Aldosterone induces rapid apical translocation of ENaC in early portion of renal collecting system: possible role of SGK

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Aldosterone stimulates Na⁺ reabsorption across its target epithelia, which are located in the distal part of excretory organs, such as kidney, colon, and salivary and sweat glands. The aldosterone target cells of the kidney are the segment-specific cells, which in the case of the collecting duct are also called principal cells, that form the epithelium of the aldosterone-sensitive distal nephron (ASDN) together with intercalated cells (which have no segment-specific characteristics). The ASDN includes the second half of the distal convoluted tubule (DCT2), the connecting tubule (CNT), the cortical collecting duct (CCD), and the medullary collecting duct (MCD). Na⁺ is reabsorbed across segment-specific cells of the ASDN by a two-step process consisting of apical influx via the epithelial Na⁺ channel (ENaC) and basolateral extrusion by the Na⁺ pump (Na-K-ATPase) (8, 17, 25, 40–42). These cells are also characterized by a high abundance of mineralocorticoid receptor and 11β-hydroxysteroid dehydrogenase type 2 (3, 11).

The action of aldosterone has been shown to depend on transcription and translation. On the basis of studies made in model epithelia, its effect has been operationally divided into three major phases: a lag period of 20–60 min, followed by an early phase of Na⁺ transport increase mediated by aldosterone-induced regulatory proteins acting on the preexisting transport machinery (39), and a late/very late phase starting ~3 h after hormone addition, during which a further increase in transport activity correlates with an increase in channels, pumps, and other elements of the transport machinery (34, 42). Early effects of aldosterone, as early as in in vitro models, have been observed in vivo on renal Na⁺ and K⁺ excretion in adrenalectomized (Adx) animals and on the activity/number of Na-K-ATPase in isolated CDs (4, 9, 16, 19).

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No early aldosterone effects have been documented in vivo at the level of ENaC as yet, although Na\(^+\) influx through this channel represents the major limiting step in Na\(^+\) reabsorption (17). Functionally, the number of active ENaCs was shown to be extremely low in the apical plasma membrane of isolated CCDs from animals on a standard laboratory diet. This number was drastically increased by a long-term, low-salt diet, which also induced an increase in circulating aldosterone (31). A similar treatment was recently shown to induce a shift of the three ENaC subunits from an intracellular localization toward the apical surface of segment-specific cells of the distal nephron (25, 27).

As yet, two gene products, K-Ras2 and serum and glucocorticoid-regulated kinase (SGK), which are potential mediators of the early stimulatory action of aldosterone on ENaC function in vivo, have been isolated (6, 17, 28, 30, 36). Both are rapidly regulated by aldosterone and have been shown to activate coexpressed ENaC in the Xenopus laevis oocyte expression system and, in the case of K-Ras2, in A6 cell epithelia (6, 28, 30, 38). The effect of SGK on ENaC-mediated Na\(^+\) transport in X. laevis oocytes was recently shown to depend on an intact SGK kinase site (21) and to be caused by an increase in channel surface expression (1).

SGK is a serine/threonine kinase known to be expressed in several tissues (43, 45). Its physiological target(s) likely includes ENaC; other targets are not yet known. The activity of SGK has been shown to depend on its own phosphorylation, for instance, by PDK1, a phosphatidylinositol-3-phosphate-dependent kinase (20, 32).

SGK was shown to be induced very rapidly and strongly at the mRNA and protein levels by dexamethasone or aldosterone in A6 epithelia (6). In the kidney of Adx rats, in situ hybridizations indicated that SGK mRNA is induced within 4 h by aldosterone in structures that probably correspond to the collecting system (6). This in vivo induction has been corroborated by in vitro studies on rabbit CCD primary cell cultures showing a rapid SGK mRNA induction by both mineralocorticoid receptor- and glucocorticoid receptor-specific agonists (30).

In the present study we performed in vivo (rat kidney) and in vitro (X. laevis oocytes) studies to localize α-ENaC and SGK induction by aldosterone in kidney tubules and address the question of how their induction might relate to ENaC surface expression.

METHODS

**Animals for tubule dissection and kidney morphology.** Adult male Adx Wistar rats, with a body weight of 180–200 g, were purchased from Iffa Credo (l’Arbresle, France) and kept on a normal diet and water supplemented with 9 g/l NaCl. Aldosterone was injected subcutaneously (50 μg/100 g) 1 wk after adrenalectomy. The animals were anesthetized with thiopental (10 mg/100 g body wt) or pentobarbital sodium (5 mg/100 g body wt ip).

