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Disinhibitory pathways for control of sodium transport: regulation of ENaC by SGK1 and GILZ

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Bhalla, Vivek, Rama Soundararajan, Alan C. Pao, Hongyan Li, and David Pearce. Disinhibitory pathways for control of sodium transport: regulation of ENaC by SGK1 and GILZ. *Am J Physiol Renal Physiol* 291: F714–F721, 2006. First published May 23, 2006; doi:10.1152/ajprenal.00061.2006.—Regulation of ENaC occurs at several levels. The principal hormonal regulator of ENaC, aldosterone, acts through the mineralocorticoid receptor to modulate ENaC-mediated sodium transport, and considerable attention has focused on defining the components of the early phase of this response. Two genes, SGK1 and GILZ, have now been implicated in this regulation. While the functional significance of SGK1 in mediating aldosterone effects is well established, new evidence has enhanced our understanding of the mechanisms of SGK1 action. In addition, recent work demonstrates a novel role for GILZ in the stimulation of ENaC-mediated sodium transport. Interestingly, both SGK1 and GILZ appear to negatively regulate tonic inhibition of ENaC and thus use disinhibition to propagate the rapid effects of aldosterone to increase sodium reabsorption in tight epithelia.

epithelial sodium channel; Nedd4-2; 14-3-3; ERK; trafficking

APPROPRIATE COORDINATION OF signaling pathways that regulate distal nephron ion transport is essential for physiological control of blood pressure and electrolyte composition. Sodium (Na⁺), as the principal extracellular cation, is a major target of this regulation, primarily through the control of activity and/or apical plasma membrane abundance of the epithelial sodium channel (ENaC) (14, 15). ENaC constitutes the rate-limiting step in Na⁺ translocation and represents the convergence of multiple activating and inhibitory regulatory factors. Aldosterone, which is the key hormonal mediator of the response to variation in dietary Na⁺, chloride (Cl⁻), and potassium (K⁺), acts primarily in the distal nephron to stimulate transport of these essential ions. The reabsorption of Na⁺ and Cl⁻ in particular establish osmotic gradients, which, in the presence of antidiuretic hormone, drive aquaporin-mediated water reabsorption, thus sustaining extracellular fluid volume and blood pressure (61). Aldosterone-regulated K⁺ secretion is central to the control of extracellular and intracellular concentrations of this essential ion. Other hormones, including insulin, vasopressin, atrial natriuretic peptide, and γ -MSH, also act in the distal nephron to modulate ion transport.

Regulation of ENaC, composed of three genetically distinct subunits (α , β , and γ), occurs at multiple levels, including gene transcription and several stages of posttranslational modification (34, 59), which alter channel open probability (74, 76), intracellular trafficking, and degradation (13). Aldosterone induces early- and late-phase responses to increase transepithelial Na⁺ transport in the collecting duct, which are largely,

or possibly solely, mediated through effects on gene transcription. For example, target genes encode the transporters themselves, which tend to be induced relatively slowly, and probably play a role in the consolidation (“late”) phase of the response (78) (e.g., ENaC- α and Na⁺-K⁺-ATPase α -subunit). However, regulatory factors are rapidly induced (16, 68) and mediate the early phases of the response by modulating the trafficking and possibly the open probability of already synthesized channels and transporters. ENaC, in particular, appears to be strongly regulated by changes in trafficking, although some evidence also supports effects of regulators such as K-Ras on open probability (70). Aldosterone induces an increase in channel density in the apical membrane within 2 h without altering expression of either the β - or γ -subunit of ENaC, in both cultured cells and intact animals (6, 43, 47). Moreover, heterologous expression of rapidly induced genes, such as serum and glucocorticoid kinase 1 (SGK1) and glucocorticoid-induced leucine zipper protein (GILZ), increases cell surface expression of ENaC and, concomitantly, Na⁺ transport (3, 16, 68). This review focuses on recent studies that have shed new light on the complex intracellular signaling cascades by which aldosterone stimulates Na⁺ current and alters the cell surface expression of ENaC.

