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Abstract—The epithelial Na^+ channel (ENaC) is an essential channel responsible for Na^+ reabsorption in the aldosterone-sensitive distal nephron. Consequently, ENaC is a major effector impacting systemic blood volume and pressure. We have shown recently that Rac1 increases ENaC activity, whereas Cdc42 fails to change channel activity. Here we tested whether Rac1 signaling plays a physiological role in modulating ENaC in native tissue and polarized epithelial cells. We found that Rac1 inhibitor NSC23766 markedly decreased ENaC activity in freshly isolated collecting ducts. Knockdown of Rac1 in native principal cells decreased ENaC-mediated sodium reabsorption and the number of channels at the apical plasma membrane. Members of the Wiskott-Aldrich syndrome protein (WASP) family play a central role in the control of the actin cytoskeleton. N-WASP functions downstream of Cdc42, whereas WAVEs are effectors of Rac1 activity. N-WASP and all 3 isoforms of WAVE significantly increased ENaC activity when coexpressed in Chinese hamster ovary cells. However, wiskostatin, an inhibitor of N-WASP, had no effect on ENaC activity. Immunoblotting demonstrated the presence of WAVE1 and WAVE2 and absence of N-WASP and WAVE3 in $mpkCCD_{c14}$ and M-1 principal cells. Immunohistochemistry analysis also revealed localization of WAVE1 and WAVE2 but not N-WASP in the cortical collecting duct of Sprague-Dawley rat kidneys. Moreover, patch clamp analysis revealed that Rac1 and WAVE1/2 are parts of the same signaling pathway with respect to activation of ENaC. Thus, our findings suggest that Rac1 is essential for ENaC activity and regulates the channel via WAVE proteins. **(*Hypertension*. 2011; 57:996-1002.) • Online Data Supplement**

Key Words: aldosterone ■ aldosterone-sensitive distal nephron ■ epithelial transport ■ Wiskott-Aldrich syndrome protein ■ actin cytoskeleton

Long-term control of blood pressure involves Na^+ homeostasis through the precise regulation of the epithelial Na^+ channel (ENaC) in the aldosterone-sensitive distal nephron. ENaC dysfunction leads to disturbances in total body Na^+ homeostasis associated with abnormal regulation of blood volume, blood pressure, and lung fluid balance.[1–3] For example, partial loss of function in ENaC produces pseudohypoaldosteronism type 1, characterized by salt wasting. In contrast, a gain-of-function mutation leads to Liddle syndrome.[4]

Ion channels are recognized to be important physiological effectors of small G proteins.[5] Small G proteins are molecular switches that control the activity of cellular and membrane proteins and regulate a wide variety of cell functions, including cytoskeleton reorganization. Dysfunction in small G proteins of the Rho family, including Rac1, Cdc42, and RhoA, contributes to the pathology of renal and cardiovascular diseases, such as hypertension and proteinuric kidney disease.[6,7] A cellular mechanism for this process is obscure. Rac1 plays a role in some renal and cardiovascular diseases, possibly influencing systemic Na^+ and water balance. Shibata et al[8] have established recently that signaling cross-talk between Rac1 and the mineralocorticoid receptor modulates receptor activity and identified Rac1 as a therapeutic target for chronic kidney disease. Silva and Garvin[9] and Takemura et al[10] also recently identified an important role for Rac1 in NaCl-induced superoxide generation in the medullary thick ascending limb[9] and in the alveolar epithelium.[10]

Members of the Wiskott-Aldrich syndrome protein (WASP)/WAVE family (N-WASP, WASP, and WAVE1, -2, -3 isoforms) share similar domain structure and activate Arp2/3-mediated actin polymerization, leading to distinct downstream effects. N-WASP inducibly binds Arp2/3 in response to Cdc42, whereas WAVE proteins constitutively interact with Arp2/3 and are effectors of Rac1 activity.[11] We have shown recently that wild-type and constitutively active (QL) Rac1 significantly increase ENaC activity. However, neither coexpression with wild-type nor with constitutively active $Cdc42^{G12V}$ had an effect on ENaC activity.[12] In the present study we report that Rac1 is required for ENaC activity in native principal cells. Fur-

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thermore, we report here that WAVE1/2 and Rac1 are parts of the same signaling mechanism with respect to ENaC. Thus, these results reveal a new signaling pathway mediating the regulation of ENaC.

