Serum and glucocorticoid inducible kinases in the regulation of the cardiac sodium channel SCN5A

Christoph Boehmer, Viktoria Wilhelm, Monica Palmada, Sabine Wallisch, Guido Henke, Heinrich Brinkmeier, Philip Cohen, Burkert Pieske, Florian Lang

Department of Physiology I, University of Tübingen, Gmelinstrasse 5, D-72076 Tübingen, Germany
Institute of Pathology, University of Greifswald, Greifswald, Germany
MRC Protein Phosphorylation Unit, School of Life Sciences, University of Dundee, Dundee, UK
Department of Internal Medicine, University of Göttingen, Göttingen, Germany

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Abstract

The serum and glucocorticoid inducible kinase SGK1 and its isoform SGK3 are both expressed in cardiac tissue. One of the functions of SGK1 is the phosphorylation and inactivation of the ubiquitin ligase Nedd4-2, which in turn could be shown to downregulate the voltage-gated Na⁺ channel SCN5A (hH1). The present study has been performed to test for a role of SGK1 and SGK3 in the regulation of SCN5A. To this end cRNA encoding the human Na⁺ channel SCN5A was injected into X. laevis oocytes with or without cRNA encoding the wild-type kinases SGK1, the constitutively active SGK1, the inactive form SGK1 or the wild-type SGK3. SCN5A currents were activated by coexpression of either wild-type SGK1 or SGK3 or the constitutively active SGK1. In contrast, the inactive mutant SGK1 significantly decreased the currents. Moreover, coexpression of SGK3 significantly altered SCN5A gating, i.e. it hyperpolarized the activation threshold and depolarized the prepotential required for 50% availability of the channel. Opposite shifts of gating properties were elicited by mutation of serine to alanine in the SGK consensus sequences of SCN5A. The present observations disclose a role of the kinases SGK1 and SGK3 in the regulation of cardiac Na⁺ channels. As SGK1 is upregulated by glucocorticoids, mineralocorticoids and a variety of inflammatory mediators and both kinases are activated by insulin and IGF1, the kinases could mediate effects of those hormones and mediators on cardiac function.

Keywords: Arrhythmia (mechanisms); Growth factors; Hormones; Infection/inflammation; Ion channels; Membrane currents; Membrane potential; Na-channel; Protein kinases; Signal transduction

1. Introduction

As shown previously, the cardiac voltage-gated Na⁺ channel SCN5A (hH1) is regulated by the ubiquitin ligase Nedd4 [1]. The binding of ubiquitin prepares the channel for subsequent clearance from the cell membrane. A similar mechanism has been described most recently for the regulation of the epithelial Na⁺ channel ENaC by Nedd4-2 [2–4]. It has further been shown that the effect of Nedd4-2 has been reversed by the serum and glucocorticoid inducible kinase SGK1 [2,4]. This kinase has originally been cloned as a glucocorticoid sensitive gene from rat mammary tumor cells [5,6]. The human isoform has been identified as a cell volume sensitive gene upregulated by cell shrinkage [7]. Subsequent studies revealed that the expression of SGK1 is stimulated by mineralocorticoids [8–10], gonadotropins [11–14], and a number of cytokines [15]. More recently, two isoforms of SGK1 have been identified, the SGK2 and SGK3 [16]. Unlike SGK1 those two kinases are not genomically encoded.
regulated by glucocorticoids and mineralocorticoids [15]. All three kinases are activated by IGF1 and insulin through PI3 kinase and PDK1 [16–18], an effect involving phosphorylation of the serine at position 422. Replacement of this serine by aspartate (K127N SGK1) leads to a constitutively active kinase [17]. Substitution of lysine at position 127 for asparagine (K127N SGK1) destroys the catalytic domain of the protein kinase and leads to enzyme inactivation.