**Preparation of rat tubule RNA.** Single proximal convoluted tubules (PT) and CCDs (1–2 mm each) were isolated by microdissection as described previously (12) and frozen in groups of two or three at −80°C. Yeast tRNA (10 μg; Fluka, Buchs, Switzerland) was added to the tubules with 500 μl TRIzol Reagent (GIBCO-BRL), and RNA was extracted according to the manufacturer’s protocol. RNA was resuspended in H\(_2\)O at a concentration of 0.1 μg/μl and 1/100 of the input RNA (corresponding to 9–24 cells) was reverse transcribed for each real-time PCR reaction.

**Real-time RT-PCR.** RNA was first reverse transcribed with MultiScribe reverse transcriptase (PE Applied Biosystems, Foster City, CA) according to the manufacturer’s instructions by using 2.5 μM random hexamers (with tissue RNA) or oligo(dT)\(_{16}\) (with tubule RNA) for priming and a total RNA concentration of 10 ng/μl. Fluorescence changes during real-time quantitative PCR were measured with an ABI Prism 7700 sequence detector (PE Biosystems). All reactions were performed by using the TaqMan PCR kit according to the manufacturer’s recommendation (PE Biosystems). The PCR primers used were the following: 5′-GCCAGGATAGACGCCCTATC (actin); 3′-ACTGCGGCTGCTCACTAGCA (actin); 5′-GAGGAGCCTCTCTCCTT (SGK); 3′-ACCCATGGCTCTGTGGCA (SGK); 5′-CTGCATGTGCATGTGTG (a-ENaC); 3′-CCACGCGACCCTCAAGG (a-ENaC); 5′-GATGCATGTGCTTGTGGAG (b-ENaC); 3′-GAGTTGCTCCCACTGGTAGATAAG (α-ENaC); and 3′-GGACCTCGAGCCGCAAT (γ-ENaC). The fluorescent oligonucleotide probes that hybridize to the template between the PCR primers were covalently labeled at their 5′ ends with the reporter dye 6-carboxyfluorescein (FAM) and at their 3′ ends via a linker arm with the quencher 6-carboxytetramethylrhodamine (TAMRA; PE Biosystems). These probes were for actin (CCATGAAGATCAAGATCATTGCCTCCTTCTCCTT); SGK (CCCCGTGCTCTGGCTCTAAGCA); α-ENaC (TGAAGCCACCACCATCCTAAAGGCCAG); β-ENaC (CGGAGGGCAGAGTCCACGTAC); and γ-ENaC (AAATGGACACTGACGACCGTGGC). Standard curves were generated by using rat colon total RNA as template. The detection limit for actin, SGK, α-ENaC, and γ-ENaC was <80 pg of the RNA. The ratio of the different signals to that of actin was calculated for every sample. The results obtained for the different test conditions are given normalized to the control values (fractional change in mRNA × expression, relative to actin). The validity of the method was verified by comparing the results obtained by real-time RT-PCR with those obtained by Northern blotting on the same tissue RNA samples (r = 0.904, data not shown).

**Immunohistochemistry on kidney sections.** Two and four hours after injection of vehicle (2 Adx rats/time point) or aldosterone (3 Adx rats/time point), kidneys were fixed by vascular perfusion and processed for immunohistochemistry as previously described (23). Serial cryosections (4–8 μm) were incubated overnight at 4°C with either a polyclonal, affinity-purified anti-SGK antiserum (dilution 1:500) or polyclonal rabbit antisera against α-ENaC (dilution 1:1,000), β-ENaC (dilution 1:1,000), or γ-ENaC (dilution 1:20,000). In some experiments, sections were coincubated with a mouse monoclonal antibody (Ab) against the vascular H\(^+\) ATPase (dilution 1:4). All antibodies have been characterized previously (5, 8, 18). The binding sites of the primary antibodies were revealed with a Cy3-conjugated donkey anti-rabbit IgG (Jackson Immuno Research Labs, West Grove, PA) diluted 1:1,000 and a FITC-conjugated goat anti-mouse IgG (Jackson Immuno Research Laboratories) diluted 1:40, respectively. Subsequently, the sections were processed according to routine procedures. Digitized images were acquired with a Visi- cam charge-coupled device camera (Visitron, Puchheim, Germany) and processed by Image-Pro Plus v3.0 software (Media Cybernetics, Silver Spring, MD). In control experiments, the
primary antibodies were omitted or replaced by a non-immune rabbit serum. All control experiments yielded no immunofluorescent staining.

