Regulation of the voltage-gated K^+ channels KCNQ2/3 and KCNQ3/5 by serum- and glucocorticoid-regulated kinase-1

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Schuetz F, Kumar S, Poronnik P, Adams DJ. Regulation of the voltage-gated K^+ channels KCNQ2/3 and KCNQ3/5 by serum- and glucocorticoid-regulated kinase-1. Am J Physiol Cell Physiol 295: C73–C80, 2008. First published May 7, 2008; doi:10.1152/ajpcell.00146.2008.—The voltage-gated KCNQ2/3 and KCNQ3/5 K^+ channels regulate neuronal excitability. We recently showed that KCNQ2/3 and KCNQ3/5 channels are regulated by the ubiquitin ligase Nedd4-2. Serum- and glucocorticoid-regulated kinase-1 (SGK-1) plays an important role in regulation of epithelial ion transport. SGK-1 phosphorylation of Nedd4-2 decreases the ability of Nedd4-2 to ubiquitinate the epithelial Na^+ channel, which increases the abundance of channel protein in the cell membrane. In this study, we investigated the mechanism(s) of SGK-1 regulation of M-type KCNQ channels expressed in Xenopus oocytes. SGK-1 significantly upregulated the K^+ current amplitudes of KCNQ2/3 and KCNQ3/5 channels ~1.4- and ~1.7-fold, respectively, whereas the kinase-inactive SGK-1 mutant had no effect. The cell surface levels of KCNQ2-hemagglutinin/3 were also increased by SGK-1. Deletion of the KCNQ3 channel COOH terminus in the presence of SGK-1 did not affect the K^+ current amplitude of KCNQ2/3- or KCNQ3/5-mediated currents. Coexpression of Nedd4-2 and SGK-1 with KCNQ2/3 or KCNQ3/5 channels did not significantly alter K^+ current amplitudes. Only the Nedd4-2 mutant Nedd4-2 exhibited a significant downregulation of the KCNQ2/3/5 K^+ current amplitudes. Taken together, these results demonstrate a potential mechanism for regulation of KCNQ2/3 and KCNQ3/5 channels by SGK-1 regulation of the activity of the ubiquitin ligase Nedd4-2.

KCNQ channel; M current; potassium channel; ubiquitin ligase; Xenopus oocyte

HETEROMERS OF THE KCNQ3 channel with the KCNQ2 or KCNQ5 channel underlie the M current, which plays an important role in regulating neuronal excitability (41). The M current is a noninactivating muscarine-sensitive K^+ current with a low voltage-gated threshold. In contrast, homomeric KCNQ1, KCNQ4, and KCNQ5 channels have been reported to exhibit time- and voltage-dependent inactivation (22, 36). Inhibition of the M current causes repetitive firing in neurons, and mutations in KCNQ3 and KCNQ2 subunits lead to benign familial neonatal convulsions (23). The KCNQ channels belong to the Kv7 family, and each subunit contains six transmembrane domains (S1–S6), a pore-forming loop between S5 and S6, a short NH2 terminus, and a large intracellular COOH terminus. Considerable effort has been devoted to unraveling the signaling pathways that regulate channel opening by muscarinic agonists. A major focus has been on the phosphoinositide-phospholipase C cycle and the phosphatidylinositol 4,5-bisphosphate depletion hypothesis, leading to the suppression of the M current in superior cervical ganglion sympathetic neurons (7, 18). Other regulatory mechanisms include Ca^{2+}, PKC, cADP-ribose, src tyrosine kinase, and PKA. Just as reduction of the M current can increase susceptibility to epilepsy, increasing the current should be protective against convulsions.

Despite its key role in regulating excitability, surprisingly little is known about the molecular mechanisms that regulate M current amplitude at the level of channel trafficking, i.e., insertion and removal from the plasma membrane. Similar to many ion channels, there exist significant subplasmalemmal pools of KCNQ channels that may serve as reservoirs for the rapid recruitment of the channels to the membrane (7). In this context, we previously reported that the ubiquitin ligase Nedd4-2 limits the cell surface levels of heterologously expressed KCNQ2/3 and KCNQ3/5 channels, thereby reducing M current density (9). Another recent report showed that calmodulin was involved in the trafficking of the KCNQ subunits from the endoplasmic reticulum (10). Thus, in addition to short-term regulation by signaling intermediates, intracellular trafficking is likely to play an important and additional role in regulating the M current.

