Original Paper

SGK3 Sensitivity of Large-Conductance Ca^{2+} -Activated K^+ Channel

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Neuronal excitation • Serum and glucocorticoid inducible kinase 3 • Cell volume

Abstract

Background/Aims: Large conductance Ca^{2+} -activated K^+ channels (maxi K^+ channels or BK channels) are rapidly activated by increase of cytosolic Ca^{2+} activity. The channels participate in the regulation of diverse functions including neuronal excitation and cell volume. The BK channels may be modified by kinases. Channel regulating kinases include the serum & glucocorticoid inducible kinase 3 (SGK3). The present study explored whether SGK3 modifies the activity of BK channels. **Methods:** cRNA encoding the Ca^{2+} insensitive BK channel mutant $\text{BK}^{\text{M513I}+\Delta 899-903}$ was injected into *Xenopus laevis* oocytes without or with additional injection of cRNA encoding wild-type SGK3, constitutively active $^{\text{S419D}}$ SGK3, or catalytically inactive $^{\text{K191N}}$ SGK3. K^+ channel activity was measured utilizing dual electrode voltage clamp. **Results:** BK channel activity in $\text{BK}^{\text{M513I}+\Delta 899-903}$ expressing oocytes was significantly increased by co-expression of SGK3 or active $^{\text{S419D}}$ SGK3, but not by coexpression of inactive $^{\text{K191N}}$ SGK3. **Conclusion:** SGK3 is a novel positive regulator of BK channels, and thus participates in the regulation of cell volume and excitability.

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The pleiotropic effects of the serum & glucocorticoid inducible kinase SGK3 include up-regulation of several transporters [1-9] and ion channels [10-13], including Ca^{2+} channels [10, 14] and voltage-gated K^+ channels [15-19].

At least in theory, SGK3 may similarly modify the large conductance Ca^{2+} -activated K^+ channels (maxi K^+ channels or BK channels, KCNMA1), which are ubiquitously expressed [20, 21] and participate in the tuning of a wide variety of functions including regulation of neuronal excitability [22-44], cell volume [45-47], vascular tone and blood pressure [48, 49], urinary bladder contraction [50], as well as erection [51].

In neurons [20], BK channels contribute to repolarization and fast afterhyperpolarization of action potentials [52, 53] and thus influence dendritic Ca^{2+} spikes [54], and neurotransmitter

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release [55-58]. They are involved in motor coordination [59], learning and memory [28, 60-62], circadian rhythm [63-66], regulation of respiration [67-69], hearing [70], and pain [71-74]. BK channels confer neuroprotection [75-78]. Defective function of BK channels may lead to epilepsy and paroxysmal dyskinesia [79, 80], mental retardation [81, 82], autism [83], and schizophrenia [84].

The channels are activated by increase of cytosolic Ca^{2+} activity [20] and are highly sensitive to cell membrane potential [20]. The channel properties are modified by accessory subunits [20, 48], BK channel protein abundance and activity could be modified by posttranslational modification including protein phosphorylation, lipidation, glycosylation, and ubiquitination [85-89].

The present study thus explored, whether wild-type SGK3, constitutively active $\text{S}^{419\text{D}}$ SGK3, or catalytically inactive $\text{K}^{191\text{N}}$ SGK3 modify the function of BK channels. To this end, the Ca^{2+} insensitive BK channel mutant $\text{BK}^{\text{M513I}+\Delta 899-903}$ was expressed in *Xenopus laevis* oocytes without or with additional expression of wild type SGK3 and/or constitutively active $\text{S}^{419\text{D}}$ SGK3, or catalytically inactive $\text{K}^{191\text{N}}$ SGK3. The BK channel activity in those oocytes was determined by dual electrode voltage clamp.

Materials and Methods

Ethical Statement

All experiments conform with the 'European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes' (Council of Europe No 123, Strasbourg 1985) and were conducted according to the German law for the welfare of animals and the surgical procedures on the adult *Xenopus laevis* frogs were reviewed and approved by the respective government authority of the state Baden-Württemberg (Regierungspräsidium) prior to the start of the study (Anzeige für Organentnahme nach §36).

Constructs

Constructs encoding mouse Ca^{2+} -insensitive BK channel ($\text{BK}^{\text{M513I}+\Delta 899-903}$) [59, 90], wild type SGK3 [91], constitutively active $\text{S}^{419\text{D}}$ SGK3 [92] or inactive $\text{K}^{191\text{N}}$ SGK3 [92] were used for generation of cRNA as described previously [93-97].