Materials and Methods

cDNA Constructs and Cell Culture

All of the chemicals and materials were purchased from Fisher Scientific, Sigma, or CalBiochem unless noted otherwise. NSC23766 was obtained from Tocris Biosciences. Wiskostatin was from Enzo Life Sciences (Plymouth Meeting, PA). Chinese hamster ovary (CHO) and M-1 cells were obtained from American Type Culture Collection and maintained with standard culture conditions (DMEM, 5% FBS, 1× penicillin-streptomycin, 37°C, 5% CO_2). $MpkCCD_{c14}$ cells were kindly provided by Dr A. Vandewalle (Institut National de la Santé et de la Recherche Médicale, Paris, France) and described previously.[13,14] For electrophysiological experiments, principal cells were grown on permeable supports (Costar Transwells; 0.4-μm pore, 24-mm diameter) and maintained with FBS and corticosteroids allowing them to polarize and form monolayers with high resistance and avid Na^+ reabsorption. The mammalian expression vectors encoding mouse α-, β-, and γ-ENaC have been described previously.[15,16] The expression vectors encoding Wiskott-Aldrich syndrome-like (SC111092), WAS protein family, member 1 (WASF1) (SC117699), WASF2 (SC126670), and WASF3 (SC115933) were from OriGene (Rockville, MD). The expression vectors encoding constitutively active and dominant-negative Rac1 (QL and T17N, respectively) were kindly provided by Drs A. Chan (Medical College of Wisconsin) and C. DerMardirossian on behalf of G.M. Bokoch (Scripps Research Institute, La Jolla, CA).

Isolation of Collecting Ducts

Patch clamp electrophysiology was used to assess ENaC activity in isolated, split-open rat cortical collecting duct (CCD). CCDs were isolated from salt-restricted (Na^+-deficient diet for 1 week) Sprague-Dawley rats as described previously.[12,14,17] Kidneys were cut into thin sections (<1 mm) with sections placed into ice-cold physiological saline solution (pH 7.4). CCDs were mechanically isolated from these sections by microdissection using forceps under a stereomicroscope. CCDs were split opened with a sharpened micropipette controlled with a micromanipulator to gain access to the apical membrane. Animal use and welfare procedures adhered to the National Institutes of Health Guide for the Care and Use of Laboratory Animals following a protocol reviewed and approved by the Institutional Animal Care and Use Committee.

Transfection and Rac1 Knockdown

For electrophysiological experiments, CHO cells were seeded on sterile 4×4-mm cover glass and transfected using Polyfect reagent (Qiagen, Valencia, CA), as described previously.[12,16] Mouse ENaC (mENaC) was reconstituted by coexpressing α-, β-, and γ-channel subunits together (0.1 to 0.3 μg of each cDNA per 35-mm dish).

Rac1 short hairpin (sh)RNA lentiviral particles (sc-36352-V; Santa Cruz Biotechnology) were used for the inhibition of Rac1 expression in principal cells. Control shRNA lentiviral particles (sc-108080; Santa Cruz Biotechnology) were used to confirm the selectivity of lentiviruses. After transduction with Santa Cruz transfection reagent (sc-108061), stable cell line expressing the shRNA was isolated via selection with puromycin.

Electrophysiology

Single-channel and whole-cell current data were acquired and subsequently analyzed with an Axopatch 200B amplifier (Axon Instruments) interfaced via a Digidata 1440A to a PC running the pClamp 10.2 suite of software (Axon Instruments). For single channel recordings, currents were filtered with an 8-pole, low-pass Bessel filter LPF-8 (Warner Institute, Hamden, CT) at 0.2 kHz. Typical bath solution was (in millimoles): 150 NaCl, 1 $CaCl_2$, 2 $MgCl_2$, and 10 HEPES (pH 7.4). Pipette solutions for cell attached and whole cell configurations were (in millimoles): 140.0 LiCl, 2.0 $MgCl_2$, and 10.0 mmol/L of HEPES (pH 7.4) and 120.0 CsCl, 5.0 NaCl, 2.0 $MgCl_2$, 5.0 EGTA, 2.0 Mg-ATP, 0.1 GTP, and 10 mmol/L of HEPES (pH 7.4), respectively. Single-channel analysis was performed as described previously.[12] Whole-cell macroscopic current recordings of mENaC expressed in CHO cells were made under voltage-clamp conditions using standard methods.[12,16]

Immunohistochemistry and Western Blot Analysis

The Sprague-Dawley rat kidneys were fixed in Zinc formalin and processed for paraffin embedding. The kidney sections were cut, dried, and deparaffinized for subsequent labeled streptavidin-biotin immunohistochemistry. After deparaffinization, the slides were treated with a citrate buffer (pH 6.0) for a total of 35 minutes. The slides were blocked with a peroxidase block (DAKO), Avidin Block (Vector Laboratories), Biotin Block (Vector Laboratories), and serum-free protein block (DAKO). Tissue sections were incubated for 90 minutes in 1:200, 1:400, or 1:500 concentrations of N-WASP (AB1963), WAVE1 (#07-037), or WAVE2 (4226; Millipore, Temecula, CA), respectively. Secondary detection was performed with goat anti-rabbit biotinylated IgG (Biocare) followed by streptavidin horseradish peroxidase (Biocare) and visualized with diaminobenzidine (DAKO). All of the slides were counterstained with Mayers hematoxylin (DAKO), dehydrated, and mounted with permanent mounting media (SAKURA).

