Original Paper

Up-Regulation of the Large-Conductance Ca^{2+} -Activated K^+ Channel by Glycogen Synthase Kinase GSK3 β

Myriam Fezai^a Musaab Ahmed^a Zohreh Hosseinzadeh^{a,b} Florian Lang^{a,c}

^aDepartment of Cardiology, Vascular Medicine and Physiology, ^bCentre for Ophthalmology, Institute for Ophthalmic Research, University of Tuebingen, Tuebingen, ^cDepartment of Molecular Medicine II, Medical Faculty, Heinrich Heine University, Duesseldorf, Germany

Key Words

Large conductance Ca^{2+} -activated K^+ channel • Glycogen synthase kinase 3 β • Voltage clamp • Lithium • Neuronal excitation

Abstract

Background/Aims: The pleiotropic functions of the large conductance Ca^{2+} -activated K^+ channels (maxi K^+ channel or BK channels) include regulation of neuronal excitation and cell volume. Kinases participating in those functions include the glycogen synthase kinase GSK3 β which is under negative control of protein kinase B (PKB/Akt). GSK3 β is inhibited by the antidepressant Lithium. The present study thus explored whether GSK3 β modifies the activity of BK channels. **Methods:** cRNA encoding the Ca^{2+} insensitive BK channel mutant BK^{M513I+Δ899–903} was injected into *Xenopus laevis* oocytes without or with additional injection of cRNA encoding wild-type GSK3 β , inactive K85R GSK3 β , or wild-type GSK3 β with wild-type PKB. K^+ channel activity was measured utilizing dual electrode voltage clamp. **Results:** BK channel activity in BK^{M513I+Δ899–903} expressing oocytes was significantly increased by co-expression of GSK3 β , but not by co-expression of K85R GSK3 β . The effect of wild type GSK3 β was abrogated by additional co-expression of wild-type PKB and by 24 hours incubation with Lithium (1 mM). Disruption of channel insertion into the cell membrane by brefeldin A (5 μM) was followed by a decline of the current to a similar extent in oocytes expressing BK and GSK3 β and in oocytes expressing BK alone. **Conclusion:** GSK3 β may up-regulate BK channels, an effect disrupted by Lithium or additional expression of PKB and possibly participating in the regulation of cell volume and excitability.

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Introduction

The large conductance Ca^{2+} -activated K^+ channels (maxi K^+ channel or BK channels) serve a variety of functions including regulation of neuronal excitability [1-23] and cell volume [24-26].

Prof. Dr. Florian Lang

Department of Physiology, Cardiology and Vascular Medicine, University of Tuebingen, Gmelinstr. 5, D-72076 Tuebingen, (Germany)
Tel. +49 7071/2972194, Fax +49 7071/295618, E-Mail florian.lang@uni-tuebingen.de

Kinases implicated in the regulation of neuronal excitability [27] and cell size [28] include the glycogen synthase kinase GSK3 β . The kinase is phosphorylated and down-regulated by protein kinase B (PKB/Akt) [29]. GSK3 β is inhibited by the antidepressant Lithium [30].

The present study thus explored, whether GSK3 β modifies the activity of BK channels. To this end, the Ca²⁺ insensitive BK channel mutant BK^{M513I+Δ899–903} was expressed in *Xenopus laevis* oocytes without or with additional expression of wild-type GSK3 β , inactive mutant K85R GSK3 β or wild-type GSK3 β together with wild type PKB. 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. 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 Ca²⁺-insensitive BK channel (BK^{M513I+Δ899–903}) [31, 32] (kindly provided by J Lingle), wild-type human GSK3 β [33], inactive mutant K85R GSK3 β [34], and wild-type PKB [35] were used for generation of cRNA as described previously [36–40]. The Ca²⁺-insensitive BK mutant was utilized because the activity of wild-type BK requires an increase in the intracellular Ca²⁺ level in oocytes, which leads to likely side effects interfering with the measurement [41, 42].