Voltage clamp in *Xenopus laevis* oocytes

Xenopus laevis oocytes were prepared as previously described [98, 99]. 20 ng cRNA encoding BK channels and 10 ng of cRNA encoding wild-type, constitutively active or inactive kinase were injected on the same day after preparation of the oocytes [94, 100-102]. The oocytes were maintained at 17°C in ND96-A, a solution containing (in mM): 88.5 NaCl, 2 KCl, 1 MgCl_2 , 1.8 CaCl_2 , 2.5 NaOH, 5 HEPES, 5 Sodium pyruvate, Gentamycin (100 mg/l), Tetracycline (50 mg/l), Ciprofloxacin (1.6 mg/l), Theophiline (90 mg/l). The solution was titrated to pH 7.4 by addition of NaOH [99, 103]. The voltage clamp experiments were performed at room temperature 3 days after the first injection. BK channel currents were elicited by 1 s pulses from -150 to +190 mV in 2 s increments of 20 mV steps from a holding potential of -60 mV. The data were filtered at 1 kHz and recorded with a Digidata A/D-D/A converter (1322A Axon Instruments) [104]. The Clampex 9.2 software was used for data acquisition and analysis (Axon Instruments). The control superfusate (ND96) contained (in mM): 93.5 NaCl, 2 KCl, 1.8 CaCl_2 , 1 MgCl_2 , 2.5 NaOH and 5 HEPES, pH 7.4. The flow rate of the superfusion was approx. 20 ml/min, and a complete exchange of the bath solution was reached within about 10 s [105, 106].

Statistical analysis

Data are provided as means $\pm$ SEM, n represents the number of oocytes investigated. As different batches of oocytes may yield different results, comparisons were always made within a given oocyte batch. All voltage clamp experiments were repeated with at least 3 batches of oocytes; in all repetitions qualitatively similar data were obtained. Data were tested for significance using ANOVA or t-test, as appropriate. Results with $p < 0.05$ were considered statistically significant.

Results

The present study explored whether the serum & glucocorticoid inducible kinase isoform SGK3 influences the large conductance Ca^{2+} -activated K^+ channels (maxi K^+ channels or BK channels). To this end, cRNA encoding Ca^{2+} -insensitive BK channel ($\text{BK}^{\text{M513I}+\Delta 899-903}$) was injected into *Xenopus* oocytes with or without additional injection of cRNA encoding SGK3 and the voltage-gated K^+ current determined by dual electrode voltage clamp experiments.

As shown in Fig. 1, the injection of cRNA encoding $\text{BK}^{\text{M513I}+\Delta 899-903}$ into *Xenopus* oocytes was followed by a substantial and significant increase of large voltage-gated K^+ currents as compared to water-injected oocytes. The additional injection of cRNA encoding wild-type SGK3 was followed by a modest but significant further increase of the voltage gated current (Fig. 1).

The effect of wild type SGK3 was mimicked by the constitutively active mutant S419D SGK3. The additional injection of cRNA encoding S419D SGK3 into $\text{BK}^{\text{M513I}+\Delta 899-903}$ expressing *Xenopus* oocytes was again followed by a significant further increase of the voltage gated current (Fig. 2).

Fig. 1. Co-expression of wild-type SGK3 increases the K^+ current in BK expressing *Xenopus laevis* oocytes. A: Representative original tracings showing currents in *Xenopus* oocytes injected with water (a), expressing BK alone (b) or expressing BK with additional co-expression of wild-type SGK3 (c). The voltage protocol is shown. Currents were activated by depolarization from -150 to +190 mV from a holding potential of -60 mV. B: Arithmetic means $\pm$ SEM of the current (I) as a function of the potential difference across the cell membrane (V) in *Xenopus* oocytes injected with water (grey diamonds, n = 9), or expressing BK without (white circles, n = 26) or with (black circles, n = 26) additional co-expression of wild-type SGK3. C: Arithmetic means $\pm$ SEM of the normalized peak outward current at +190 mV in *Xenopus* oocytes injected with water (stippled bar, n = 9), or expressing BK without (white bar, n = 26) or with (black bar, n = 26) additional co-expression of wild-type SGK3. *** ($p < 0.001$) indicates statistically significant difference from oocytes expressing BK alone.

Fig. 2. The effect of SGK3 is mimicked by the constitutively active $S419D$ SGK3. A: Representative original tracings showing currents in *Xenopus* oocytes expressing BK alone (a) or with additional co-expression of wild type SGK3 (b) or additional co-expression of constitutively active $S419D$ SGK3 (c). The voltage protocol is shown. Currents were activated by depolarization from -150 to +190 mV from a holding potential of -60 mV. B: Arithmetic means $\pm$ SEM of the normalized peak outward current at +190 mV in *Xenopus* oocytes expressing BK without (white bar, n = 12) or with additional co-expression of wild-type SGK3 (black bar, n = 16) or of constitutively active $S419D$ SGK3 (grey bar, n = 16). **($p < 0.01$), *** ($p < 0.001$) indicates statistically significant difference from oocytes expressing BK alone.

Fig. 3. The effect of SGK3 is disrupted by the inactivating mutation $K191N$ SGK3. A: Representative original tracings showing currents in *Xenopus* oocytes expressing BK alone (a) or with additional co-expression of wild type SGK3 (b) or additional co-expression of inactive $K191N$ SGK3 (c). The voltage protocol is shown. Currents were activated by depolarization from -150 to +190 mV from a holding potential of -60 mV. B: Arithmetic means $\pm$ SEM of the normalized peak outward current and at +190 mV in *Xenopus* oocytes expressing BK without (white bar, n = 13) or with additional co-expression of wild-type SGK3 (black bar, n = 9) or of inactive $K191N$ SGK3 (grey bar, n = 10). *** ($p < 0.001$) indicates statistically significant difference from oocytes expressing BK alone.