Expression of proteins was analyzed in $mpkCCD_{c14}$ and M-1 cells by immunoblotting with antibodies to the N-WASP, WAVE/Scar, WAVE-2, and WAVE-3 (#09-145; all Millipore). Control cell lysate for WAVE1 derived from 3T3/A31 cell line was also obtained from Millipore (#12-305). Equal amounts of cell lysate were subjected to Western blot analysis using antibodies against corresponding proteins and β-actin, as was described previously.[12,18]

3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide Cell Viability Assay

For the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, $mpkCCD_{c14}$ cells were seeded as was done previously[19] into 12-well cluster plates at a subconfluent levels and allowed to grow for ≥1 week. After treatment, MTT (Sigma-Aldrich) was added (0.5 mg/mL) and cells were incubated for 30 minutes. The medium was aspirated and replaced with isopropanol to solubilize the formazan products followed by optical density readings at 570 nm. The cell viability as a percentage of the untreated cells was calculated from the absorbance values.

Statistics

All of the summarized data are reported as mean±SEM. Data are compared using either the Student (2-tailed) t test or a one-way ANOVA (post hoc comparison with Bonferroni multiple correction), and $P<0.05$ is considered significant.

Results

Rac1 Is Essential for ENaC Activity and ENaC-Mediated Sodium Reabsorption

We and others have shown previously that several small G proteins including Rac1 regulate ENaC activity through different mechanisms.[5,10,12,15,16,20–22] Rac1 markedly enhanced ENaC activity when coexpressed in CHO cells.[12,15] Surprisingly, coexpression of neither wild-type nor constitutively active (G12V) mutant Cdc42 had any effect on ENaC activity.[12] Herein, we tested the hypothesis that Rac1 is also essential for physiological regulation of the channel in native and cultured principal cells. As shown in Figure 1A, the addition of NSC23766 (100 μmol/L), a selective inhibitor of the Rac1-GEF interaction,[23] significantly decreased ENaC

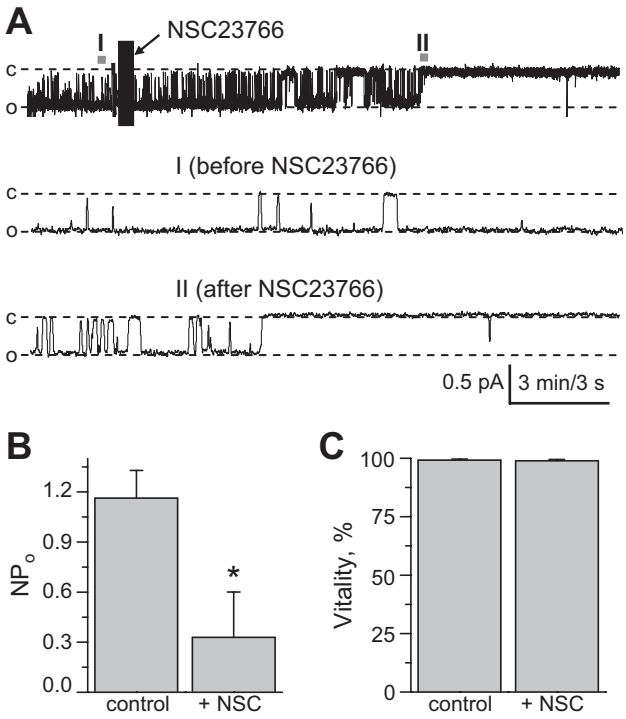

Figure 1. Inhibition of Rac1 results in a rapid and marked decrease of epithelial Na^+ channel (ENaC) activity in native principal cells. **A**, Continuous current trace from a representative cell-attached patch that contained ENaC and was made on the apical membrane of principal cells in isolated split-open rat cortical collecting duct (CCD) before and after treatment with NSC23766 (100 μmol/L). Areas before (I) and after (II) treatment are shown below with an expanded time scale. This patch was held at a −60 mV test potential during the course of the experiment. c and o denote closed and open current levels, respectively. **B**, Summary graphs of NP_o in cell-attached patches from freshly isolated CCDs before and after NSC23766 (NSC; N=7). Data are mean±SEM, *vs before application of NSC23766. **C**, Inhibitory effects of NSC23766 on viability of $mpkCCD_{c14}$ cells. Cells were treated with NSC23766 (100 μmol/L) for 30 minutes. Grow inhibition was monitored by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay. Absorbance at 495 nm was measured, and the percentage of growth against untreated cells was calculated. Data are represented as a viable fraction (percentage) of untreated cells from ≥3 experiments.