Criteria for cortical distal segment identification on kidney sections. The initial segments of the ASDN, DCT2, and CNT are situated in the cortical labyrinth. The “landmark” for the CNT is its close vicinity to the cortical radial vessels. The CCDs are identified in the medullary rays.

Expression in oocytes and two-electrode, voltage-clamp measurements. *X. laevis* ENaC cDNAs in pSDeasy with a FLAG epitope sequence (α- and β-ENaC<sup>39</sup> for binding and immunofluorescence) or without (electrophysiology) were used. After plasmids were ligated into pSDeasy, PCR-amplified sequences were verified by PCR together with a silent A→G restriction site (CTTAAG replacing CTCAAA) located 18 nucleotides downstream of the lysine-alanine mutation (nucleotide sequence GCA replaced GGA). Amplicons containing the mutations were digested with AflII and flanking enzymes (BamHI for the upstream and XhoI for the downstream fragment) and ligated into pSDeasy. PCR-amplified sequences were verified by DNA sequencing and SGK protein expression by Western blot (not shown). cDNA encoding the human ribosomal protein L28 was used as control (14). After plasmids were linearized with *Bgl*II (β-ENaC, XSGK), AflIII (α- and γ-ENaC), or *XhoI* (L28), capped cRNA was synthesized by using SP6 or T3 (L28) RNA polymerase, as previously described (13, 28). The vector pSDeasy/SGK has been described (6). The K130A mutation (with an alanine replacing lysine in the putative ATP binding pocket) was introduced by PCR together with a silent A→G restriction site (CTTAAG replacing CTCAAA) located 18 nucleotides downstream of the lysine-alanine mutation (nucleotide sequence GCA replaced GGA). Amplicons containing the mutations were digested with AflII and flanking enzymes (BamHI for the upstream and XhoI for the downstream fragment) and ligated into pSDeasy. PCR-amplified sequences were verified by DNA sequencing and SGK protein expression by Western blot (not shown). cDNA encoding the human ribosomal protein L28 was used as control (14). After plasmids were linearized with *Bgl*II (β-ENaC, XSGK), AflIII (α- and γ-ENaC), or *XhoI* (L28), capped cRNA was synthesized by using SP6 or T3 (L28) RNA polymerase, as previously described (28).

Iodination of *M<sub>a</sub>Ab and binding assay. The iodination and binding procedures were as previously shown (28) and corresponded essentially to those described by Firsov et al. (13). The specific activity of the labeled *M<sub>a</sub>* anti-FLAG monoclonal Ab (Kodak, Rochester, NY) ranged between 0.3 and 0.7 × 10<sup>18</sup> cpm/mol, where cpm is counts/min. Background binding measured on H<sub>2</sub>O-injected oocytes amounted to 33% of the total binding on oocytes injected with ENaC cRNAs alone and was subtracted.

Immunohistochemical detection of ENaC in *X. laevis* oocytes. Fixation (24 h after injection with cRNA), embedding, and cryosectioning were as described previously (28). A tyramide signal amplification (TSA-Direct) kit (NEN, Boston, MA) was used for immunofluorescence according to the manufacturer’s instructions, and ENaC<sup>γ</sup> was detected with the *M<sub>a</sub>* anti-FLAG IgG Ab as described (28). No staining was observed in experiments where the primary Ab was omitted or replaced by a non-immune rabbit serum. All control experiments yielded no immunofluorescent staining.

Statistics. Data are expressed as means ± SE. The difference between control and test values was evaluated by using Student’s *t*-test (2 tailed, unpaired, or 1 sample).

RESULTS

Rapid induction of α-ENaC by aldosterone in ASDN. To quantitate the effect of aldosterone on tubular ENaC subunit mRNAs, PTs and CCDs were microdissected from Adx rats and Adx rats that had received a single subcutaneous injection of aldosterone (50 µg/100 g) 2 h before. As shown in Fig. 1, the mRNA of α-ENaC was induced in CCDs by a factor of 1.92 ± 0.25 (*n* = 12, from 4 rats/condition), whereas those for β- and γ-ENaC subunits were not significantly altered. ENaC mRNA was not detected in PTs.

