Epithelial Na\(^+\) channel activation and processing in mice lacking SGK1

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Epithelial Na\(^+\) channel activation and processing in mice lacking SGK1. Am J Physiol Renal Physiol 294: F1298–F1305, 2008. First published April 2, 2008; doi:10.1152/ajprenal.00579.2007.—Amiloride-sensitive Na\(^+\) channel activity was examined in the cortical collecting ducts of a mouse line (SGK1\(^{-/-}\)) deficient in the serum- and glucocorticoid-dependent protein kinase SGK1. This activity was correlated with changes in renal Na handling and in the maturation of epithelial Na\(^+\) channel (ENaC) protein. Neither SGK1\(^{-/-}\) mice nor paired SGK1\(^{+/+}\) animals expressed detectable channel activity, measured as amiloride-sensitive whole-cell current (I\(_{Na}\)), under control conditions with standard chow. Administration of aldosterone (0.5 \(\mu\)g/h via osmotic minipump for 7 days) increased I\(_{Na}\) to a similar extent in SGK1\(^{-/-}\) (375 \(\pm\) 61 pA/cell at -100 mV) and in SGK1\(^{+/+}\) (350 \(\pm\) 57 pA/cell) animals. However, the maturation of ENaC, assessed as the ratio of cleaved to full-length forms of \(\gamma\)-ENaC, was more pronounced in SGK1\(^{+/+}\) mice. The SGK1\(^{-/-}\) animals exhibited a salt-wasting phenotype when kept on a low-Na diet for up to 2 days, losing significantly more Na in the urine than wild-type mice. Under these conditions, I\(_{Na}\) was enhanced more in SGK1\(^{-/-}\) (94 \(\pm\) 14 pA/cell) than in SGK1\(^{+/+}\) (23 \(\pm\) 5 pA/cell) genotypes. Despite the larger currents, the ratio of cleaved to full-length \(\gamma\)-ENaC was lower in the knockout animals. The mice also expressed a smaller amount of Na\(^+\)-Cl\(^-\) cotransporter protein under Na-depleted conditions. These results indicated that SGK1 is essential for optimal processing of ENaC but is not required for activation of the channel by aldosterone.

**METHODS**

Generation of targeting construct. An ~30-kb RamI fragment of a bacterial artificial chromosome (BAC) clone (obtained from Genome Systems, St. Louis, MO) that contains the entire SGK1 gene, as judged by Southern blotting using a 5' probe ~700 bp upstream from the transcription initiation site and a 3' probe located at the extreme 3' region of the SGK1 gene, was subcloned into pSLS301 vector. We introduced three loxp sites into the introns in the sgk1 locus (Supplemental Fig. S1). The first loxp site was introduced into intron 1, using a BsaHI restriction site, while the second and third loxp sites were inserted into intron 6 at the XcmI site bracketing a neo expression cassette. We also introduced two FRT sites [recognition sites for the FLP recombinase (25)] flanking the neo cassette close to the second and third loxp sites (Supplemental Fig. S1). These sites allow removal of the neo cassette in vivo by crossing mice with the floxed SGK1 and neo cassette with FLPf deleter mice (31). After linearization, the targeting construct was electroporated into R1129/Sv \(\times\) 129/Sv-C57BL6-derived embryonic stem (ES) cells. Recombinant clones were enriched by selection with G418, and clones with the desired recombination were identified by Southern blotting.

Generation of SGK1 knockout mice. All experimental protocols were approved by the Institutional Animal Use and Care Committees of Dartmouth Medical School and the Weill Medical College of Cornell University, and all procedures using experimental animals adhered to the American Physiological Society’s “Guiding Principles in the Care and Use of Animals.” Floxed SGK1 mice were generated by the Transgenic and Knock-out Mouse Core Facility at Dartmouth. Positive ES clones were microinjected into C57BL6-derived blastocysts and implanted into pseudopregnant foster females. Progeny were identified by PCR analysis of tail DNA (PCR conditions are available upon request), and Southern blot analysis was used to verify integration of the transgene. Figure 1 shows a Southern blot of DNA from wild-type mice (+/+) and from mice heterozygous for the floxed SGK1 allele (+/−). After germ line transmission of the

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A mouse line lacking SGK1 exhibited transient urinary Na wasting on a low-Na diet, although the phenotype was rather mild (42). In particular, it was considerably weaker than that of global deletion of the mineralocorticoid receptor (4) or of \(\alpha\)-ENaC (21). This suggests that in addition to stimulating Na\(^+\) channels through SGK1 induction, the hormone acts through other mechanisms and/or on other transport systems. We generated an independent but similar SGK1 knockout mouse line to further investigate the role of SGK1 in regulation of Na\(^+\) channels. We found that channels can be activated in the absence of this protein. However, SGK1 seems to be required for full processing of ENaC and by inference for optimal trafficking of the channels to the apical membrane.

