of these channels, respectively, and $t_n$ is the fractional open time spent at each of the observed current levels. $P_o$ was calculated by dividing $NP_o$ by the number of active channels within a patch as defined by all-point amplitude histograms. For calculating $P_o$ in paired experiments, $N$ was fixed as the greatest number of active channels observed in control or experimental conditions. To estimate total ENaC activity ($fNP_o$) under particular physiological condition, we normalized $NP_o$ to the frequency of observing patches with at least one active channel ($f = \text{number of patches with active channels} / \text{total number of patches}$). ENaC activity was continuously monitored during period of time 60-120 sec. Using previously described analysis\textsuperscript{4}, we can reliably (P<0.05) estimate the maximal number of functional ENaC in a patch using this time span. For representation, current traces were corrected for a slow baseline drifts as necessary.

Urinary sodium excretion. Prior to electrophysiological assessment of ENaC activity, urine was collected from bladders of sacrificed animals and frozen at -20°C until analyzed. The time of urine sampling was the same (10AM-11AM) for all animals. Urinary sodium excretion was calculated as the ratio of urinary sodium to creatinine concentrations. Urinary
sodium concentration was measured using PFP7 Flame photometer (Techne, Burlington, NJ). Urinary creatinine concentration was assessed with Hitachi 7000 HPLC System (Pleasanton, CA, USA).
REFERENCES


Figure S1. Genetic deletion of BK receptors diminishes natriuretic effects of ACE inhibition. Summary graph of urinary sodium excretion from mice used for patch clamp experimentation in Figure 4. Urinary sodium excretion was calculated as the ratio (mM/mM) of urinary sodium and urinary creatinine. Samples are taken directly from bladder. #-significant increase versus Wild Type control. ##-significant decrease versus Wild Type+captopril.