Nedd4-2 is regulated by other factors, including serum- and glucocorticoid-regulated kinase (SGK). Indeed, SGK-1 is known to regulate channel abundance in the plasma membrane by inhibition of the ubiquitin ligase Nedd4-2 as a result of direct phosphorylation of Nedd4-2 (6), a mechanism that underlies the glucocorticoid-mediated stimulation of the epithelial Na^+ channel (ENaC), or by interaction with trafficking molecules (28, 45). SGK-1 was originally identified in a screen to identify genes that increased dramatically when cells were exposed to serum and/or glucocorticoids (42). SGK-1 (and related kinases SGK-2 and SGK-3) is a member of the “AGC” subfamily, which includes PKA, PKG, and PKC, with a catalytic domain that is similar to PKB. SGK-1 is ubiquitously expressed and has been shown to be involved in the regulation of the activity of a wide variety of ion channels (e.g., ENaC, ROMK, KCNE1/KCNQ1, KCNQ4, Kv1.3, Kv1.5, Kv4.3, CIC2, and GluR6) and transporters/exchangers (e.g., EAAT1-5, GLUT-1, and Na^+-K^+-ATPase) (24). SGK can regulate target proteins by direct phosphorylation of an SGK-1 consensus sequence (RXRXXS), as in the case of the KCNQ4 channel (35). Taken together, SGK-1 is implicated in a wide variety of physiological functions, such as ion transport regulation, hormone release, neuronal excitability, cell proliferation, and apoptosis (24).
The present study arises from our previous findings that KCNQ2/3 and KCNQ3/5 channels are regulated by Nedd4-2 (9) and that SGK-1 and KCNQ2/3/5 channels are found in the same regions of the brain, e.g., the hippocampus and cortex (3, 4, 19, 26, 27, 37, 41, 46). The M current is involved in spatial memory and learning (11, 15), regulating neuronal excitability (5, 47) and epilepsy (benign familial neonatal convulsions) (23). Taken together, these data support the need for an investigation of the possibility that KCNQ2/3 and KCNQ3/5 channels are targets of SGK-1.

MATERIALS AND METHODS

Plasmids and expression constructs. The rat KCNQ2 (Kv7.2) and KCNQ3 (Kv7.3) clones were provided by Prof. D. McKinnon (State University of New York, Stony Brook, NY), the rat KCNQ2-HA clone by Prof. T. J. Jentsch, and the rat KCNQ5 (Kv7.5) clone by Prof. O. Pongs (both affiliated with Hamburg University, Zentrum für Molekulare Neurobiologie, Hamburg, Germany). The human Nedd4-2 clones [wild-type (WT), S342A, S448A, and S342A and S448A] have been described previously (25). The human SGK-1 clone, its enzymatic inactive K127QSGK-1 clone, and the ligase-deficient inactive mutant C922SNedd4-2 were obtained from Dr. C. Yun (Emory University, Atlanta, GA). Two different truncated KCNQ3 subunits deleting two-thirds of the COOH terminal were produced using the restriction enzymes Pvu II and Stu I (1,536 and 1,592 bp, respectively; New England Biolabs). The total length of the KCNQ3 subunit was 2,591 bp, and the length of the COOH terminal was ~1,562 bp. Capped RNA for the plasmids KCNQ2/3/5, Nedd4-2 (WT, S342A, S448A, and S342A and S448A), SGK-1, and K127QSGK-1 were synthesized using the appropriate restriction enzymes and mMessage mMACHINE in vitro transcription kit (Ambion).