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modified allele, heterozygous floxed SGK1 mice were inbred to generate mice homozygous for the floxed SGK1 allele.

To generate SGK1 knockout mice, we bred homozygous floxed SGK1 mice with global Cre-deleter mice [BALB/c-Tg(CMV-cre)1Cgn/J, obtained from Jackson Laboratory, Bar Harbor, ME]. In the offspring, nucleotides 121–1615 (encompassing exons 2–6) of SGK1 are eliminated. Mice were screened by PCR using the following primers. For the wild-type allele the forward primer (5'-CTCAGTCTTTTGGGCTCTTT-3') and reverse primer (3'-CCATCTCCCCTTTTCCCTCTT-5') generate a 243-bp amplicon, while for the SGK1 knockout allele the same forward primer and the reverse primer (3'-TIGGTITGGGTITGAAGCTTTT-5') generate a 411-bp amplicon.

SGK1 mRNA levels were determined by quantitative PCR using the forward primer 5'-CTCAGTCTTTTGGGCTCTTT-3' and the reverse primer 5'-TTCCTCTTTGGGTGTGCCTTC-3'. These primers span nucleotides 32–482 in the mouse sgk1 gene and amplify a 450-bp product only from the wild-type gene. SGK1 knockout mice were then backcrossed to 129/SvJ wild-type mice for five generations. Male SGK1 knockout mice were transferred to Weill-Cornell and mated with wild-type 129/SvJ wild-type mice for five generations. The heterozygote offspring were mated to produce matched SGK1 heterozygous and SGK1 homozygous animals used for the experiments.

**Experimental procedures.** Animals were fed either a sodium-deficient rodent diet or a matched control diet that contained 1% NaCl (MP Biomedicals, Solon, OH). Some animals fed the 1% NaCl diet were implanted subcutaneously with osmotic minipumps (model 2001, Alza, Palo Alto, CA) for 6–7 days to increase levels of circulating aldosterone. Aldosterone was dissolved in polyethylene glycol 300 at 2 mg/ml to give a calculated infusion rate of 0.5 μg/h.

For measurements of urinary Na⁺ and K⁺ excretion, mice were placed individually in metabolic cages for timed urine collections. They had free access to food and to water sweetened with 3% sucrose to increase water consumption and urine flow. Urine was collected twice per day, in the early morning and in the early evening. Urine Na and K concentrations were measured with a flame photometer (Instrumentation Lab model 943).

**Electrophysiology.** Mice were killed, kidneys were excised, and cortical collecting ducts (CCDs) were isolated, split open, and prepared for patch-clamp measurements as described previously (9). Whole cell currents were measured in principal cells of the split tubules, identified visually. The tubules were superfused with solution prewarmed to 37°C and containing (in mM) 135 Na methanesulfonate, 5 KCl, 2 Ca methanesulfonate, 1 MgCl₂, 2 glucose, 5 mM Ba methanesulfonate, and 10 N-2-hydroxyethylpiperazine-N'-2-ethane-sulfonic acid (HEPES) adjusted to pH 7.4 with NaOH. Amiloride was added to the bath solution to a final concentration of 10 μM. Patch-clamp pipettes were pulled to a resistance of 2–6 MΩ and were filled with a solution containing (in mM) 7 KCl, 123 aspartic acid, 20 CsOH, 20 TEAOH, 5 EGTA, 10 HEPES, 3 MgATP, and 0.3 NaGDPβS, with pH adjusted to 7.4 with KOH.