activity in freshly isolated rat CCD. ENaC activity was recorded for ≥3 minutes before application of NSC23766, and we used patches with stable ENaC open probability only. Application of vehicle had no effect on ENaC activity (data not shown). A continuous trace is shown at the top. Segments before and after NSC23766 are shown below at expanded time scales. As is apparent from this representative trace and summarized in Figure 1B, inhibition of Rac1-GEF interaction resulted in a rapid decrease of ENaC activity in this native preparation. To test for possible cytotoxic effect of NSC23766, we performed MTT cell viability assay. This assay measures the conversion of MTT of living cells, and a decrease in cellular MTT formazan could be the index of grow inhibition and cell toxicity. Data obtained revealed that 100 μmol/L of NSC23766 had no effect on cell vitality of the $mpkCCD_{c14}$ cells (Figure 1C).

To further establish the role of Rac1 in ENaC-mediated sodium reabsorption in principal cells, we used shRNA against Rac1. Antimouse Rac1 shRNA lentiviral particles were used to downregulate mRNA levels of Rac1 in the M-1 cells. These cells retain many characteristics of native CCD cells, including morphology and antigens, and are routinely used to study ENaC activity and sodium reabsorption.[24–26] Efficient downregulation of Rac1 expression in M-1 cells stably expressing anti-Rac1 shRNA lentivirus was verified by Western blot analysis (Figure 2A and 2B). Functional consequences of Rac1 silencing were examined with transepithelial electric measurements and patch clamp analysis. Rac1 knockdown significantly decreased basal transepithelial Na^+ flux in the M-1 cells (Figure 2C). Control (scrambled shRNA) and Rac1 knockdown M-1 cells were seeded simultaneously at the same concentration and grown in the same conditions. Amiloride (10 μmol/L) was added to the apical membrane at the end of experiments to confirm that short-circuit current was mediated by ENaC. Furthermore, as demonstrated by patch clamp analysis, Rac1 knockdown decreased ENaC activity in the M-1 cells (Figure 2D). For these experiments, cell-attached seals were formed on the apical membrane of control M-1 cells and cells with knockdown of Rac1. Single-channel analysis revealed that Rac1 knockdown results in a decrease of the number of active channels (*N*) and has no effect on the channel open probability (Figure 2E and 2F).

N-WASP and WAVE Proteins Increase ENaC Activity

To investigate the actions of the N-WASP and WAVE proteins on ENaC, we reconstituted mouse ENaC in CHO cells in the absence and presence of coexpressed N-WASP, WAVE1, WAVE2, and WAVE3. Figure 3A shows typical currents before (arrows) and after treatment with amiloride (10 μmol/L) from voltage clamp experiments performed on CHO cells containing ENaC alone or coexpressed with WAVE1. For these experiments, cells were held at a holding potential of 40 mV with voltage ramps (300 ms) from 60 mV down to −100 mV used to elicit current (Figure 3B). Figure 3C shows a summary graph of experiments testing the role of N-WASP and WAVE proteins in regulation of ENaC activity. These results demonstrate that coexpression of N-WASP and WAVEs with ENaC in CHO cells markedly increases channel activity.

WAVEs But Not N-WASP Are Expressed in Principal Cells and Localized in Rat CCD and Regulate ENaC-Mediated Sodium Transport

Using Western blot analysis, we examined the expression profile of N-WASP/WAVE proteins in the cultured principal cells. We found high expression levels of WAVE1 and WAVE2 in both mpkCCDc14 and M-1 cells. In contrast, neither WAVE3 nor N-WASP was detected in these cell lines (Figure 4A). Immunohistochemistry analysis also revealed localization of only WAVE1 and WAVE2 in the CCD of Sprague-Dawley rat kidneys. Shown in Figure 4B are images from kidney immunohistochemically stained for N-WASP, WAVE1, and WAVE2 at ×60 magnifications. Negative control (stained with secondary antibodies in the absence of primary antibodies) is also shown. Additional negative control experiments (stained without primary and secondary

