Serum- and Glucocorticoid-inducible Kinases (SGK) regulate KCNQ1/KCNE potassium channels

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The stress reaction includes the release of stress hormones such as cortisol via the HPA axis. One of the genes regulated by cortisol is the serum- and glucocorticoid-inducible kinase 1 (SGK1) a stimulator of the slow outward potassium channel KCNQ1/KCNE1—one of the major mediators of cardiac repolarization. Apart from KCNE1, several other KCNE β subunits including KCNE3 and KCNE5 have been detected at the mRNA level in cardiac tissue as well as in the inner ear and the gastro-intestinal tract. Here, we extend our previous investigations to KCNQ1/KCNE3 channels and their modulation by SGKs. We show that these channels are not stimulated by any of the three SGK isoforms when expressed in a heterologous expression system. 3D docking simulations suggest that crucial residues within KCNQ1 and KCNE1 are co-localized to a region close to the putative inner phase of the membrane, suggesting a key region important for channel complex sorting into vesicles. Identification of the KCNQ1/KCNE recycling pathway and its modulation by SGK provides a mechanistic insight into stress-induced modulation of KCNQ1/KCNE channels.

The cardiac action potential duration is adapted to stress via both the sympathetic nervous system and the hypothalamic-pituitary-adrenal (HPA) axis.

Emotional stress is a common precursor to sudden cardiac death and is mediated by the sympathetic nervous system.

and the release of stress hormones such as cortisol via the HPA axis. One of the genes regulated by cortisol is the serum- and glucocorticoid-inducible kinase 1 (SGK1). According to in vitro experiments, SGK1 stimulates the slow outward potassium current $I_{Kr}$. This current—which is conducted by channels composed of KCNQ1 α subunits and KCNE1 β subunits (KCNQ1 is also called $K_v$LQT1 or $K_v$7.1; KCNE1 was originally named IsK or minK)—is also increased during a stress reaction, where it is one of the major mediators of cardiac repolarization. Accordingly, gain of function polymorphisms of SGK1 are associated with shortening of the QT interval, an electrocardiographic measure of ventricular repolarization time. On the other hand, mutations in KCNQ1 or KCNE1 that impair channel function lead to prolongation of the QT interval, resulting in the long QT syndrome (LQTS), an inherited cardiac arrhythmia associated with increased risk of sudden cardiac death. Therefore, SGK1-mediated regulation of KCNQ1/KCNE1 might be particularly important in patients with KCNQ1 or KCNE1 mutations who are prone to fatal cardiac arrhythmias triggered by physical and psychological stress.

Recently, we demonstrated that the exocytosis of KCNQ1 to the plasma membrane requires the small GTPase RAB11, while its endocytosis is dependent on RAB5. We further showed that SGK1 enhances the exocytosis of KCNQ1/KCNE1, which is controlled by RAB GTPases via a pathway involving the phosphorylation of phosphoinositide 3-phosphate 5-kinase (PIKfyve) and the generation of PI(3,5)P2. SGK1 sensitivity of most LQTS-associated mutant $I_{Kr}$ channels remains intact. However, channels containing the disease-associated mutants KCNQ1(Y111C), KCNQ1(L114P) or KCNE1(D76N) were unexpectedly downregulated by SGK1 as a consequence of a disruption in RAB11-dependent recycling of channels. Those channels were sensitive to RAB7, indicating that these channels pass late endosomes.

Apart from KCNE1, several other KCNE β subunits including KCNE3 and KCNE5 have been detected at the mRNA level in cardiac tissue as well as in the inner ear. Furthermore, KCNQ1
SGK regulates $K_v7.1$/KCNE