**Immunoblots.** Polyclonal antibodies against the γ-subunit of the rat ENaC were described previously (12, 13). Whole kidneys were homogenized with a glass homogenizer (Wheaton, Millville, NJ) in ice-cold lysis buffer containing 250 mM sucrose, 10 mM triethanolamine adjusted to pH 7.4 with NaOH, 1 μg/ml leupeptin, and 0.1 mg/ml phenylmethylsulfonyl fluoride (Sigma-Aldrich, St. Louis, MO). In some cases the kidney cortex and medulla were homogenized and analyzed separately. For this, each kidney was cut into five or six coronal slices, which were divided into cortical and medullary components with a razor blade. The homogenate was centrifuged at 1,000 g for 10 min to sediment unbroken cells and nuclei. The supernatant was processed for immunodetection. Total protein was measured by the Bio-Rad Bradford assay (Bio-Rad, Hercules, CA). Urinary aldosterone was obtained from Chemicon (Millipore, Bedford, MA) and used at a dilution of 1:1,000. A polyclonal antibody against the Na⁺–Cl⁻ cotransporter (NCC) was obtained from Chemicon (Millipore, Bedford, MA) and used at a dilution of 1:2,000. Anti-rabbit IgG conjugated with alkaline phosphatase was used as a secondary antibody. The sites of antibody-antigen reaction were visualized with a chemiluminescence substrate (Western Breeze, Invitrogen) before exposure to X-ray film (Biomax ML, Kodak, Rochester, NY). Band densities were quantitated with a Quantity One densitometer and acquisition system (Bio-Rad Laboratories, Hercules, CA).

**Aldosterone excretion.** Urinary aldosterone concentrations were determined by ELISA using a monoclonal antibody (generous gift of Dr. Celso Gomez-Sanchez, University of Mississippi Medical Center, Jackson, MS) as previously described (15). Urinary aldosterone was normalized to creatinine, determined with the Jaffe reaction.

**RESULTS**

Cre-mediated excision eliminates nucleotides 121–1615 encompassing exons 2–6 from the mouse SGK1 gene. This region includes codon 127, whose presence is essential for the kinase activity of SGK1 (17). Elimination of exons 2–6 also results in a frameshift for the downstream coding region, resulting in missense mutations starting at codon 139, and a premature stop codon at 159. Thus, after Cre-mediated excision, the mutant SGK1 gene encodes a 46-amino acid peptide, from which only amino acids 1–25 correspond to wild-type SGK1.

Figure 2 illustrates the PCR products obtained with tail DNA from homozygous (SGK1+/−) and heterozygous (SGK1+/+) SGK1 knockout and wild-type (SGK1+/+) mice. The expected size of the SGK1+/− amplicon is 243 bp, while that of the amplicon from SGK1/−/− mice is 411 bp. We also tested the expression of SGK1 mRNA from the kidneys of SGK1+/− and SGK1/−/− mice, using a primer pair that spans nucleotides 32–486 in the mouse SGK1 mRNA, a region that is missing after Cre-mediated excision of the floxed sgk1 gene. As shown in Fig. 3, no product was amplified from two SGK1/−/− mouse kidneys with these primers, while an amplicon with the expected size was amplified from RNA obtained from a
SGK1+/+ mouse kidney. Homozygous SGK1 knockout mice are viable and fertile (albeit the litter sizes were small) and do not exhibit any gross phenotype.

Representative whole cell currents in the presence and absence of amiloride for principal cells in the CCD of SGK−/− mice are shown in Fig. 4. Under control conditions, with animals fed normal (1% NaCl) rodent chow and untreated with hormone, whole-cell conductances were small and amiloride had no detectable effect (Fig. 4A). This is consistent with previous findings in wild-type mice under similar conditions (9), and the results were indistinguishable from those obtained with paired SGK1+/+ mice (Fig. 5). In most cases an amiloride-sensitive current of 10 nA/cell, measured at −100 mV, could have been detected.

To see whether increasing levels of mineralocorticoids could upregulate Na+ channels in SGK1−/− mice, we implanted animals with osmotic minipumps to deliver aldosterone at a constant rate for 1 wk. In this case all cells studied had significant amiloride-sensitive currents (INa), as illustrated in Fig. 4B. Although the magnitude of INa was variable, the mean values were not different in SGK1+/+ and SGK−/− animals (Fig. 5). Thus SGK1 is not essential for upregulation of Na+ channels by chronic mineralocorticoid treatment.

We also examined the abundance of γ-ENaC in the kidneys of the mice. Previous studies showed that mineralocorticoid treatment, as well as other maneuvers that increased Na+ channel activity, shifted γ-ENaC protein in rat kidney from the full-length form of ~80 kDa to a smaller molecular mass of ~65 kDa (12, 13, 23). This smaller form presumably represents proteolytic cleavage of the subunit (20). Similar to results in rats, we found that chronic aldosterone administration increased the total amount of cleaved γ-ENaC as well as the ratio of cleaved to full-length protein in both SGK1+/+ and SGK1−/− mice (Fig. 6). However, in contrast to the effects on overall channel activity, the mice lacking SGK1 had significantly less cleaved γ-ENaC (Fig. 7). Similar blots made with anti-β-ENaC antibody revealed no detectable difference in staining between wild-type and knockout animals (data not shown). An anti-α-ENaC antibody raised against the NH2-terminal sequence of the rat did not give satisfactory staining of specific bands in mouse kidney extracts.