Electrophysiological recordings of outward K⁺ currents mediated by the heteromers KCNQ2/3 and KCNQ3/5 expressed in Xenopus oocytes. Oocytes (stage V–VI) were removed from Xenopus laevis. The protocols for our experiments were submitted to and approved by the Animal Ethics Committee of the University of Queensland, an independent review committee, and are in accordance with the guidelines for care and use of laboratory animals of the Institute for Laboratory Animal Research. After defolliculation with collagenase (Sigma type I), oocytes were injected with KCNQ2/3 (7.2 ng/oocyte) and KCNQ3/5 (or their appropriate deletion plasmids, 7.2 ng/oocyte) alone or in combination with SGK-1, K127QSGK-1, Nedd4-2 (WT, S342A, S448A, or S342A and S448A, 3.6 ng/oocyte). Similar results were obtained with different amounts of RNA for the KCNQ2/3 (7.2 ng/oocyte) + SGK-1 (1.8 ng or 3.6 ng/oocyte) + Nedd4-2 (3.6 ng or 1.8 ng/oocyte) experiment. As a positive control to confirm that SGK-1 was functional in this system, another known target of SGK-1, Fig. 1. Serum- and glucocorticoid-regulated kinase-1 (SGK-1) upregulates K⁺ currents mediated by KCNQ2/3 and KCNQ3/5 channels expressed in Xenopus oocytes. A: representative family of depolarization-activated K⁺ currents mediated by KCNQ2/3 heteromers and recorded from Xenopus oocytes in the absence and presence of SGK-1 or kinase-inactive K127QSGK-1. Oocytes were held at −80 mV and depolarized from −70 to +20 mV in 10-mV increments for 1 s. B: representative family of depolarization-activated K⁺ currents recorded from Xenopus oocytes expressing the KCNQ3/5 heteromer in the absence and presence of SGK-1 or the kinase-inactive K127QSGK-1. Oocytes were held at −80 mV, and voltage steps were applied from −70 to +20 mV in 10-mV increments for 1 s. C and D: relative K⁺ current amplitude (I/I_{control}) obtained at +50 mV in oocytes expressing KCNQ2/3 and KCNQ3/5 channels in the absence and presence of SGK-1 and K127QSGK-1. Data were normalized to wild-type KCNQ2/3 and KCNQ3/5 channels and are expressed as means ± SE of 30–31 oocytes from 3 different batches. ***P < 0.001 vs. control. E: representative current (I_{K})-voltage (I-V) relationships of KCNQ2/3 channels in the absence and presence of SGK-1 expressed in 1 batch of Xenopus oocytes. Values are means ± SE of 6–10 oocytes/group.
Kv1.3, was injected along with SGK-1. If upregulation of the Kv1.3 currents was observed as previously described by Henke et al. (17), the experiments were carried out. Oocytes were incubated at 18°C in ND96 solution containing (in mM) 96 NaCl, 2 KCl, 1 CaCl₂, 1 MgCl₂, and 5 HEPES supplemented with 5 mM pyruvic acid, 50 μg/ml gentamycin, and 40 μl enroflaxacin (Baytril) with or without 50 ml of horse serum (GIBCO). The solution was changed daily. After 3 days, whole cell K⁺ currents were recorded from oocytes with use of the two-electrode voltage-clamp technique. The microelectrodes were filled with 3 M KCl, and their resistance varied from 0.2 to 2 MΩ. During recordings, the bath solution was ND96 without supplements, and experiments were conducted at room temperature (22–24°C). Data were low pass filtered at 1 kHz using a Gene Clamp 500B amplifier and a Digidata 1322A interface. Data were digitized at 10 kHz and analyzed with pCLAMP 8 (Axon Instruments, Union City, CA) and GraphPad Prism 4 software.

Cell surface expression levels of KCNQ2/3 channels. The method of Zerangue et al. (48) was used to determine the cell surface expression levels of KCNQ2/3 channels, as previously described for ClC5 channels (20). Briefly, oocytes expressing hemagglutinin (HA)-tagged KCNQ2 channel were incubated for 1 h at 4°C in ND96 solution supplemented with 1% BSA. They were then incubated at 4°C with the primary antibody (monoclonal mouse anti-HA antibody, 1 μg/ml; Roche Molecular Biochemicals) and then with a secondary antibody (horseradish peroxidase-conjugated goat anti-mouse antibody). The oocytes were then washed and transferred in individual wells in a 96-well plate that contained 50 μl of Supersignal ELISA.
Femto maximum-sensitivity substrate solution (Pierce) and incubated for 5 min at room temperature, and the chemiluminescence was quantified using a Fluostar Optima microplate reader (BMG Technologies, Offenburg, Germany). Uninjected oocytes and injected oocytes without an HA epitope were used as negative controls to subtract background signals from real data.