has been shown to be coexpressed with different KCNE subunits in the gastro-intestinal tract to generate cAMP-modulated, largely voltage-insensitive potassium channels.\textsuperscript{16-20} Previously we had studied the SGK-mediated modulation of KCNQ1/KCNE1 channels only. Here we extend our investigations to KCNQ1/ KCNE3 channels and their modulation by SGK. We show that these channels are not stimulated by any of the three SGK isoforms when expressed in \textit{Xenopus laevis} oocytes (Fig. 1). This suggests that KCNE3 may inhibit SGK1-3 sensitivity of KCNQ1 channel complexes in the heart, the inner ear and the gastro-intestinal tract—in contrast to KCNE1 that stimulates SGK sensitivity. This differential modulation of SGK sensitivity by the different KCNE channel subunits would functionally influence the response of KCNQ1-dependent processes to stress hormones like glucocorticoids. Among these processes are $Cl^-$ secretion into the intestinal lumen and the $H^+$ secretion into the stomach.\textsuperscript{19,20} Stimulation of intestinal $Cl^-$ secretion by stress-induced glucocorticoid release may favor diarrhea, since SGK-sensitive complexes (KCNQ1/KCNE1/ KCNE2) are present in the intestine and could be translocated to the plasma membrane upon activation by SGK. Reflux disease as a result of increased gastric $H^+$ secretion, on the other hand, will probably not be affected by glucocorticoids, as KCNE1 is absent and KCNE3 is heavily expressed in the stomach. Future testing of the effects of SGK1 on KCNQ1 channels coexpressed with KCNE2, KCNE4 and KCNE5 will be helpful to estimate effects on native channels in tissues such as the pancreas, the kidneys and the gastro-intestinal tract.

The in vitro observations presented in Figure 1 are in agreement with the hypothesis that the $\alpha$ subunit KCNQ1 contains the trafficking components and the $\beta$ subunit KCNEx modulates them, possibly by presenting or hiding them. In this regard, several studies have indicated that the determinants of channel protein trafficking are positioned in intracellular regions of KCNQ1.\textsuperscript{12-16} However, KCNE1 subunits mutated in the HxNDP(73-77) motif within the intracellular C-terminus bear the potential to heavily modulate SGK1 sensitivity of KCNQ1 channels.\textsuperscript{12} KCNE3 contains a homologous but subtly different motif within its C terminus: KxSDP(87-91). It is well possible that the amino acid exchanges at the first and third position of this motif abolished SGK1 sensitivity, as the mutations H73C and N75C of KCNE1 reported in our previous study did.\textsuperscript{12} Key to the proposed molecular modulation of channel targeting may be the interaction of regions KCNQ1(73–77) and KCNQ1(111–117).\textsuperscript{12} In order to gain further insight into the possible localization of the residues crucial for SGK1-stimulated recycling we performed 3D modeling. In the 3D docking simulations, the crucial residues co-localized to a region close to the putative inner phase of the membrane, suggesting a key region important for channel complex sorting into vesicles (Fig. 2).

Identification of the KCNQ1/KCNE recycling pathway and its modulation by SGK provides a mechanistic insight into stress-induced modulation of KCNQ1/KCNE1, KCNQ1/KCNE3 and KCNQ1/KCNE1/KCNE3 channels that might influence cardiac repolarization as well as $Cl^-$ secretion into the intestinal lumen, observations of potentially important clinical significance.

\section*{Methods}

\textbf{Molecular biology.} The molecular biological procedures have been described previously.\textsuperscript{25} Clones used were subcloned into oocyte expression vectors, a modified pcDNA3 vector or pSGEM. In vitro synthesis of poly-A-tailed, capped cRNA was performed with SP6 and T7 mMessage mMACHINE kits (Ambion, Austin, TX).

\textbf{Electrophysiology.} \textit{Xenopus laevis} oocytes were obtained according to German law as described before.\textsuperscript{24} Stage V oocytes were collected and injected with 30–50 nl of cRNA. Oocytes were injected with 1 ng or 5 ng of KCNQ1 cRNA alone or with 1 ng of KCNQ1 cRNA plus 5 ng of KCNE3 cRNA. To test for
Figure 2. Localization within KCNQ1 of key residues accounting for SGK1 sensitivity. We generated a KCNQ1 3D model based on the coordinates of the solved crystal structure of Kv1.2. In addition we modeled the extended KCNE1 transmembrane domain as an alpha-helix. Flexibility at central glycines allowed the KCN1 alpha helix to bend. Subsequently, KCNE1 was docked on the KCNQ1 tetrameric channel. The upper figures show the tetrameric channel model including KCNE1 in top view and side view. The four KCNQ1 subunits are colored in magenta, yellow, green and orange, respectively, whereas KCNE1 extended transmembrane domains are blue. The lower figure shows a magnification of the residues KCNQ1(91-129) and KCNE1(65-81). Key residues in KCNQ1 for recycling are shown in green, whereas the residues in KCNE1 are colored orange (S73), pink (N75), red (D76) and magenta (P77).

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