Both, SGK1 and SGK3 are expressed in cardiac tissue [7,16]. Thus, those kinases could be considered potential regulators of the cardiac voltage gated Na+ channels. The present study has been performed to possibly disclose an influence of SGK1 and SGK3 on the cardiac Na+ channel. To this end, the cardiac Na+ channel SCN5A has been expressed in Xenopus oocytes with and without coexpression of the wild-type kinase SGK1, the constitutively active kinase S422D SGK1, the inactive form K127N SGK1 or the wild-type SGK3.

2. Methods

cRNA encoding wild-type SGK1 [7], constitutively active SGK1 (S422D SGK1) [17], inactive SGK1 (K127N SGK1) [17], wild-type SGK3 [16] wild-type SCN5A [19,20] and mutant SCN5A (S483A SCN5A and S663A SCN5A) were synthesized in vitro as previously described [21]. The S483A SCN5A and S663A SCN5A mutants were made by site directed mutagenesis (Stratagene, Amsterdam, The Netherlands). The polymerase chain reaction primers used for mutagenesis are: S483A SCN5A, sense (5’ gaa aac gga tgt ctc cag gaa cgg ag 3’), antisense (5’ ctc ctc ctt gaa aga tct cta 3’). S663A SCN5A, sense (5’ gga cgc tga ctg cgg cga ggg ccc 3’), antisense (5’ cta ctt gtc cca aca 3’). The complete constructs were sequenced to prove the correct nucleotide exchange and to exclude any additional mutations.

Dissection of Xenopus laevis ovaries, collection and handling of the oocytes have been described in detail elsewhere [21]. The investigation conforms with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised, 1996). Oocytes were injected with 5 ng of human SCN5A and/or 7.5 ng of the respective human kinases. Control oocytes were injected with H2O. All experiments were performed at room temperature 4–5 days after injection of the respective cRNAs. In two-electrode voltage-clamp experiments currents were recorded following a step change of the holding potential from –120 mV to a –20 mV test potential. The data were filtered at 10 kHz, and recorded with MACLAB digital to analog converter and software for data acquisition and analysis (ADInstruments, Castle Hill, Australia). The control bath solution (ND96) contained 96 mM NaCl, 2 mM KCl, 1.8 mM CaCl2, 1 mM MgCl2, and 5 mM HEPES, pH 7.4. The final solutions were titrated to the pH indicated using HCl or NaOH. The flow-rate of the superfusion was 20 ml/min and a complete exchange of the bath solution was reached within about 10 s. Inactivation kinetics were analyzed by plotting the normalized peak current versus the prepulse voltage. Activation was analyzed by plotting normalized slope-conductance versus test pulse voltage.

Polymerase chain reaction was used to test for endogenous expression of Nedd4-2, SGK1 and SGK3 in Xenopus oocytes. To this end total RNA was isolated with RNasy RNA isolation kit (Qiagen, Germany) following the manufacturer’s instructions. A 1 µg amount of RNA was reversed transcribed into cDNA using Advantage RT-for-PCR Kit (Clontech, Palo Alto, CA, USA). The oligonucleotide sequences used for amplification of xNedd4-2, xSGK1 and xSGK3 were as follows: xNedd4-2, sense (5’ cac cct gaa ccc aaa atg gaa tg 3’); xNedd4-2, antisense (5’ ctt ctt ctt cct cca ctc cat tgt 3’); xSGK1, sense (5’ ttc ttc ttc cag aca act age 3’); xSGK1, antisense (5’ tag atg tag tgc cat tgg g 3’); xSGK3, sense (5’ ctt ctt gta aca cgg aaa cgg gat g 3’); xSGK3, antisense (5’ ccc ctc cta taa cca aat cca gaa c 3’). 1 µl of xNedd4-2, xSGK1 and xSGK3 primers were added to 25 µl reaction containing 50 mM KCl, 10 mM Tris–HCl pH 8.4, 15 mM MgCl2, 2 mg/ml BSA, dNTP mix, each 0.2 mM. The mixture was denatured at 95 °C for 1 min, followed by 36 cycles, each consisting of denaturation at 95 °C for 30 s, annealing at 61.2 °C (for xNedd4-2), 56.3 °C (for xSGK1) and 57.9 °C (for xSGK3) for 30 s and extension at 72 °C for 1 min. PCR products were then resolved by electrophoresis in 2% agarose (Boehringer Ingelheim, Germany) and recorded digitally.