To determine the effect of aldosterone on ENaC protein abundance and subcellular localization, ENaC subunits were disclosed by immunohistochemistry in the kidneys of Adx rats and of Adx rats 2 and 4 h after a single subcutaneous aldosterone injection (Fig. 2). In control rats, α-ENaC was undetectable in CCDs, whereas β- and γ-ENaC were readily visible in the cytoplasm of the segment-specific CCD cells. Two and four hours after aldosterone injection, the presence of α-ENaC was indisputable in the cytoplasm of CCD cells, indicating that aldosterone rapidly increased its abundance. The amount of immunohistochemically revealed γ-ENaC and the subcellular localization of ENaC subunits in the CCD cells were not affected by the single aldosterone injection. The β-ENaC signal slightly decreased after 4 h. Changes similar to those in CCD occurred in MCD (see Fig. 5B).

Aldosterone shifts ENaC toward the apical plasma membrane in initial portions of the ASDN. Immunohistochemistry disclosed that, in the initial portions of the ASDN, aldosterone not only increased the abundance of α-ENaC but also profoundly affected the subcellular localization of all three ENaC subunits (Fig. 3). In Adx rats without aldosterone administration, β- and γ-subunits of ENaC were found in a fine granular staining pattern exclusively throughout the cytoplasm of all segment-specific cells, and α-ENaC was undetectable (Fig. 3). Two hours after aldosterone injection, α-ENaC was visible in the cytoplasm of the segment-specific cells of the CNT. At that time point, the intracellular staining for all three ENaC subunits was more condensed in the upper third of most CNT cells. At 4 h, ENaC subunits were shifted from the diffuse intracellular localization toward the apical plasma membrane. The aldosterone-induced translocation of ENaC was most obvious for α-ENaC and was seen predominantly in the initial ASDN (DCT2 and the early CNT). Apical labeling decreased progressively along the CNT and was absent in the CCD (Fig. 2).
SGK is rapidly induced by aldosterone in the ASDN. Our previous in situ hybridization data suggested that, in Adx rats, aldosterone induces SGK selectively in the distal nephron (6). Now, we quantified the induction of SGK mRNA in microdissected PTs and CCDs from Adx rats by using real-time RT-PCR. Two hours after aldosterone administration, SGK was increased by a factor of 3.6 ± 0.5 in CCDs, whereas it remained unchanged in PTs (Fig. 4). These results confirm that SGK mRNA is rapidly induced by aldosterone on the mRNA level in rat CCDs.

To test whether aldosterone in vivo rapidly induces SGK on the protein level as well, we performed immunohistochemistry on kidney sections from Adx rats treated as above. Consecutive cryosections from renal cortex (Fig. 5A) and medulla (Fig. 5B) were incubated with anti-SGK and anti-β-ENaC antibodies, respectively. In untreated Adx animals, SGK was not detectable in ENaC-positive tubular profiles of kidney cortex (Fig. 5A) and medulla (Fig. 5B). In contrast, 2 h after aldosterone injection, SGK was highly abundant in all ENaC-expressing, segment-specific cells of the ASDN. Four hours after aldosterone injection, SGK protein was still visible in the ASDN, but the staining intensity had already declined. Along the entire ASDN, induced SGK was homogenously distributed throughout the cytoplasm and always extranuclearly, compatible with a cytosolic localization. SGK was not seen in intercalated cells that were identified by their strong binding to H-ATPase antibodies (Fig. 6).

SGK increases ENaC activity in X. laevis oocytes by a mechanism involving its kinase activity. We and others have previously shown that SGK expression in X. laevis oocytes increases the transport activity of coex-
pressed ENaC (6, 30). We have now tested whether the kinase activity of \textit{X. laevis} SGK might be required to mediate this effect by selectively mutating a single amino acid (K130A) known to be required for the function of its consensus kinase site (32). Although expression of wild-type SGK increased the amiloride-sensitive current fourfold, expression of the kinase-dead mutant SGK decreased it (Fig. 7A). The lack of ENaC activity upregulation by the kinase-dead SGK indicates that its kinase activity is required for this action. The twofold lower current observed in the presence of kinase-dead SGK further suggests a competition of this overexpressed mutant protein with an endogenous one (dominant negative effect). The fact that coexpression of an unrelated protein (ribosomal protein L28) did not interfere with the ENaC-mediated sodium current (data not shown) excludes the possibility that the effect of kinase-dead SGK was due to competition at the translational level.