SGK1 is an Important Aldosterone-Induced Gene, Which Controls ENaC-Mediated Na⁺ Transport

SGK1 was originally identified in a mammary tumor cell line as a serine-threonine kinase of unknown function (82). It was subsequently independently cloned by several groups in screens for genes induced by osmotic shock (80), brain injury (37), and aldosterone (16, 53). In amphibian collecting duct-like cells (A6) and in native rat kidney, SGK1 mRNA levels are upregulated by aldosterone within 30 min, protein levels

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increase within 45 min, and maximal protein levels are observed within 6 h (16). SGK1 is expressed ubiquitously in mammalian tissues but is strongly induced in collecting duct, and heterologous expression of SGK1 with ENaC in *Xenopus laevis* oocytes markedly stimulates amiloride-sensitive Na⁺ transport (3, 16). This mechanism has also been demonstrated in several epithelial cell lines, including mammalian collecting duct (54). In addition, SGK1 knockout mice have elevated aldosterone levels and are unable to conserve Na⁺ appropriately on a low-salt diet (84). Furthermore, immunohistochemical staining shows that despite ENaC expression levels, which are comparable to those of wild-type, apical membrane expression of ENaC is diminished.

SGK1 is one of six members of the Sgk1/Akt family of serine-threonine kinases, which are known to be activated in a phosphatidylinositol 3'-kinase (PI3K)-dependent fashion. Insulin signals through PI3K and has been shown in cell culture experiments to activate SGK1 (58). Conversely, pharmacological inhibition of PI3K deactivates SGK1 and Na⁺ transport in various epithelial cell lines. These data support the paradigm that SGK1 is found at the convergence of two extracellular signals (aldosterone and insulin) and regulates distal nephron Na⁺ transport (23, 81). Physiological data in SGK1 knockout mice further support this idea. Recent evidence also suggests that the PI3K-dependent activity of SGK1 may be regulated by interaction with WNK1 [with no lysine(K) isoform 1] and that this interaction may lead to increased Na⁺ transport through ENaC (85, 86). Intronic deletions in WNK1 which lead to upregulation of the gene product have been identified recently in patients with type II pseudohypoaldosteronism (Gordon's syndrome) (83), and more recently WNK1 gene polymorphisms have been linked to hypertension in the general population (73). WNK1 may therefore cause hypertension through PI3K and SGK1, but this remains to be fully substantiated.

While no monogenetic forms of hypertension have been linked to SGK1, several human genetic studies have linked single-nucleotide polymorphisms in SGK1 to elevated systolic and diastolic blood pressure, as well as hypertension associated with hyperinsulinemia (11, 12, 79). It remains to be seen whether mutations that increase SGK1 activity cause hypertension, or whether inhibition of SGK1 activity in select populations (for example, patients with the metabolic syndrome or type II diabetes) could ameliorate hypertension.

SGK1 is the Physiologically Relevant Isoform that Stimulates Aldosterone-Induced Na⁺ Transport

The Na⁺-wasting phenotype of SGK1 knockout mice is clearly milder than the severe Na⁺ wasting observed in mice lacking either the mineralocorticoid receptor (7), ENaC- α (35), or ENaC- β (49). Potential compensatory elements include two other SGK isoforms, SGK2 and SGK3, which were identified by homology cloning (18, 41), as well as non-SGK/Akt family mediators. SGK2 and SGK3 share 80% homology with SGK1 in their kinase domains and, like SGK1, are activated through the PI3K signaling pathway (40). SGK2 and SGK3 are expressed in the kidney (18, 41), share in vitro substrates with SGK1 (41), and appear to stimulate ENaC (25) and Na⁺-K⁺-ATPase (31) in *X. laevis* oocytes. However, SGK2 and SGK3 are not corticosteroid regulated in cultured cells (41, 54) or in

the kidney (2), suggesting that they do not play significant roles in aldosterone-regulated Na⁺ reabsorption.

Consistent with this notion, mice lacking SGK3 display normal Na⁺ balance even when Na⁺ restricted and do not display the aldosterone resistance observed in SGK1 knockout mice (48). Furthermore, SGK1-SGK3 double knockout mice do not show a more severe Na⁺-wasting phenotype than SGK1 knockouts (26). Surprisingly, the principal phenotype of SGK3 null mice is a striking defect in postnatal hair follicle development (48). Moreover, SGK2 mRNA levels are not upregulated in compound SGK1 and SGK3 knockout mice, suggesting that SGK2 does not compensate for SGK1 at the transcriptional level. Taken together, SGK1 appears to be the relevant SGK isoform in the response to Na⁺ restriction. Interestingly, one recent study suggests that SGK1 may also be implicated in mediating the natriuretic and hypertensive effects of insulin in states of insulin resistance (33). It remains to be determined whether other SGK/Akt isoforms mediate these natriuretic actions as well. In contrast to SGK2 and SGK3, Akt1 does not stimulate ENaC-mediated Na⁺ transport in model systems (4).