Data are provided as means±S.E.M., n represents the number of oocytes investigated. All experiments were repeated with at least three batches of oocytes; in all repetitions qualitatively similar data were obtained. All data were tested for significance by unpaired comparison between groups using the Student t-test, and only results with P<0.05 were considered as statistically significant.

3. Results

RT-PCR analysis reveals that intrinsic SGK1, SGK3 and Nedd4-2 message is expressed in Xenopus laevis oocytes (Fig. 1).

In SCN5A (hH1) expressing cells depolarization from –120 mV to –20 mV triggered a voltage-gated inward current (I Na) of 5.92±0.32 µA (n = 61) while in water injected cells no significant depolarization-activated currents could be detected (0.07±0.01 µA, n = 10). Coexpression of wild-type SGK1 or wild-type SGK3 increased the SCN5A mediated sodium currents to 10.39±1.26 µA (n = 27) and to 9.07±0.53 µA (n = 61), respectively (Fig. 2).
Fig. 1. RT-PCR on noninjected oocytes. PCR products were amplified with primers specific for xNedd4-2, xSGK1 and xSGK3. All three messages could be detected in noninjected oocytes.

The stimulating effect of the wild-type SGK1 was mimicked by the constitutively active S422D SGK1 (8.01±0.70 μA, n=38) but not by the inactive mutant K127N SGK1 (4.65±0.41 μA, (n=23) Fig. 3). Treatment of SCN5A expressing oocytes with 10 μM dexamethasone for 48 h was without any effect on channel activity [untreated: 6.50±0.29 μA, (n=35), treated: 5.81±0.30 μA, (n=37)].

In addition to increasing the absolute current, coexpression of SGK3 slightly but significantly altered SCN5A gating. As illustrated in Fig. 4, coexpression of SGK3 shifted the inactivation curve towards more positive voltages [SCN5A alone $V_{1/2\text{ (inact)}}$: $-86.21\pm0.67$ mV, (n=64), SCN5A+SGK3 $V_{1/2\text{ (inact)}}$: $-82.99\pm1.05$ mV, (n=30)] and the activation kinetics to more negative voltages [SCN5A alone $V_{1/2\text{ (act)}}$: $-39.43\pm1.18$ mV, (n=60), SCN5A+SGK3 $V_{1/2\text{ (act)}}$: $-46.16\pm1.07$ mV, (n=34), Fig. 5]. The SCN5A gating was not significantly modified by coexpression of the inactive mutant [K127N SGK1 $V_{1/2\text{ (inact)}}$: $-87.48\pm1.45$ mV, (n=24); $V_{1/2\text{ (act)}}$: $-35.67\pm1.55$ mV, (n=24)].

SCN5A deletion mutants of putative SGK phosphorylation sites mirrored the SGK3 effects on channel kinetics. The S483A SCN5A mutant displays gating kinetics that are shifted towards more negative voltages for inactivation [wild-type SCN5A $V_{1/2\text{ (inact)}}$: $-88.24\pm0.89$ mV, (n=33), S483A SCN5A $V_{1/2\text{ (inact)}}$: $-93.60\pm1.08$ mV, (n=20)] and the activation kinetics to more positive voltages [wild-type SCN5A $V_{1/2\text{ (act)}}$: $-36.88\pm0.70$ mV, (n=35), S483A SCN5A $V_{1/2\text{ (act)}}$: $-26.86\pm1.37$ mV, (n=20), Fig. 6].

The S663A SCN5A activation kinetics were similarly shifted towards more positive voltages [S663A SCN5A $V_{1/2\text{ (act)}}$: $-31.01\pm0.79$ mV, (n=20)]. There was no significant difference in inactivation kinetics of the S663A SCN5A mutant [$V_{1/2\text{ (inact)}}$: $-88.35\pm1.10$, (n=20)] compared to wild-type SCN5A (Fig. 6).