Statistics. Statistical significance was determined using an unpaired Student’s t-test for comparison of two groups (e.g., one group compared with control) or one-way ANOVA if more groups were compared with each other. Statistical significance was set at *P < 0.05, **P < 0.01, or ***P < 0.001.

RESULTS

SGK-1 increases KCNQ2/3- and KCNQ3/5-mediated K⁺ current amplitudes in Xenopus oocytes. Depolarization-activated K⁺ currents were recorded from Xenopus oocytes expressing KCNQ2/3 and KCNQ3/5 heteromers. The KCNQ currents featured similar current-voltage (I-V) relationships and showed sensitivity to linopirdine (data not shown), as reported previously (41, 43). When SGK-1 was expressed, there was a significant increase in the KCNQ2/3 current amplitude to 140 ± 7% (n = 31, P < 0.001) compared with control (i.e., KCNQ2/3 expressed alone), as shown in Fig. 1, A and C. Similar effects were observed for the KCNQ3/5 heteromers, with the current amplitudes increasing to 170 ± 14% (n = 30, P < 0.001) compared with control (Fig. 1, B and D). The I-V relationships of KCNQ2/3 channels were not altered by SGK-1 (Fig. 1E), and similar results were obtained for KCNQ3/5 channels (data not shown). These data suggest that SGK-1 was acting on the channel directly or on an intermediate accessory protein. To demonstrate that the kinase activity of SGK-1 was responsible for the effects on current amplitude, the experiments on KCNQ2/3 and KCNQ3/5 channels were repeated with a kinase-inactivated mutant, K1270SGK-1. Expression of K1270SGK-1 resulted in currents through KCNQ2/3 and KCNQ3/5 channels that were not significantly different from the currents observed for KCNQ2/3 (Fig. 1, A and C) or KCNQ3/5 (Fig. 1, B and D) channels expressed alone.

**SGK-1 does not alter K⁺ current amplitude of KCNQ2 or KCNQ5 homomultimers.** The subunit specificity of the SGK-1 effect was investigated. The homeric KCNQ2, KCNQ3, and KCNQ5 channels were expressed alone or with SGK-1. As is well characterized for KCNQ channels (41), in the absence of KCNQ3 channels, the K⁺ current amplitudes through KCNQ2 and KCNQ5 monomers were 5- to 10-fold smaller than those in the presence of KCNQ3 channels, whereas the currents through the KCNQ3 homomers were effectively not detectable. In these experiments, SGK-1 had no effect on the amplitude of the K⁺ currents mediated by KCNQ2 (110 ± 12% compared with control, n = 10) or KCNQ5 (99 ± 14.5% compared with control, n = 20) homomers. These data indicate that the COOH termini of KCNQ2 or KCNQ5 channels are not involved, and it is the KCNQ3 subunit that is required for the SGK-1-dependent upregulation of KCNQ2/3 and KCNQ3/5 channels.

**SGK-1 and Nedd4-2 regulate cell surface levels of KCNQ2/3 channels.** We then determined whether the SGK-1-induced changes in K⁺ currents were due to changes in the cell surface levels of the channels. The KCNQ2 channel tagged with an HA epitope at the extracellular region was coexpressed with the