SGK1 Stimulates ENaC Through Phosphorylation of Nedd4-2

SGK1 kinase activity is required for all known SGK1 functions, including ENaC regulation (10, 20, 42). The best characterized target of SGK1 in the context of Na⁺ transport is the E3 ubiquitin ligase Nedd4-2 (for neural precursor cell expressed, developmentally downregulated protein Nedd4-2), a close relative of the "WW domain"-containing protein Nedd4-1, which was identified by a two-hybrid screen as an interacting partner for the COOH-terminal tail of the β -subunit of ENaC (71). Both Nedd4-1 and Nedd4-2 are expressed in kidney and have both been shown to bind ENaC (through direct interaction of tryptophan-rich WW domains with proline- and tyrosine-containing "PY" motifs within ENaC cytoplasmic tails), but only Nedd4-2 has been shown to alter ENaC-mediated Na⁺ transport in *X. laevis* oocytes (38). Overexpression of Nedd4-2 leads to decreased cell surface expression and decreased Na⁺ transport (1). Lack of interaction between Nedd4-2 and Liddle's variants of ENaC (which lack functional PY motifs) is an important mechanism of increased distal Na⁺ reabsorption in patients with Liddle's syndrome, a rare monogenetic form of hypertension.

Nedd4-2 appears to play a physiological role in aldosterone-mediated Na⁺ transport because, in contrast to Nedd4-1, it is a target of SGK1 (20, 66). Overexpression of SGK1 results in phosphorylation of Nedd4-2, and disruption of Nedd4-2's tonic inhibition of ENaC can indirectly stimulate an increase in Na⁺ transport. Moreover, recent work from Flores et al. (23) has demonstrated that, after acute treatment with aldosterone, the rise in phosphorylated Nedd4-2 parallels the increase in SGK1 in a cortical collecting duct cell line. In addition, targeted gene (siRNA) knockdown of Nedd4-2 in cultured cells has suggested that Nedd4-2, but not Nedd4-1, is required for aldosterone-mediated Na⁺ transport (67). However, this issue remains unsettled as Vecchione and colleagues (77) have shown that specific WW domains, found in both isoforms, may functionally inhibit ENaC, and it seems possible that either or both isoforms may be used in a context-dependent fashion. Interestingly, recent evidence has suggested that Nedd4-2 can also

ubiquitinylate SGK1 and induce its proteasome-mediated degradation (88). Thus SGK1-mediated phosphorylation of Nedd4-2 may shift the ligase's substrate specificity from ENaC to SGK1 itself. This model of codependent regulation may allow for feedback inhibition and more finely tuned regulation of Na⁺ transport.

14-3-3 Proteins Inhibit Nedd4-2 to Stimulate Aldosterone-Mediated Na⁺ Transport

Recent studies have begun to shed light on the mechanistic basis of SGK1-mediated modulation of Nedd4-2, by implicating phosphorylation-induced interaction with 14-3-3 proteins (8, 36). 14-3-3 Proteins are small scaffolding proteins, which are highly conserved in eukaryotic organisms and modulate a remarkable variety of cellular processes, including nuclear localization of transcription factors such as FKHL1, the activity of enzymes such as serotonin-*N*-acetyltransferase, and progression of ion channels such as KCNK3 from the endoplasmic reticulum (ER) to the Golgi apparatus (9, 56, 57). There are seven known mammalian isoforms of 14-3-3 (β , γ , ϵ , η , σ , θ , and ζ) encoded by distinct genes, which bind as homo- or heterodimers to single or paired motifs within target proteins (52). The modulation of target protein function is accomplished through several different types of activities, including exposure of nuclear export signals, alteration of active site conformation, displacement of interacting proteins, or bridging of target proteins to induce interaction (75).