4. Discussion

We demonstrate here for the first time stimulation of the cardiac voltage gated Na⁺ channel by the serum and glucocorticoid inducible kinases SGK1 and SGK3. Earlier findings indicated that the surface expression of the cardiac voltage gated Na⁺ channel SCN5A (hH1) is under the control of the ubiquitin ligase Nedd4. Coexpression of Nedd4 reduced the peak currents induced by SCN5A expressed in Xenopus oocytes [1]. Later, it has been shown that Nedd4-2 similarly downregulates the epithelial Na⁺
channel ENaC [2–4]. It has further been shown that SGK1 phosphorylates Nedd4-2, an effect leading to Nedd4-2 inactivation [2,4]. *Xenopus* oocytes do express endogenous Nedd4-2 activity which can similarly be inhibited by SGK1. The coexpression of wild-type SGK1 or constitutively active S422D SGK1 reverses the action of the endogenous Nedd4-2 and thus increases channel activity. The inactive K127N SGK1 mutant, where the catalytic subunit has been destroyed [15] rather inhibits the channel activity. The K127N SGK1 mutant presumably displaces endogenous SGK without being able to phosphorylate the target protein. As a result, endogenous Nedd4-2 is disinhibited and the channel expression is downregulated.

In addition to modification of peak currents by SGK1 and SGK3, our observations point to an effect of the SGK3 on the gating properties of the channel. The influence of the kinase on gating is not likely the result of Nedd4-2 phosphorylation and subsequent increase of channel abundance but may rather reflect a more direct influence on channel properties. Accordingly, a Y1980A mutation of the Na⁺ channel prevented the downregulation by Nedd4-2 but did not significantly modify the voltage dependence of steady state inactivation and activation [1].

The present observations disclose a completely novel signaling mechanism in the regulation of cardiac function. This mechanism may be important in the cardiac action of several hormones. As the expression of SGK1 is markedly stimulated by both, glucocorticoids [5,22,23] and mineralocorticoids [8–10], it could participate in the effects of both hormones on cardiac ion channels. It is noteworthy that dexamethasone has recently been shown to partially correct the clinical phenotype of a patient carrying a G514C mutation of SCN5A [24]. Moreover, dexamethasone partially reversed the electrophysiological defect of the mutated channel [24]. In contrast to SGK1, the isoform SGK3 has not been shown to be under strong transcriptional control of glucocorticoids and mineralocorticoids [15] and is thus less likely to mediate effects of those hormones. In oocytes glucocorticoids do not significantly
modify the channel activity. Presumably they do not sufficiently enhance SGK1 levels in the *Xenopus* oocyte expression system.

As SGK1 is regulated by gonadotropins [11–14], it could participate in the regulation of cardiac function during pregnancy [25]. SGK1 is further upregulated by a number of inflammatory cytokines including TGFβ [15,26]. As TGFβ is upregulated in myocarditis [27–32], altered channel regulation by SGK1 could contribute to the pathophysiology of this disease. Again, transcription of SGK3 appears not to be sensitive to those hormones and mediators [15,16].

Both kinases, SGK1 and SGK3 require activation to become functional. They are activated by IGF1 and insulin through PI3 kinase and PDK1 [16–18]. Thus, the effects of insulin and IGF1 may involve stimulation of Na⁺ channel activity through SGK1 and SGK3. The regulation of Na⁺ channel activity by SGK1 allows the integration of genomic and post-translational regulation. Glucocorticoids, mineralocorticoids, gonadotropins and inflammatory cytokines stimulate the expression of SGK1 which is, however, not necessarily active. Insulin and growth hormone (or IGF1) are effective through activation of the kinase. The effect requires both, expression and activation of the kinase and thus, the control of Na⁺ channels by hormones and mediators.
References