![Fig. 4. SGK-1-dependent regulation of KCNQ2/3 and KCNQ3/5 channels requires Nedd4-2 phosphorylation sites. A: representative steady-state current traces of KCNQ2/3 channels in the absence and presence of SGK-1 and the S342A,S448A Nedd4-2 mutant in which both SGK-1 phosphorylation sites were altered from serine to alanine. Xenopus oocytes were held at −80 mV and depolarized in 10-mV increments from −70 to +20 mV for 1 s. B: representative current traces from KCNQ3/5 channels in the absence and presence of the S342A,S448A Nedd4-2 mutant and SGK-1. C: normalized current amplitudes of KCNQ2/3 channels at +50 mV from Xenopus oocytes in the absence and presence of S342A,S448A Nedd4-2 mutant and SGK-1. **P < 0.01 vs. control (Tukey’s 1-way ANOVA). D: normalized current amplitudes of KCNQ3/5 channels at +50 mV from Xenopus oocytes in the absence and presence of S342A,S448A Nedd4-2 mutant and SGK-1. **P < 0.01 vs. control (Tukey’s 1-way ANOVA).
KCNQ3 channel in *Xenopus* oocytes, and the cell surface channels were labeled with an antibody against the extracellular HA epitope. The anti-HA antibody was labeled with a luciferase-conjugated secondary antibody, and the luminescence intensity was measured in a luminometer. Expression of SGK-1 caused a significant increase in the cell surface levels of KCNQ3/2-HA to 145 ± 10% compared with control (n = 20 from 3 separate batches). This effect was not observed when kinase-inactivated K127Q-S GK-1 was used (88 ± 14%, n = 10; Fig. 2). Ekberg et al. (9) previously reported that Nedd4-2 reduces KCNQ currents and used confocal immunofluorescence microscopy to show that Nedd4-2 caused a decrease in the membrane-associated levels of epitope-tagged KCNQ channels. Here we determined the effects of Nedd4-2 on the cell surface of KCNQ3/2-HA in *Xenopus* oocytes. When Nedd4-2 was expressed, there was a pronounced decrease in the levels of KCNQ3/2-HA to 27 ± 10% compared with control (n = 15). This effect was not observed when the ligase-deficient C922S-Nedd4-2 was used (Fig. 2), consistent with our previous findings (9). Thus the effects of SGK and Nedd4-2 on current amplitudes are due to changes in the cell surface levels of KCNQ3/3 channels.

**SGK-1 regulates KCNQ current amplitudes by inactivation of Nedd4-2.** The best-characterized mode of action of SGK-1 on ion channels is inactivation of Nedd4-2 by phosphorylation at SGK-1 consensus sites on Nedd4-2 (6, 25, 38). We therefore investigated whether the action of SGK-1 on KCNQ channels was due to inactivation of Nedd4-2. In these experiments, SGK-1 increased the current amplitude through KCNQ2/3 and KCNQ3/5 channels by 143 ± 10% and 178 ± 9%, respectively (n = 30; Fig. 3), and Nedd4-2 inhibited the KCNQ2/3 and KCNQ3/5 currents to 55 ± 11% and 45 ± 5% of control, respectively (n = 30; Fig. 3). However, when SGK-1 and Nedd4-2 were coexpressed, there was no significant effect on the current amplitudes through either channel compared with the control [110 ± 10% for KCNQ2/3 + SGK-1 + Nedd4-2 (n = 30) and 95 ± 10% for KCNQ3/5 + SGK-1 + Nedd4-2 (n = 30); Fig. 3]. This result suggested that when SGK-1 and Nedd4-2 were coexpressed, SGK-1 may be phosphorylating Nedd4-2, thereby nullifying any effect on cell surface levels of KCNQ3 channels and resulting in no significant change in current density.

To characterize this effect, we performed additional experiments using mutants of Nedd4-2 that were resistant to phosphorylation by SGK-1. Nedd4-2 has two SGK-1 phosphorylation consensus motifs (RXRXXS) at serine 342 and serine 448. Constructs were used in which these residues were mutated to alanines to determine the effect of SGK-1. First, we coexpressed a double-mutant, S342A,448ANedd4-2, with SGK-1 and found a significant reduction in the current amplitudes through KCNQ2/3 and KCNQ3/5 channels (Fig. 4). The same effect was observed when SGK-1 was coexpressed with S448ANedd4-2, with SGK-1 and found a significant reduction in the current amplitudes through KCNQ2/3 and KCNQ3/5 channels (Fig. 4). The same effect was observed when SGK-1 was coexpressed with S448ANedd4-2, whereas S342A-Nedd4-2 had no effect (Fig. 5). These results demonstrated that, in this system, serine 448 on Nedd4-2 was phosphorylated by

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**Fig. 5.** Nedd4-2 phosphorylation site at serine 448 appears essential for the SGK-1-dependent regulation of KCNQ2/3 and KCNQ3/5 channels. **A**: representative current amplitudes of KCNQ2/3 and KCNQ3/5 channels in the absence and presence of SGK-1, S342ANedd4-2, S448ANedd4-2, and S342A,S448ANedd4-2 in *Xenopus* oocytes. Oocytes were held at −80 mV and depolarized to +50 mV for 1 s. **B**: normalized steady-state K⁺ current amplitudes at +50 mV from oocytes expressing KCNQ2/3 and KCNQ3/5 channels in the absence and presence of SGK-1, S342A,Nedd4-2, S448ANedd4-2, and S342A,S448ANedd4-2. Values are means ± SE of 10 oocytes from 3 batches. *P < 0.05; **P < 0.01 vs. control (Tukey’s 1-way ANOVA).
SGK-1, resulting in its inactivation and the subsequent increase in current amplitude.