The functionally critical SGK1 target motif within Nedd4-2, surrounding serine 444 (*X. laevis* numbering) (20), conforms perfectly to the 14-3-3 consensus motif, while two other sites match it partially (24, 65). One of these, serine 338, has been shown to be of secondary importance in the response to SGK1. The third site has not been well characterized. These SGK1 sites are conserved in vertebrate species, from *Xenopus* to human, and SGK1-mediated phosphorylation of Nedd4-2 (20, 66) has been demonstrated *in vivo* and *in vitro* at both motifs (20). In particular, Nedd4-2 mutants with alanine substitution at serine 444 inhibit ENaC as strongly as wild-type but do not respond to SGK1; furthermore, SGK1-mediated phosphorylation is markedly reduced.

Importantly, 14-3-3 proteins interact in cultured cells and *in vitro* with wild-type, but not mutant, Nedd4-2 in an SGK1-dependent fashion (Fig. 1A). Moreover, endogenous Nedd4-2 and 14-3-3 interact in collecting duct principal cells, and this interaction is stimulated by mineralocorticoids and blunted by inhibitors of PI3K. 14-3-3 Proteins also play a functional role in SGK1-mediated Na⁺ transport in heterologous systems (Fig. 1B). Thus 14-3-3 proteins may be necessary to coordinate SGK1 regulation of Nedd4-2 and ENaC, but further *in vivo* studies are needed to determine whether 14-3-3 proteins are necessary and/or sufficient to control Nedd4-2 activity in the context of physiologically relevant Na⁺ transport. Moreover, which 14-3-3 isoform(s) is implicated in Nedd4-2 regulation of ENaC is uncertain; evidence supporting roles for two different isoforms has been adduced (8, 36). Figure 2 shows the simplest interpretation of the 14-3-3 effect: SGK1 phosphorylation of Nedd4-2 induces binding of a 14-3-3 dimer, resulting in attenuation of the Nedd4-2-ENaC interaction.