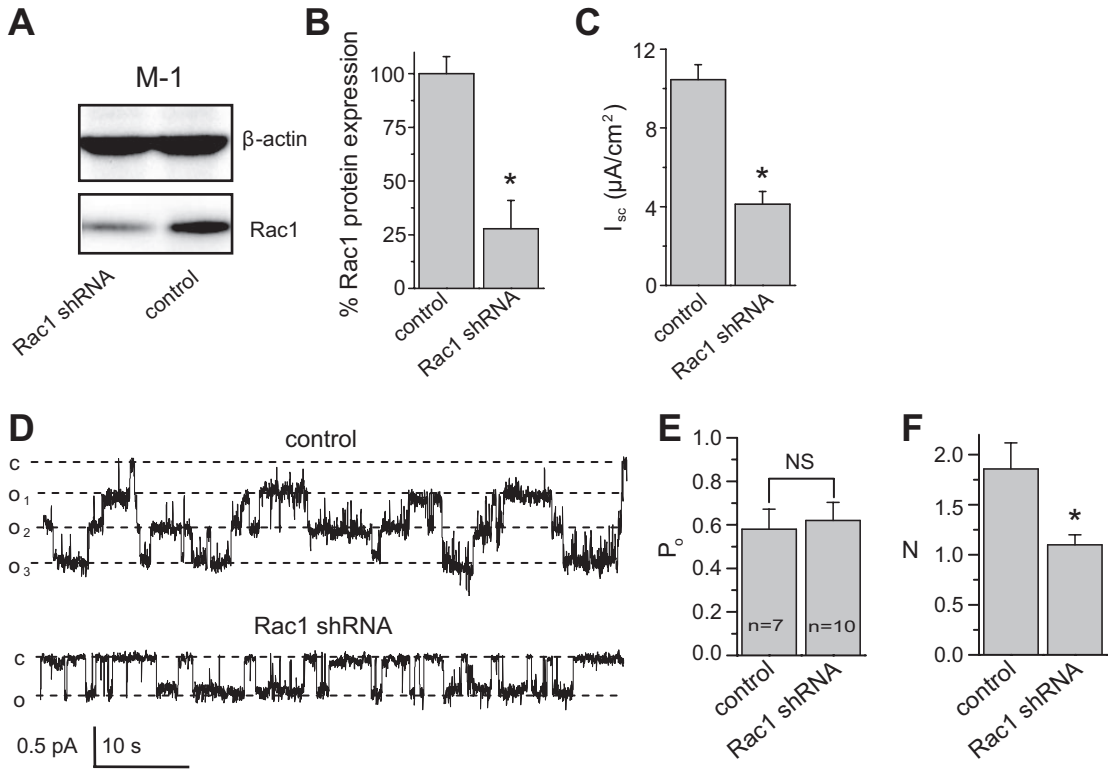

Figure 2. Short hairpin (sh)RNA–mediated silencing of Rac1 inhibits transepithelial current and the number of channels in M-1 cells. **A**, Western blot from control M-1 cells (scrambled shRNA) or a stable cell line expressing shRNA versus Rac1 (Rac1 shRNA). Cell lysates were analyzed using anti-Rac1 antibodies (bottom). Equal loading was verified by anti–β-actin antibodies (top). Shown are representative data from 5 experiments. **B**, Densitometric analysis of relative Rac1 protein expression in control and Rac1 knocked-down (KD) M-1 cells. **C**, Summary graph of amiloride-sensitive short-circuit current values in control and Rac1 KD M-1 cells. **D**, Representative current traces from cell-attached patches that were made on the apical membrane of control (upper trace) and Rac1 KD M-1 cells (lower trace). These patches were held at a −60 mV test potential during the course of the experiment. c and o denote closed and open current levels, respectively. **E** and **F**, Summary graphs showing the effect of Rac1 knockdown on ENaC P_o (**E**) and the mean number of ENaCs within patches (**F**) under each condition. *vs control shRNA.

antibodies) also did not show any staining (data not shown). As clear from the immunohistochemical staining, WAVE1 and WAVE2 are localized in the CCD (shown by arrows). Surprisingly, we did not observe any staining for N-WASP in the CCD (Figure 4B). Thus, these results demonstrate that WAVE but not N-WASP proteins are expressed in cultured principal cell lines and localized in CCD. To confirm this finding, we used wiskostatin, a selective inhibitor of N-WASP.[27] We tested an effect of wiskostatin on ENaC activity when the channel was overexpressed in CHO cells either alone or with N-WASP or WAVE1. However, wiskostatin had no effect on ENaC activity. Similarly, wiskostatin