In addition, cell surface expression experiments were performed on KCNQ2/3 heteromers. Using the chemiluminescence assay and KCNQ2-HA, we found that $^{S342A,S448A}$Nedd4-2 and $^{S448A}$Nedd4-2 resulted in significant decreases in cell surface levels when coexpressed with SGK-1, whereas $^{S342A}$Nedd4-2 had no effect (Fig. 6).

**SGK-1 modulation requires the COOH terminus of KCNQ3.** We previously showed that the interaction of Nedd4-2 with KCNQ3 requires the intracellular 520-amino acid COOH terminus (9). We therefore performed deletion analysis to demonstrate that the action of SGK-1 on KCNQ involved COOH- terminal interactions. The COOH terminus of KCNQ3 channels was truncated using the restriction enzymes Pvu II and Stu I (Fig. 7A). These enzymes truncate the COOH terminus of KCNQ3 channels at the proximal end by approximately two-thirds. The $\bar{I}_{K}$/H11001 current amplitudes of the truncated KCNQ3 heteromers (KCNQ2/3Δ and KCNQ3Δ5) were not significantly different from control (KCNQ2/3 and KCNQ3/5) $\bar{I}_{K}$ currents, indicating that heteromers were expressed and functional with the truncated COOH-terminal KCNQ3 subunit. When the truncated KCNQ3 (KCNQ3Δ3) channel was expressed, the currents through KCNQ2/3Δ or KCNQ3Δ5 channels were observed. When the truncated KCNQ3Δ3 subunit was coinjected with KCNQ2 or KCNQ5 channels and coexpressed with SGK-1, the current amplitudes were not different from the truncated controls [105 ± 15% for KCNQ2/3Δ + SGK-1 (n = 20) and 90 ± 15% for KCNQ3Δ5 + SGK-1 (n = 20); Fig. 7A]. The $I-V$ relationships of the truncated KCNQ3Δ3 subunit coexpressed with KCNQ2 or KCNQ5 channels (Fig. 7, B and C) exhibited no differences from the $I-V$ relationships recorded from the truncated KCNQ2/3Δ or KCNQ3Δ5 heteromers coexpressed with SGK-1. Furthermore, SGK-1 had no effect on the currents through the KCNQ2 or KCNQ5 homomeric channels.
channels (data not shown). These results confirm that the upregulation by SGK-1 of K⁺ currents through KCNQ channels requires the COOH terminus of KCNQ3 channels.

**DISCUSSION**

The M current plays a significant role in normal brain physiology as well as in disease states. In the present study, we identify SGK-1 as a regulator of the cell surface levels of KCNQ2/3 and KCNQ3/5 channels and, hence, a potential mediator of the M current. When expressed with KCNQ subunits, SGK-1 increased the current amplitudes mediated by these channels. These data demonstrate that the paradigm for SGK-1 regulation of ion channels and transporters (24) extends to the channels that constitute the M current. The catalytically inactive K127Q SGK-1 has been shown to inhibit cardiac Na⁺ channel activity (2); however, it does not affect KCNQ2/3 and KCNQ3/5 channel activity (Figs. 1 and 2), suggesting that the endogenous SGK-1 in *Xenopus* oocytes does not interact with KCNQ2/3 and KCNQ3/5 channels. We previously reported that Ned4-2 downregulates KCNQ2/3 and KCNQ3/5 current amplitudes in *Xenopus* oocytes (9). In the present study, using surface labeling techniques, we demonstrate that this is in fact due to a 50% reduction in the number of KCNQ channels at the cell surface. No increase in KCNQ2/3 or KCNQ3/5 current amplitudes was observed in the presence of C922S Ned4-2, suggesting that the endogenous Ned4-2 in *Xenopus* oocytes does not regulate KCNQ2/3/5 channels, as shown previously for neuronal voltage-gated Na⁺ channels (12). Importantly, we show that the action of SGK-1 on KCNQ is due to the phosphorylation of Ned4-2 on serine 448. In recent studies, it has been shown that it is serine 444 on Xenopus Ned4-2 and serine 428 on human Ned4-2 that are important for the regulation of ENaC by SGK-1 (6, 25). The mechanism for the effect of SGK-1 has been recently shown to involve the phosphorylation of Ned4-2 by SGK-1. This results in the binding of 14-3-3 to Ned4-2, which serves to sequester Ned4-2 into an inactive pool (1, 21).