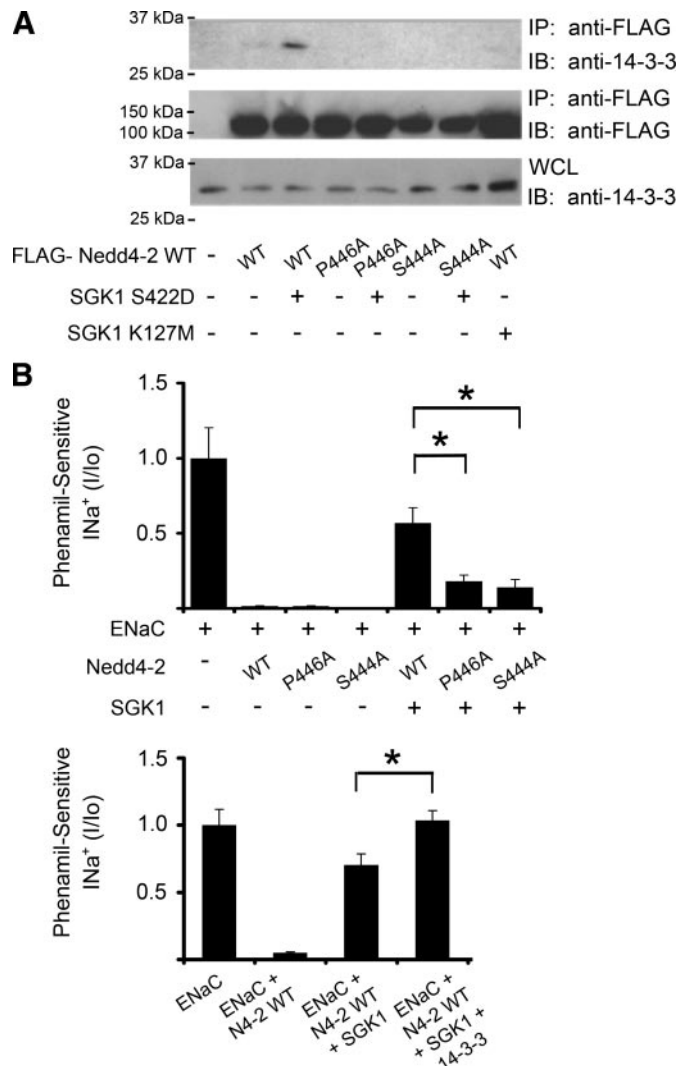


Fig. 1. 14-3-3 Induces an inhibitory interaction with SGK1 on phosphorylation of Nedd4-2. **A**: SGK1 stimulates Nedd4-2-14-3-3 interaction. Wild-type (WT) or mutant FLAG-tagged Nedd4-2 was expressed in HEK 293 cells with or without constitutively active SGK1 (S422D) as shown. Coimmunoprecipitation of endogenous 14-3-3 with Nedd4-2 was markedly stimulated by SGK1 (lane 3). Mutation of Nedd4-2 at Pro446 or Ser444 abrogated the SGK1-mediated interaction of Nedd4-2 and 14-3-3. Kinase-dead SGK1 (K127M) was unable to stimulate interaction. IP, immunoprecipitated; IB, immunoblotted. **B**: interaction of Nedd4-2 and 14-3-3 augments SGK1-stimulated Na⁺ transport. Nedd4-2 mutations that disrupt 14-3-3 interaction reduce SGK1-mediated epithelial Na⁺ channel (ENaC) activation in *Xenopus laevis* oocytes (top). 14-3-3 Overexpression further stimulates ENaC-mediated Na⁺ current in the presence of SGK1 (bottom). ENaC-mediated Na⁺ current was measured by 2-electrode voltage clamp in the presence and absence of phenamil and is depicted relative to ENaC alone. The statistical significance between different conditions was compared as indicated. See Ref. 8 for details. **P* < 0.01.

SGK1 May Increase Cell Surface Expression of ENaC

Taken together, the available data suggest the schematic model shown in Fig. 2. Aldosterone may or may not affect ENaC open probability (29, 39), and although there is evidence that SGK1 phosphorylates ENaC itself (21), the majority of evidence favors the hypothesis that SGK1 substrates are regulatory proteins, such as Nedd4-2, or components of the trafficking machinery. In addition, aldosterone and SGK1 clearly have substantial effects on channel plasma membrane

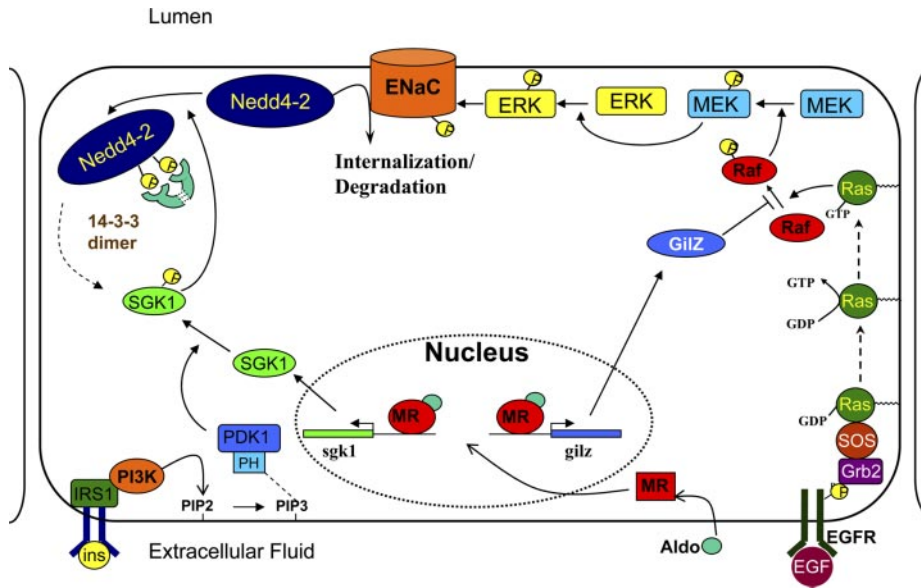


Fig. 2. Schematic depiction of the role of SGK1 and GILZ in hormonal regulation of ENaC activity in an epithelial cell. SGK1 and GILZ abundance is regulated by aldosterone (Aldo). SGK1 activity is regulated by insulin through phosphatidylinositol 3'-kinase (PI3K). According to our hypothesis, GILZ acts in parallel to inhibit ERK signaling. Together, they coordinately block Nedd4-2 inhibition of ENaC. See text for details. For simplicity, only some of the proposed and/or demonstrated interacting complexes are shown. Small circled P, phosphate; Ins, insulin. See the text for definitions of other abbreviations.