In contrast to the injection of cRNA encoding wild-type SGK3 or $S419D$ SGK3, the additional injection of cRNA encoding the inactive mutant $K191N$ SGK3 did not significantly modify the voltage gated current in BK^{M513I+Δ899-903} expressing *Xenopus* oocytes (Fig. 3).

A further series of experiments was performed to elucidate whether the effect of SGK3 required Na⁺/K⁺ ATPase activity. To this end, the oocytes were exposed to 1 mM ouabain. As illustrated in Fig. 4, similar to the effects of SGK3 in the absence of ouabain, in the continued presence of ouabain, the additional injection of cRNA encoding SGK3 into BK^{M513I+Δ899-903}

Fig. 4. The effect of wild-type SGK3 on K⁺ current in BK expressing *Xenopus laevis* oocytes did not depend on Na⁺/K⁺ATPase activity. **A:** Representative original tracings showing currents in ouabain (1 mM) treated *Xenopus* oocytes expressing BK alone (a) or expressing BK with additional co-expression of wild-type SGK3 (b). The voltage protocol is shown. Currents were activated by depolarization from -150 to +190 mV from a holding potential of -60 mV. **B:** Arithmetic means ± SEM of the normalized peak outward current at +190 mV in *Xenopus* oocytes expressing BK without (white bar, n = 19) or with (black bar, n = 19) additional co-expression of wild-type SGK3. *** (p<0.001) indicates statistically significant difference from oocytes expressing BK alone.

expressing *Xenopus* oocytes was followed by a significant further increase of the voltage gated current (Fig. 4).

Discussion

The present study uncovers a novel function of the serum & glucocorticoid inducible kinase isoform SGK3, i.e. the up-regulation of large conductance Ca²⁺-activated K⁺ channels (maxi K⁺ channel or BK channels). Co-expression of the wild-type SGK3 and of the constitutively active ^{S419D}SGK3 [92], but not coexpression of the inactive ^{K191N}SGK3 [92] was followed by a significant increase of the voltage gated current in *Xenopus* oocytes expressing the Ca²⁺-insensitive BK channel BK^{M513I+Δ899-903}.

The present observations did not attempt to define the cellular mechanisms involved in the up-regulation of BK channel activity by SGK3. In theory, SGK3 could be effective by direct phosphorylation of the channel protein and/or by phosphorylating and thus modifying the activity of other signalling molecules, which in turn influence BK channels. BK channel regulating kinases include AMP activated kinase [107], protein kinase A [108, 109], protein kinase C [108, 110, 111], cGMP-dependent protein kinase (PKG) [112], and cyclin-dependent kinase isoform CDK5 [113]. BK channels are further regulated by WNK4, which downregulates BK channels, an effect requiring ERK and p38 kinase [89]. Interestingly, the effect was abolished by coexpression of SGK1 [89]. SGK3 influences some ion channels by phosphorylating and thus inactivating the ubiquitin ligase Nedd4-2, which otherwise

ubiquitinates target proteins thus labelling them for degradation [15, 114-117]. However, whether the large conductance Ca^{2+} -activated K^+ channels are targeted by Nedd4-2, remains uncertain. In theory, SGK3 could have been effective by activation of the Na^+/K^+ ATPase [6], which may up-regulate some K^+ channels [118]. However, Na^+/K^+ ATPase inhibitor ouabain did not appreciably blunt the effect of SGK3 on BK.

The functions sensitive to the activity of large conductance Ca^{2+} -activated K^+ channels include regulation of neuronal excitability [26]. Activation of K^+ channels is expected to hyperpolarize neurons and thus to inhibit excitation. SGK3 contributes to the regulation of neuronal excitability further by regulating glutamate receptors [11, 12, 119, 120] and glutamate transporters [2, 3, 121]. To which extent the modest upregulation of BK channels contributes to SGK3 sensitive neuronal excitation, remains elusive.

Several K^+ channels including BK channels are further decisive for regulatory cell volume decrease [122-124]. In several cell types cell swelling leads to activation of Ca^{2+} permeable cation channels, increase of cytosolic Ca^{2+} activity, activation of large conductance Ca^{2+} -activated K^+ channels, K^+ exit, hyperpolarization and potential-driven Cl^- exit [122-124]. To which extent SGK3-sensitivity of BK channels contributes to cell volume regulation, remains to be shown, however.

In conclusion, SGK3 elicits a modest stimulating effect on large conductance Ca^{2+} -activated K^+ channels, an effect possibly modifying cell volume regulation and neuroexcitability.

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Disclosure Statement

The authors of this manuscript state that they do not have any conflict of interests and nothing to disclose.

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