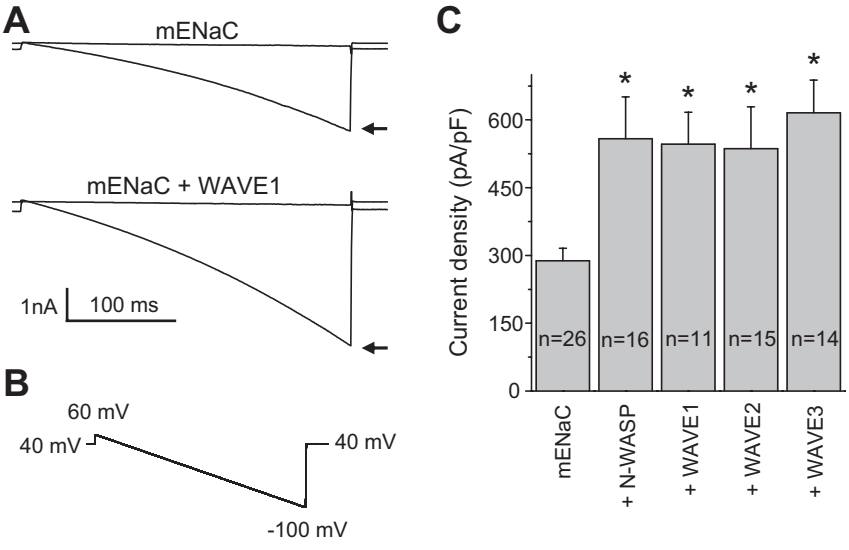

Figure 3. N-Wiskott-Aldrich syndrome protein (WASP) and WAVEs increase epithelial Na^+ channel (ENaC) activity. **A**, Overlays of typical macroscopic current traces before (arrow) and after 10 μmol/L of amiloride from voltage-clamped Chinese hamster ovary cells transfected with α, β, and γ subunits of mENaC alone (top) or coexpressed with WAVE1 (bottom). Currents evoked with a voltage ramp (60 to −100 mV from a holding potential of 40 mV). **B**, Diagram of experimental protocol for voltage steps used to measure amiloride-sensitive current in Chinese hamster ovary cells. **C**, Summary graph of amiloride-sensitive current density at −80 mV for Chinese hamster ovary cells expressing mENaC alone or coexpressed with N-WASP or WAVE1, 2, or 3, respectively. The number of observations for each group is shown. *vs mENaC alone.

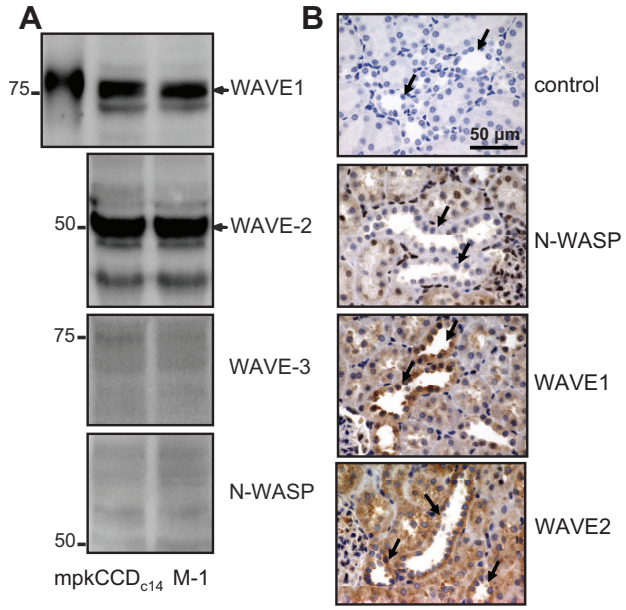

Figure 4. Expression profiles of N-Wiskott-Aldrich syndrome protein (WASP)/WAVEs in the rat kidney tissue and in epithelial cells. **A**, Expression profiles of N-WASP/WAVEs in principal cells. Figure shows Western blot analysis of endogenous N-WASP/WAVEs in mouse principal cells (mpkCCD$_{c14}$ and M-1). Western blot analysis was repeated 4 times with similar results. Left lane for WAVE1 represents a control for cell lysate derived from 3T3/A31 cell line. **B**, Representative immunohistochemical staining for N-WASP and WAVE1 detection in kidney cortical sections of Sprague-Dawley rats at ×60 magnification. Negative control tissue (control) stained with secondary antibodies in the absence of primary antibodies is also shown. Cortical collecting ducts (CCD) are shown by arrows.

had no effect on Na^+ reabsorption across mpkCCD$_{c14}$ principal cell monolayers (Figure S1, available in the online Data Supplement at http://hyper.ahajournals.org).