We found that when serine 448 was mutated to an alanine, the SGK-1 response was lost (Fig. 6), indicating that the upregulation of KCNQ currents was due to a mechanism similar to that observed for ENaC. Further evidence for this model comes from the experiments in which SGK-1 was coexpressed with Ned4-2. In this case, we failed to observe any change in the current amplitude through KCNQ2/3 or KCNQ3/5 channels, presumably because each protein exerts a dominant-negative or scavenging effect (Figs. 3 and 4), such that the individual actions are cancelled out.

The fact that only the COOH terminus of the KCNQ3 channel is required for this regulation by SGK-1 (Fig. 7) highlights the central role of the KCNQ3 channel in the intricate regulation of the KCNQ heteromeric complexes. The COOH terminus of Kv7 channels appears to play a crucial role in many different regulatory processes (14). We previously showed that Ned4-2 binds to the COOH terminus of KCNQ channels (9). These present data are consistent with a model whereby phosphorylation of Ned4-2 by SGK-1 prevents its interaction with the COOH terminus of the KCNQ3 channel, resulting in increased levels of the KCNQ3 channel at the cell surface and larger currents. SGK-1 can also alter ion channel activity by direct phosphorylation of the channel (35). Interestingly, the KCNQ3 channel does contain an SGK-1 consensus motif (RPRTPS) in the NH₂ terminus. The fact that we localize the effect of SGK-1 to the COOH terminus shows that this NH₂-terminal motif is not involved in the response. Similarly, there is a potential SGK-1 phosphorylation site in the COOH terminus of the KCNQ5 channel. However, SGK-1 had no effect on the currents through the KCNQ5 homomeric channels. Further studies are required to elucidate the roles of the COOH-terminal tails of KCNQ2 and KCNQ5 channels in the regulation of the heteromeric channel complexes.

SGK-1 and KCNQ subunits are found in similar locations in structures such as the hippocampus and cortex (3, 4, 19, 26, 27, 37, 41, 46). It is clear that modulation of the M current is required in spatial memory formation, learning, neuronal excitability, neuronal survival, neuroprotection from apoptosis, and neuronal development (5, 11, 13, 15, 31, 32, 34, 44, 47). In addition, genetic variants in Ned4-2 have been identified in patients with photosensitive epilepsy, and although these mutations do not affect the Ned4-2 actions on neuronal Na⁺ channels (8), these mutations may alter the regulation of Ned4-2 by downstream kinases such as SGK-1.

Strategies to maintain elevated levels of SGK-1 in selected brain regions may be of use in the treatment of neuronal hyperexcitability, including epilepsy or migraine or, in the peripheral nerves, in the treatment of neuropathic pain (30, 39). Pharmacological agents, such as retigabine, that enhance the M current are known to have an analgesic effect in animal models of inflammatory pain (29), and inhibition by linopirdine has been reported to enhance cognition (11, 34). SGK-1 is part of the Akt/PKB family of kinases that are the subject of considerable interest as therapeutic targets (16, 33, 40). The identification of the molecular mechanisms that regulate M current density will extend our understanding of neuronal plasticity and provide information about the development of cell type-specific therapeutic treatments. In conclusion, we have identified SGK-1, via its inhibitory effect on Ned4-2, as a potential key regulator of the M current. These findings highlight the growing complexity of the mechanisms by which excitable cells can regulate critical currents involved in the maintenance of the action potential.

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**REFERENCES**


15. Fontanata DJ, Inouye GT, Johnson RM.