In a previous study using a similar strain of mice, knockout of SGK1 resulted in a significant natriuresis compared with wild type when animals were salt restricted for 2–3 days (42). This salt wasting eventually disappeared after 7 days on the low-Na diet, at which time both knockout and wild-type animals reduced urinary Na+ nearly to zero, suggesting that the kinase might be more important in the early response to salt depletion. We confirmed this phenotype in our mice as shown in Fig. 8A. We measured Na+ excretion during the day and during the night. As expected (40), there was a clear diurnal pattern of excretion, with much higher levels achieved during the night, when most of the daily food consumption occurred.
When the food was switched from high to low Na in the early evening, Na+ excretion decreased overnight, instead of the normal increase, and continued to fall over the next 2 days in both sets of mice. However, excretion was higher in SGK1+/− compared with wild-type animals between 12 and 45 h after the beginning of salt restriction. The excess Na+ excretion was 20–25 nmol Na/min, or ~0.1% of the estimated filtered Na load. During this early phase of Na restriction K+ excretion tended to be higher in the SGK1+/− mice compared with wild-type mice (Fig. 8B). The difference reached statistical significance only for the daytime period (9:00 AM to 6:00 PM) after the first night of the low-Na diet. The greater Na loss in the SGK1+/− mice during salt restriction was accompanied by a larger increase in aldosterone excretion rates (Fig. 8C). The basal excretion rate was about threefold higher in the knockout animals, although the differences were not statistically significant. During Na depletion, aldosterone excretion increased in both groups of mice and was significantly higher in the knockout animals (Fig. 5). This suggests that transporters other than the Na+ channels, or Na+ channels in segments other than the CCD, are defective in the knockout animals under these conditions.

Immunoblots for γ-ENaC after 2 days of Na depletion are shown in Fig. 10. The ratio of cleaved to full-length forms is statistically larger in the SGK1+/− mice. Again, the appearance of cleaved γ-ENaC did not correlate with channel activity as assessed with electrophysiological measurements. To assess whether the regulation of ENaC might be affected differently in different parts of the nephron, we examined γ-ENaC expression in both cortex and medulla of salt-depleted mice. The cortex samples should represent mostly connecting tubules (CNTs) and late distal convoluted tubules (DCTs), while ENaC in the medulla should arise from collecting ducts (13). As shown in Fig. 10B, the ratio of cleaved to full-length γ-ENaC was similar in cortex and medulla. While again there was a tendency for larger ratios in the SGK1+/− mice, this effect was not statistically significant in this group of animals.

Expression of the NCC in the mice is shown in Fig. 11. Two major bands were stained in the immunoblots, presumably corresponding to the monomeric (140 kDa) and dimeric (280 kDa) forms of the protein (10). Under control conditions there was no significant difference in expression (Fig. 11A). After 2 days of low-Na diet, however, both forms of the protein were significantly less abundant in the SGK1−/− mice compared with the SGK+/+ mice (Fig. 11B). The differences were larger for the putative dimeric form (Fig. 11C). Similar blots assaying for the Na+/K+2Cl− cotransporter NKCC2 and the Na+/H+ exchanger NHE3 showed no obvious differences between wild-type and knockout animals (Fig. 12).

To assess the role of misregulation of Na+ channels during this time period, we measured INa in CCDs isolated from animals during the second day of Na depletion. A typical result for an SGK−/− mouse is shown in Fig. 9. INa was easily detectable under these conditions, although much lower than for the animals chronically treated with aldosterone. The mean currents were actually larger for SGK−/− animals than for SGK1+/+ animals (Fig. 5). This suggests that transporters other than the Na+ channels, or Na+ channels in segments other than the CCD, are defective in the knockout animals under these conditions.

Fig. 5. Mean INa in principal cells of SGK1+/+ and SGK1−/− mice under different conditions: control, aldosterone (aldo) administration for 7 days (d), and low-Na diet for 2 days. Currents were measured with a cell potential of −100 mV. Data are plotted as means ± SE for ≥10 cells from ≥3 animals. *P < 0.05 for SGK1+/+ vs. SGK1−/−.