Rac1 Enhances ENaC Activity via WAVE1 or WAVE2

Results in Figure 3 demonstrate that WAVE proteins enhance ENaC activity when these proteins are coexpressed in CHO cells. Moreover, as we demonstrated previously, Rac1 also increases amiloride-sensitive current density.[12,15] To test whether Rac1 and WAVE signaling converge before activating ENaC, we probed the effects of coexpressing these proteins on ENaC activity. As summarized in Figure 5A, ENaC activity in the presence of constitutively active Rac1 (QL) and WAVE1 did not change compared with ENaC plus either Rac1 or WAVE1. Similarly, we did not observe any additive effect when ENaC was coexpressed with Rac1 and WAVE2 (Figure 5B). These results demonstrate that coexpression of downstream effectors of Rac1 recapitulates independent activation of ENaC by this small G protein. In addition we tested an effect of dominant-negative Rac1^{T17N} on the activity of ENaC coexpressed with WAVE1 or WAVE2 (Figure 5C). As seen from this Figure, dominant-negative Rac1 precludes WAVE1- or WAVE2-mediated enhancement of ENaC activity. Thus, the results in Figure 5 are consistent with the concept that Rac1 and WAVE1/2 are components of the same signaling mechanism with respect to activation of ENaC and that active Rac1 is required for WAVE proteins to increase ENaC activity.

Discussion

ENaC is regulated by small G proteins and the actin cytoskeleton.[5,12,15,16,20,28–30] Several other epithelial ion channels in the kidney are also regulated by small GTPase Rac1 through different mechanisms. For instance, Bezzerides et al[31] demonstrated that epidermal growth factor stimulation rapidly induces TRPC5 channel translocation to the plasma membrane, and this insertion is mediated via Rac1. Thebault et al[32] also reported that epidermal growth factor–mediated stimulation of TRPM6 occurs via signaling through Src kinases and Rac1, thereby redistributing endomembrane TRPM6 to the plasma membrane. The role of Rho family proteins and their regulators in the pathogenesis of high blood pressure was discussed recently.[33] WASP family proteins are emerging as central regulators of actin assembly via their interactions with small G proteins of the Rho family, particularly Rac1 and Cdc42.[11] WASP and N-WASP function downstream of Cdc42. WAVEs are essential for development

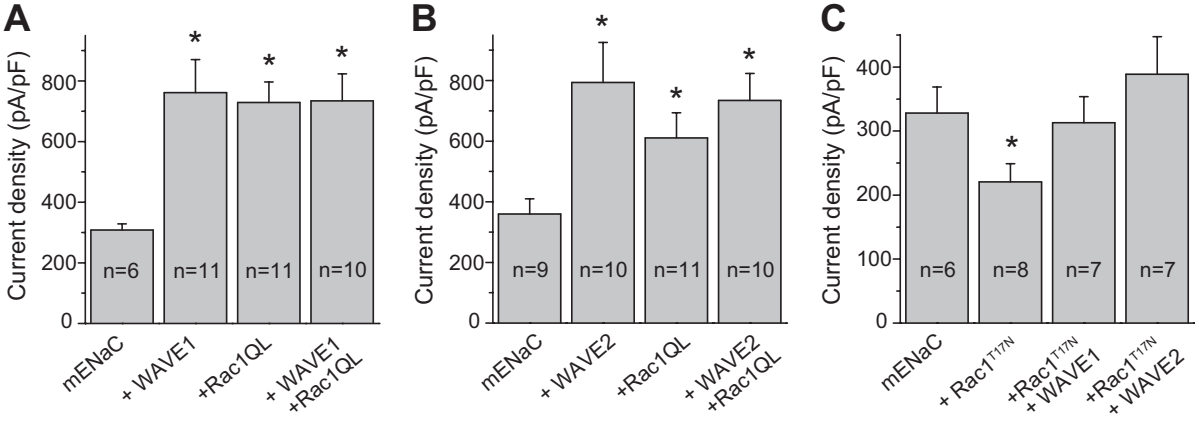

Figure 5. Rac1 increases epithelial Na^+ channel (ENaC) activity via WAVE. Summary graph of ENaC activity from Chinese hamster ovary cells overexpressed with mENaC subunits alone and channel plus constitutively active Rac1 QL and WAVE1 (**A**) or WAVE2 (**B**) separately or together. **C**, Summary graph of ENaC activity when channel is coexpressed with dominant-negative Rac1 T17N alone or in the presence of WAVE1 or WAVE2. The number of observations for each group is shown. *vs mENaC alone.

of Rac1-mediated signaling. Interestingly, our recent data revealed that ENaC is specifically regulated by Rac1 but not Cdc42.[12] Defining the role of WAVE proteins as specific regulators of ENaC greatly clarifies the regulation of ENaC by Rac1 over Cdc42.