\textit{SGK acts on ENaC activity by increasing its surface expression in X. laevis oocytes.} To test whether the functional change induced by SGK coexpression might be explained by changes in XENaC surface expression, we measured the binding of monoclonal antibodies (M2Ab) to epitope tags introduced in the extracellular

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**Fig. 4.** Real-time RT-PCR of serum and glucocorticoid-regulated kinase (SGK) on RNA extracted from microdissected proximal convoluted tubules (PT) and CCDs of kidneys from ADX rats 2 h after injection of vehicle (open bars) or aldosterone (solid bars). Values are means ± SE (n = 12, pooled from 4 animals). \( P < 0.0001 \) for ADX + aldo vs. ADX.

**Fig. 5.** Profiles of CNT (CN) in cortex (A) and of collecting duct (CD; C) in the upper third of the inner medulla (B) in kidneys from ADX rats and from ADX rats 2 and 4 h after aldosterone injection. Immunofluorescence with rabbit antisera against SGK and \( \beta \)-ENaC on pairs of consecutive cryostat sections is shown. In A, note the absence of SGK staining but the strong cytoplasmic \( \beta \)-ENaC-staining in CNT cells of ADX rats, the prominent cytoplasmic SGK staining, and the shift of \( \beta \)-ENaC toward the apical cell pole 2 h after aldosterone, and the weak SGK staining contrasting with a prominent \( \beta \)-ENaC-staining in the apical cell pole and plasma membrane of the same cells (arrows) after 4 h. Arrowheads: intercalated cells. In B, note the absence of SGK staining but the strong cytoplasmic \( \beta \)-ENaC staining in CD cells of collecting duct profiles from ADX rats (C), the presence of strong cytoplasmic staining for SGK and \( \beta \)-ENaC 2 h after aldosterone, and the weak staining for both SGK and \( \beta \)-ENaC after 4 h. Bars, ~20 \( \mu \)m.

**Fig. 6.** CNT profile in kidney of ADX rat 2 h after aldosterone injection. Double immunofluorescence on the same cryostat section with rabbit polyclonal antibody against vacuolar H\(^{-}\)-ATPase is shown. SGK is seen only in the segment-specific CNT cells (arrows) but not in the H\(^{-}\)-ATPase-positive intercalated cells (arrowheads). Bar, ~20 \( \mu \)m.
Fig. 7. Effects of wild-type (wt) and kinase-dead (mut) SGK on ENaC-mediated current and ENaC surface localization in Xenopus laevis (X) oocytes. A: opposite effects of wt and mut SGK on ENaC-mediated current. Bars, means ± SE of fractional values (test/control) of 6 independent experiments, each with 3–10 oocytes/point. For wt SGK and mut SGK vs. control, P = 0.016 and <0.0001, respectively. B: binding of radioiodinated anti-Flag antibody (M2Ab) to surface-expressed epitope-tagged α,β,γ-ENaC. Bars, means ± SE of fractional values (test/control) of 4 independent experiments each with 12 oocytes. wt SGK vs. control, P = 0.05; mut SGK is statistically not significantly different from control. C: immunostaining with M2 anti-FLAG antibody against epitope-tagged XenNaC in cryosections of oocytes. Note the weak staining in the plasma membrane of oocytes expressing XenNaC alone (left) but the drastic increase in plasma membrane staining of oocytes coexpressing XenNaC and wild-type SGK (middle), whereas XenNaC surface staining is absent in kinase-dead mutant-expressing oocytes (right).

The results of these binding and morphological experiments suggest that the opposite effects of SGK and kinase-dead SGK on the activity of coexpressed ENaC in X. laevis oocytes might be entirely mediated by a change in ENaC cell-surface expression.

DISCUSSION

Aldosterone induces α-ENaC accumulation and α,β,γ-ENaC surface expression in ASDN. Previous in vivo studies have shown that chronic elevations of plasma aldosterone levels induce in kidney aldosterone-target epithelia an increase in α-ENaC subunit expression at the mRNA and protein levels. In contrast, β- and γ-ENaC appear to be expressed constitutively in ASDN (2, 7, 10, 22, 26, 27). The present study shows that, in kidney of Adx rats, accumulation of α-ENaC protein is recognizable as early as 2 h after a single aldosterone injection and remains at approximately the same level during the following 2 h. This upregulation of α-ENaC occurs in all segment-specific cells of the ASDN. The about twofold rise in the level of α-ENaC mRNA (2 h after aldosterone injection) appears small compared with the striking increase in immunofluorescence from zero in Adx rats to bright immunostaining in Adx+aldosterone rats. This suggests that, in the mammalian distal nephron, the regulation of α-ENaC protein abundance might also involve posttranscriptional mechanisms in addition to α-ENaC transcription. A previous in vitro study on A6 epithelia suggested this possibility (29).