Fig. 6. Expression of epithelial Na+ channel (ENaC) γ-subunit in SGK−/− and SGK1+/− mouse kidney. Proteins from whole kidney homogenates were separated by SDS-PAGE and blotted with anti-γ-ENaC antibody. Each lane was loaded with 100 μg of protein from a different animal.

Fig. 7. Quantitation of the relative densities of cleaved vs. full-length γ-ENaC. The ratios of densities of the 65-kDa to 80-kDa forms of the subunit are plotted as means ± SE for ≥4 animals. *P < 0.05 for SGK1+/+ vs. SGK1−/−.
DISCUSSION

SGK1 was first identified as a serum- and glucocorticoid-regulated serine/threonine kinase in mammary epithelial cells by Firestone and colleagues (41). Subsequently, two groups independently found that the sgk1 gene is regulated by corticosteroids in renal cells. SGK1 mRNA was increased by dexamethasone in the amphibian kidney cell line A6 (8) and by aldosterone in primary cultures of mammalian collecting duct cells (27). Furthermore, coexpression of SGK1 together with ENaC subunits in Xenopus oocytes strongly enhanced amiloride-sensitive currents through the Na+/H+ channels. Subsequently, the finding of transcriptional regulation of the SGK1 gene by mineralocorticoids was confirmed in vivo. Rapid increases in both SGK1 mRNA and protein were demonstrated in the mammalian kidney itself, particularly the distal aldosterone-sensitive nephron segments, and in the colon (22, 26, 32). Other isoforms of SGK, termed SGK2 and SGK3, have been identified, but these are not induced by aldosterone (29).

The mechanism of action of SGK1 in regulating the channels is thought to involve, at least in part, effects on ENaC trafficking. One such pathway is based on the ability of SGK1 to phosphorylate the ubiquitin ligase Nedd4-2, a protein known to interact with and downregulate ENaC (11, 35). According to this scheme, phosphorylation decreases Nedd4-2 binding to the COOH-terminal PY motifs of α- and γ-ENaC, diminishing ubiquitination of the channel protein and increasing its residence time at the membrane. Consistent with this idea, Alvarez de la Rosa et al. (3) found that SGK coexpression increased the surface expression of ENaC in Xenopus oocytes. In addition, recent reports indicate that SGK1 is involved in the mineralocorticoid receptor-dependent activation (5) or de-repression (44) of α-ENaC transcription.

Fig. 8. Urinary Na+/H+ (UNaV; A), K+/H+ (UKV; B), and aldosterone (C) excretion in SGK1−/− and SGK1+/+ mice in response to dietary Na restriction for 44 h. Na+ excretion was measured in urine collected the day before and twice per day after switching from control to low-Na diet. D, daytime collection; N, nighttime collection. Data represent means ± SE for 9 animals of each genotype for Na+ and K+ excretion and for 4 animals of each genotype for aldosterone excretion. *P < 0.05 for SGK1−/− vs. SGK1+/+.
The overall importance of the induction of SGK1 in the regulation of ENaC activity by aldosterone, however, is unclear. Helms et al. (17) used genetic techniques to alter the expression of SGK1 in the M1 cell line from mouse kidney. They found that lack of the kinase blunted the hormone-dependent increase in transepithelial Na+/H+ transport as well as the basal transport rate. Alvarez de la Rosa and Canessa (2) used a similar approach in amphibian A6 cells, and they found that the effects of increasing SGK1 expression were additive to those of aldosterone. This would not be expected if SGK1 were the predominant regulator of the channels, and it indicates that other pathways are involved.

We found that SGK1 is not essential for increased Na+/H+ channel activity in the CCD of the mouse in response to chronic aldosterone administration or short-term Na depletion through dietary salt restriction. The latter observation is at odds with a previous report using another SGK1 knockout mouse line (42). In that study, transepithelial potential differences, which generally reflect apical Na+/H+ channel activity, were diminished in CCDs from Na-restricted SGK1−/− mice compared with wild type. We found that, if anything, INa were larger in the knockout animals, even though they showed a salt-wasting phenotype very similar to that of the previous study. We do not know the cause of this discrepancy, but we feel that whole-cell INa affords a more direct assay for channel activity in these cells than the transepithelial voltage. It is also worth noting that in the previously described SGK1 knockout there was no effect on Na+ channel activity or its stimulation by aldosterone in the colon (30).