abundance (20, 43, 47), although it remains uncertain whether their effects are primarily of channel insertion, retrieval, or both (19). The effects of brefeldin A (BFA) on ENaC currents may shed some light on this issue. BFA is a fungal metabolite, which potently inhibits anterograde vesicular transport from the ER to the *cis*-Golgi, and hence delivery of newly synthesized channels (or other cargo) through the Golgi to the plasma membrane. Regulatory factors that primarily stimulate steady-state transport by inhibiting the endocytic pathway will delay BFA-induced run-down in Na⁺ current, whereas factors that stimulate exocytosis should have little or no effect. One prior study did not find a substantial effect of SGK1 on BFA-induced run-down in ENaC-mediated Na⁺ current in *X. laevis* oocytes (3). In this study, Nedd4-2 was not coexpressed, and the SGK1 effect on the baseline current was modest (~2-fold). Nedd4-2-mediated ubiquitinylation increases ENaC retrieval and degradation (44, 45, 72), and SGK1 has a substantially more potent ENaC stimulatory effect when it is acting in the presence of Nedd4-2 (Fig. 3) (8, 20, 66). Interestingly, under conditions of Nedd4-2 coexpression, SGK1 markedly inhibits

BFA-induced run-down, consistent with a pronounced effect on channel internalization (Fig. 3). However, its effects may not be limited to this arm of ENaC trafficking; Nedd4-2 ubiquitinylation may also influence trafficking at the level of sorting in endosomes and *trans*-Golgi (28, 32). Moreover, SGK1 may have Nedd4-2-independent effects on trafficking of the channel. Thus SGK1 likely acts at multiple points in ENaC trafficking to control its plasma membrane abundance.

Aldosterone-Induced GILZ Negatively Regulates ERK to Stimulate Na⁺ Transport

Several lines of evidence suggest that SGK1 is not the only mediator of aldosterone action. Most notably, although SGK1 knockout mice have aldosterone resistance, their phenotype is substantially less severe than that of either mineralocorticoid receptor- or ENaC- α knockout mice (25, 84), or of adrenalectomized wild-type animals (43), suggesting that renal ENaC function and renal mineralocorticoid action are partially, but not completely, dependent on SGK1. The observation that short-term aldosterone treatment induces an accumulation of SGK1 in the entire aldosterone-sensitive distal nephron, whereas the apical insertion of ENaC takes place only in the connecting tubule and early collecting duct, indicates that other factors are probably required to drive ENaC cell surface expression and activity, and thus Na⁺ reabsorption (43). It is also notable that inhibitors of PI3K (which is responsible for activation of SGK1) do not completely abolish aldosterone-induced Na⁺ transport (81), further supporting the idea that aldosterone may act through multiple pathways to stimulate Na⁺ reabsorption, some of which are SGK1 and PI3K independent. Other regulators, including K-Ras (69) and KS-WNK1 (54), have been identified and shown to stimulate Na⁺ transport; however, aldosterone regulation has not been demonstrated in mammals, and their physiological importance has not yet been established.

Recent evidence supports the theory that GILZ represents an important mediator of aldosterone action, which acts in parallel with SGK1 to increase ENaC plasma membrane localization (68). GILZ is a member of the TSC22 (TGF- β -stimulated

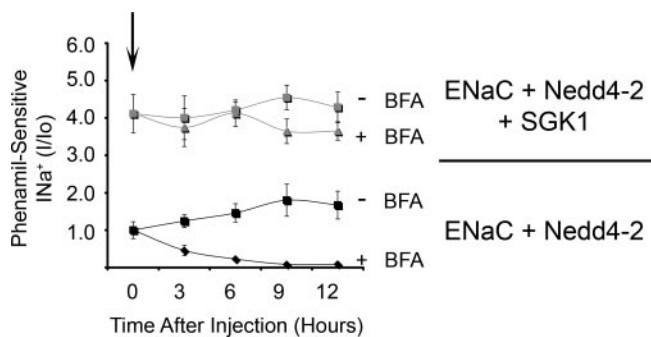


Fig. 3. SGK1 markedly blunts brefeldin A (BFA)-induced rundown of ENaC-mediated Na⁺ current. *X. laevis* oocytes were injected with cRNA for ENaC subunits and Nedd4-2 with (top 2 curves, gray) or without (bottom 2 curves, black) SGK1. Oocytes were treated with BFA (5 μ M) or vehicle (arrow), and 2-electrode voltage clamp was performed at various times after BFA exposure. At earlier time periods, washout of BFA brought current back up to pretreatment levels (not shown). Phenamil-sensitive Na⁺ current (I_{Na^+}) is expressed relative to ENaC+Nedd4-2 before injection of BFA.