Patch-clamp analysis has revealed that, in isolated CCDs from rats on control diet, the number of active ENaCs was very low but that it was dramatically increased in CCDs from rats on chronic dietary Na+ restriction (31). These functional data correlate with recent immunofluorescence studies in kidneys of rats and mice on prolonged low-salt diet that demonstrated a shift of ENaC from intracellular compartments toward the apical plasma membrane (25, 27). Yet, these studies were unable to disclose whether the translocation of ENaC to the apical membrane depends on the low-salt intake itself or on the diet-associated rise in plasma aldosterone. We now show that, in Adx rats on a salt-supplemented diet, aldosterone indeed causes a rapid shift of ENaC subunits to the apical plasma membrane. This translocation displays a pronounced axial heterogeneity along the ASDN. In the present conditions, it is apparent in the initial upstream portions of the ASDN and progressively disappears farther downstream in the CCD. A similar axial heterogeneity in the apical abundance of ENaC along the ASDN has been observed in rabbits (24) and mice on a control diet (25). However, the tubular portion with apical ENaC localization was longer after chronic dietary salt restriction than after an acute aldosterone treatment because it also included the CCDs (25). The observation of a differential translocation of ENaC along the ASDN suggests that systemic changes in aldosterone levels cannot be the sole factor for regulating aldosterone-dependent Na+ reabsorption via ENaC. It is conceivable that local factors that vary along the tubule axis must cooperate with aldosterone to promote the translocation of ENaC from an intracellular pool to the apical membrane.

Is SGK a mediator of ENaC surface expression? A recently published study (1) as well as the present
The present study shows that the kinase catalytic site of SGK needs to be intact for promoting ENaC surface expression in X. laevis oocytes. This complements the notion that SGK needs to be phosphorylated to be an active kinase and that 3-phosphoinositide-dependent protein kinase 1 (PDK1) functions as an upstream kinase of SGK (32). Furthermore, recent experiments showed that inhibition of phosphoinositol 3-kinase (PI3-kinase) activity markedly reduces SGK stimulation of ENaC activity in the oocyte system. PI3-kinase inhibition also prevented both phosphorylation of SGK and mineralocorticoid-induced Na\(^+\) transport in A6 cells (44). Together with these results, our study shows that SGK expression per se is not sufficient to promote ENaC surface expression in X. laevis oocytes as well as in epithelial cells. In the oocyte system, this effect of SGK is shown to depend on its own catalytic activity, and thus, phosphorylation.

**Role of SGK and \(\alpha\)-ENaC induction in ENaC surface expression: a hypothesis.** Together, the present in vivo and in vitro data suggest the following hypothesis for the regulation of ENaC cell surface expression by aldosterone in ASDN: SGK is rapidly induced by aldosterone and, if activated, promotes the translocation of available \(\alpha, \beta, \gamma\)-ENaC toward the surface. Activation of SGK requires its phosphorylation by a PDK1-like kinase. This phosphorylation could be controlled by an axially heterogenous mechanism that thereby restricts ENaC translocation to more or less long upstream portions of the ASDN. In the absence of aldosterone, the availability of preassembled ENaC is limited by a low level of \(\alpha\)-ENaC. Hence, by inducing an upregulation of \(\alpha\)ENaC, aldosterone also leads to a progressive increase in the pool of assembled \(\alpha, \beta, \gamma\)-ENaC available for translocation to the surface.

Whether the translocation of the three ENaC subunits observed by immunofluorescence corresponds to a transfer of ENaC toward a subapical pool or directly to the luminal surface membrane cannot be discerned at the present resolution. Furthermore, the precise role of SGK in the context of the complex regulatory network that controls ENaC surface expression and includes protein kinase A and Nedd4 (15, 35, 37), however, remains to be elucidated. In conclusion, we show that aldosterone rapidly induces the accumulation of SGK and \(\alpha\)-ENaC along the entire ENaC-expressing ASDN. We hypothesize that SGK might play an important role in the translocation of ENaC to the apical cell surface by integrating the transcriptional aldosterone action with signals that might be differentially available along the ASDN.

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