Deletion of the kinase may affect Na+ transport systems in nephron segments other than the CCD. For example, we see that the thiazide-sensitive Na++Cl− cotransporter is decreased in abundance under low-Na conditions. This is most marked in the putative dimeric form of the transporter, which is the major form in the plasma membrane as judged by biotinylation assays (G. Frindt and L. G. Palmer, unpublished observations). We do not know to what extent this difference might account for the excess Na+ excretion in the SGK−/− animals. In addition, the activity of the Na+/H+ exchanger NHE3, which is responsible for a large fraction of proximal tubule Na+ reabsorption, can also be regulated by SGK1 in fibroblasts (43). A modest decline in the activity of either of

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**Fig. 10.** Expression of γ-ENaC in SGK−/− and SGK1+/+ mouse kidney after 2 days on a low-Na diet. A: proteins from whole kidney homogenates were separated by SDS-PAGE and blotted with anti-γ-ENaC antibody. B: in a different set of animals, samples from cortex and medulla were compared. Each lane was loaded with 100 μg of protein from a different animal.

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**Fig. 11.** Expression of Na+/Cl− cotransporter (NCC) in SGK−/− and SGK1+/+ mouse kidney. Whole kidney homogenates were separated by SDS-PAGE and blotted with anti-NCC antibody. Each lane represents 80 μg of protein from a different animal. A: control conditions. B: after 2 days on a low-Na diet. C: ratios of densities for bands at 140 and 280 kDa in SGK−/− to SGK1+/+ mice after 2 days on a low-Na diet. *Statistically different from 1 (P < 0.05).

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**Fig. 12.** Expression of Na+−K++2Cl− cotransporter NKCC2 and Na+/H+ exchanger NHE3 in SGK−/− and SGK1+/+ mouse kidney. Whole kidney homogenates were separated by SDS-PAGE and blotted with anti-NKCC2 or anti-NHE3 antibody. Each lane represents 80 μg of protein from a different animal.
these transporters might also contribute to the natriuresis that we observed. The resulting reduction in plasma volume would produce the observed increase in circulating aldosterone levels (Fig. 8C), also shown previously (42), and would account for the increased channel activity. It is also possible that the lack of SGK1 reduces aldosterone-dependent ENaC activity in upstream nephron segments, such as the CNT, which probably contributes more to overall Na+ reabsorption than the CCD (14). However, the finding that K+ excretion increased, if anything, during the early phase of Na restriction is consistent with the idea that Na+ channel activity is enhanced rather than diminished, and that more proximal transporters are downregulated. Furthermore, the comparison of immunoblots from cortex (containing ENaC mainly from CNTs and late DCTs) and medulla (containing ENaC mainly from collecting ducts) does not indicate a greater effect of the SGK1 deletion in the former region (Fig. 10B). This possibility, however, cannot be completely ruled out.

In contrast to the lack of effect on overall channel activity, the absence of the kinase did diminish the degree of processing of the channel protein, as shown by a reduced amount of the small-molecular-mass form of γ-ENaC. This form presumably represents subunits that have undergone a physiological cleavage, either by membrane-associated enzymes such as prostasin or channel-activating proteases (CAPs) (37, 39) or by intracellular proteases such as furin (20). Cleavage of γ-ENaC often correlates well with channel activity. Prevention of cleavage by protease inhibitors (1, 16, 37), by genetic deletion of proteases (19), or by mutation of consensus target sequences (6, 19) all reduce channel activity, at least in part through decreases in open probability, while addition of exogenous proteases such as trypsin can open inactive channels (7).

In rats, several manipulations of the animal that resulted in the induction of channel activity in the CCD were also associated with increases in the abundance of cleaved γ-ENaC (12). Since the proteases that have been implicated are expressed mainly in the Golgi apparatus or at the cell surface, an increase in the cleaved form could, at least in part, reflect trafficking of the channels to the apical membrane, rather than enhanced enzymatic activity.

Our results are consistent with an important role of SGK1 in ENaC processing and trafficking, as suggested previously (11, 34). However, they also imply another level of regulation of channel activity that can under some conditions be dissociated from trafficking events. Thus aldosterone could have multiple effects on the channels, including induction of the subunits themselves, translocation to the apical membrane, and finally activation of channels in the membrane. The last mechanism may be of less importance in oocytes and other systems in which the kinase is well correlated with channel function. However, it may account in part for the aldosterone-dependent but SGK1-independent increases in channel activity elicited in vivo in this study.

GRANTS

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