We have demonstrated here that N-WASP, WAVE1, WAVE2, and WAVE3 upregulate ENaC activity when overexpressed in CHO cells. However, a combination of pharmacological approach with Western blotting and immunohistochemistry provides reliable evidence for the role of WAVE but not N-WASP proteins in regulation of ENaC activity. Western blot analysis revealed strong expression of WAVE1 and WAVE2 only in cultured principal cells. Immunohistochemical analysis also identified WAVE1 and WAVE2 but not N-WASP in the CCD of the kidneys of Sprague-Dawley rats. Similarly, Yamazaki et al[34] have shown that WAVE1 and WAVE2 were expressed in Madin-Darby canine kidney cells. The functions of WAVE1 and WAVE2 were redundant in this system, but WAVE2 appeared to play a more significant role. The authors have proposed that Rac-WAVE–mediated actin reorganization is required for organization and maintenance of cell-cell adhesion. Furthermore, we have shown that wiskostatin, a recently described chemical inhibitor that selectively inhibits N-WASP-mediated actin polymerization in vitro,[27,35] has no effect on ENaC either when expressed alone or when coexpressed with N-WASP or WAVE1 and that this inhibitor does not alter ENaC-mediated sodium reabsorption in mpkCCD$_{c14}$ cells. Despite a growing number of recent studies, which use wiskostatin to uncover novel cellular functions for N-WASP, the selectivity of this particular drug has not been completely determined yet. In fact, it was shown that wiskostatin treatment causes a rapid irreversible decrease in cellular ATP levels.[36]

Thus, Rac1-WAVE signaling complex might play a central role in blood pressure control in the kidney. The details of this mechanism and, in particular, the downstream signaling have not been fully delineated. For example, it was shown that the intermediary protein linking Rac1 and WAVE2 is IRSp53, the insulin receptor substrate.[37,38] Interestingly, insulin is known to increase ENaC activity. It is possible that it does so in part via IRSp53 impinging on Rac1/WAVE signaling. Moreover, mitogen-activated protein kinase and NADPH might also be involved in the effect of Rac1 on ENaC. Furthermore, WAVE proteins contain several important binding domains including a WAVE homology domain at the N-terminus, a basic region, a proline-rich region, and a C-terminal verprolin-cofilin-acidic region involved in binding actin and the Arp2/3 complex. WAVE2 exists in a multimolecular complex with several other proteins, including PIR121 or Sra-1, Nap1, Abi-1/2, and HSPC300.[39] However, exact mechanisms involved in regulation of this complex are not defined yet.

Perspectives

Long-term control of blood pressure involves Na^+ homeostasis through the precise regulation of ENaC. The role of Rac1 proteins in hypertension was also proposed. This study has identified that, in the kidney, ENaC is a regulation target for Rac1. The regulation of ENaC by Rac1 appears to be mediated via WAVE proteins. A role for Rac1 and WAVEs in the regulation of ENaC activity is a new concept. Therefore, it is important to understand this mechanism of renal blood pressure control and its role in the diseases associated with fluid imbalance and hypertension. Moreover, our data suggest that Rac1 and WAVE proteins are potential targets for the prevention of ENaC-mediated diseases. Future studies are needed to investigate the functional role, as well as the mechanisms, of Rac1/WAVE/ENaC signaling pathway in the development of such diseases in hypertensive and transgenic models. Furthermore, several important activators of Rac1 signaling, such as epidermal growth factor and angiotensin II, might be involved in this mechanism, and their role should be defined in future studies.

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Disclosures

None.

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Novel role of Rac1/WAVE signaling mechanism in regulation of the epithelial Na^+ channel (ENaC)

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Figure S1. ENaC activity and ENaC-mediated sodium reabsorption are unaffected by N-WASP inhibition with wiskostatin. A, Summary graph of amiloride-sensitive current density at -80 mV for CHO cells expressing mENaC alone or co-expressed with N-WASP or WAVE1 not treated or pretreated with wiskostatin (1-2 hrs; 25 μM). The number of observations for each group is shown. *, *versus* ENaC alone. B, Time course of relative Na^+ transport across monolayers of $mpkCCD_{c14}$ cells in the absence and presence of treatment with various concentrations of wiskostatin (10, 25 and 100 μM). Values are means ± S.E.M; n = between 5 and 9 for each concentration. $MpkCCD_{c14}$ cells were serum-starved overnight. Wiskostatin and vehicle (control) were added bilaterally at time 0 and current was normalized to the starting level. Amiloride (10 μM; arrow) was added to the apical membrane at the end of experiment.