clone 22) family of leucine zipper proteins that were initially thought to function as transcription factors (17). As its name implies, it was originally identified in T lymphocytes as a glucocorticoid early-response gene (17). It was identified subsequently as a mineralocorticoid-regulated transcript by serial analysis of gene expression and was shown to be rapidly and robustly stimulated by aldosterone in native collecting duct (51) and cultured collecting duct cells (60). In cultured T cells, GILZ inhibits IL-2 production by inhibiting ERK signaling, possibly by displacing Ras from the Ras-binding domain of Raf (5). Interestingly, in collecting duct cells, the ERK pathway has a potent inhibitory effect on ENaC (50, 64, 87), which may be due, in large measure, to direct ENaC phosphorylation, which stimulates interaction with Nedd4-1 and/or Nedd4-2 (64). This latter observation suggested a possible mechanism for GILZ stimulation of ENaC, which has been supported by recent evidence in oocytes and cultured collecting duct cells (68), and is reviewed briefly below.

It is now increasingly clear that ERK activation, for example by EGF (in cultured collecting duct cells) (22, 27, 63) or by progesterone (in ENaC-expressing *X. laevis* oocytes) (55), inhibits Na⁺ current, thereby serving as a late brake, which provides feedback inhibition to limit aldosterone-stimulatory effects (27). However, there is constitutive ERK activation in collecting duct cells (46, 68), which could also influence basal levels of Na⁺ transport, as well as electrical potential gradients. GILZ expression in progesterone-treated oocytes (to activate ERK), markedly inhibits phospho-ERK2 formation and stimulates ENaC-mediated Na⁺ current, in a manner that is similar to, and nonadditive with, MEK inhibition by U-0126 (68) (Fig. 4). Similarly, in EGF-treated mpkCCD_{c14} (murine collecting duct) cells, heterologous expression of GILZ induces parallel downregulation of activated ERK and upregulation of transepithelial Na⁺ transport. As in oocytes, MEK inhibition has a nonadditive effect, similar to GILZ expression, further suggesting that they act in the same pathway, upstream of ERK1/2. As in oocytes, most of the stimulatory effect of GILZ in mpkCCD_{c14} cells appears to reflect inhibition of ERK signaling, and hence disinhibition of Na⁺ transport. Moreover, EGF, which is known to act through EGFR to stimulate ERK1/2 in HEK 293 cells, markedly decreases cell surface ENaC expression, and heterologous expression of GILZ in EGF-treated HEK 293 cells completely reverses this effect. Together, these data strongly support the view that GILZ contributes to mineralocorticoid stimulation of ENaC-mediated Na⁺ transport by inhibiting the ERK cascade (Fig. 2).

Interestingly, these results involving GILZ and ERK are reminiscent of the mechanism by which SGK1 stimulates ENaC activity, which also involves “inhibiting an inhibitor”: Nedd4-2 (20). ERK-mediated ENaC inhibition appears to proceed through phosphorylation of the channel, which stimulates interaction with Nedd4 proteins (64), leading to a decrease in channel surface expression and subsequent degradation (30). The latter may be due to a late effect that is secondary to enhanced internalization. Although this mechanism remains to be further elucidated, it is notable that in HEK 293 cells, 1-h treatment with EGF markedly diminished cell surface ENaC without any detectable change in the total cellular pool. Here, too, the effect of GILZ on ENaC appears to be largely, if not solely, mediated by its inhibition of ERK1/2. These data support the view that the effects of ERK and GILZ, like those

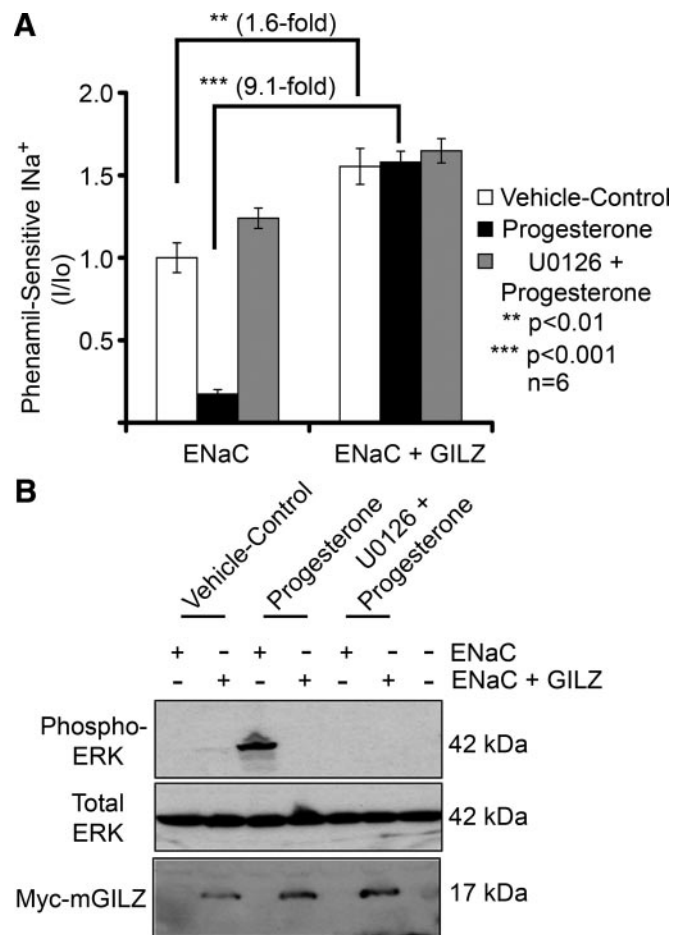


Fig. 4. GILZ stimulates ENaC activity in *X. laevis* oocytes by inhibiting ERK. **A:** *X. laevis* oocytes were coinjected with cRNA for all 3 ENaC subunits (3 ng each) either alone or in combination with GILZ cRNA (3 ng). Twenty-four hours after incubation in a low-Na⁺ medium, they were treated with 2 μ g/ml progesterone (or an equal volume of ethanol/DMSO vehicle control) to activate ERK. As a control, 1 group of oocytes was pretreated with the MEK inhibitor U-0126 for 2 h before progesterone treatment. ENaC activity was measured using 2-electrode voltage clamp and expressed relative to ENaC alone+vehicle. **B:** GILZ prevents activation of ERK in a manner similar to U-0126. Shown is Western blot analysis for active/phosphorylated (Phospho-) ERK (p42/ERK2) in GILZ-expressing oocytes (or controls) treated with progesterone or ethanol/DMSO (vehicle control). Blots were stripped and reprobed for total ERK as shown. The blot was probed with anti-Myc antibody to detect Myc-tagged GILZ expression. Adapted from Ref. 68.

of Nedd4-2 and SGK1 (20, 43), are primarily on levels of plasma membrane channel expression. During the early phase of aldosterone action, the parallel effects of SGK1 and GILZ converge on the ENaC-Nedd4-2 interaction, abrogating it by increasing Nedd4-2 phosphorylation (and hence recruitment of 14-3-3) and decreasing channel phosphorylation, respectively. This study highlights the importance of “disinhibition” as a key mechanism in the control of ENaC function, probably through modulation of trafficking and degradation (62).

Perspectives

The rapid effects of aldosterone on ENaC-mediated Na⁺ transport modulate existing PI3K-dependent and ERK-dependent signaling cascades, acting, at least in part, to disinhibit the tonic inhibition of ENaC by ubiquitin ligase Nedd4-2 (Fig. 2).

Several investigations have provided further insight into the mechanisms by which aldosterone-induced SGK1 modulates Nedd4-2 to stimulate ENaC. In addition, elucidation of mechanisms by which aldosterone-induced GILZ negatively regulates both ERK activation and the Nedd4-2-ENaC interaction suggests a parallel mechanism to SGK1 in the control of epithelial Na⁺ transport. We suggest that SGK1 and GILZ, in concert, mediate cross talk between steroid and growth factor signaling pathways, thereby providing context-appropriate regulation of Na⁺ transport.

NOTE ADDED IN PROOF

Since submission of this review, Liang et al. have shown that 14-3-3 β may specifically regulate the interaction between phosphorylated Nedd4-2 and ENaC (*J Biol Chem*. In press.).

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