Voltage clamp in *Xenopus laevis* oocytes

Xenopus laevis oocytes were prepared as previously described [38, 43, 44]. 20 ng cRNA encoding BK channels and 7.5 ng of cRNA encoding wild-type or inactive kinase K85R GSK3 β were injected on the same day after preparation of the oocytes [37, 45–47]. The oocytes were maintained at 17°C in a solution, containing (in mM): 88.5 NaCl, 2 KCl, 1 MgCl₂, 1.8 CaCl₂, 2.5 NaOH, 5 HEPES, 5 Sodium pyruvate, supplemented with Gentamycin (100 mg/l), Tetracycline (50 mg/l), Ciprofloxacin (1.6 mg/l), Theophiline (90 mg/l) and pH 7.4 [43, 48]. Lithium Chloride (1 mM or 10 mM) was added where indicated. The voltage clamp experiments were performed at room temperature 3 days after the first injection. BK channel currents were elicited every 1 s with 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 2 kHz and recorded with a Digidata A/D-D/A converter (1322A Axon Instruments) [35, 49]. The Clampex 9.2 software was used for data acquisition and analysis (Axon Instruments) [35, 47, 50, 51]. The control superfusate (ND96) contained (in mM): 93.5 NaCl, 2 KCl, 1.8 CaCl₂, 1 MgCl₂, 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 [52, 53].

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 (Tukey test). Results with $p < 0.05$ were considered statistically significant.

Results

The present study explored whether glycogen synthase kinase GSK3 β modifies the activity of the large conductance Ca²⁺-activated K⁺ channels (maxi K⁺ channel or BK channels). To this end, cRNA encoding Ca²⁺-insensitive BK channel (BK^{M513I+Δ899–903}) was injected into

Fig. 1. Co-expression of wild-type GSK3 β increases the K⁺ current in BK expressing *Xenopus laevis* oocytes. A: Representative original tracings showing currents in *Xenopus* oocytes injected with water (a), expressing GSK3 alone (b) expressing BK alone (c) or expressing BK with additional co-expression of wild-type GSK3 β (d). The voltage protocol is shown (not to scale). Currents were activated by depolarization from -150 to +190 mV from a holding potential of -60 mV. B: Arithmetic means $\pm$ SEM (n = 23-54) of the current (I) as a function of the potential difference across the cell membrane (V) in *Xenopus* oocytes injected with water (white circles), expressing GSK3 β alone (black triangles) or expressing BK without (white rectangles) or with (black rectangles) additional co-expression of wild-type GSK3 β . C, D: Arithmetic means $\pm$ SEM (n = 23-54) of the maximal current (C) and the conductance (D) calculated by linear fit of I/V-curves shown in B between +130 mV and +190 mV in *Xenopus* oocytes injected with water (dotted bar), expressing GSK3 β alone (grey bar) or expressing BK without (white bar) or with (black bar) additional co-expression of wild-type GSK3 β . *** (p < 0.001) indicates statistically significant difference from oocytes expressing BK alone.

Fig. 2. The effect of GSK3b is disrupted by the inactivating mutation ^{K85R}GSK3 β . A: Representative original tracings showing currents in *Xenopus* oocytes injected with water (a), expressing BK alone (b) or with additional co-expression of wild-type GSK3 β (c) or inactive ^{K85R}GSK3 β (d). The voltage protocol is shown (not to scale). Currents were activated by depolarization from -150 to +190 mV from a holding potential of -60 mV. B: Arithmetic means $\pm$ SEM (n = 11-29) of the current (I) as a function of the potential difference across the cell membrane (V) in *Xenopus* oocytes injected with water (white circles) or expressing BK without (white rectangles) or with additional co-expression of wild-type GSK3 β (black rectangles), or inactive ^{K85R}GSK3 β (white triangles). C, D: Arithmetic means $\pm$ SEM (n = 11-29) of the maximal current (C) and the conductance (D) calculated by linear fit of I/V-curves shown in B between +130 mV and +190 mV in *Xenopus* oocytes injected with water (white dotted bar), or expressing BK without (white bar) or with additional co-expression of wild-type GSK3 β (black bar) or inactive ^{K85R}GSK3 β (grey bar). *** (p < 0.001) indicates statistically significant difference from oocytes expressing BK alone.

Fig. 3. The effect of GSK3 β is disrupted by additional co-expression of PKB. A: Arithmetic means $\pm$ SEM ($n = 12-28$) of the current (I) as a function of the potential difference across the cell membrane (V) in *Xenopus* oocytes injected with water (white circles) or expressing BK alone (white rectangles), expressing BK with additional co-expression of wild-type GSK3 β (black rectangles) or expressing BK with additional co-expression of wild-type GSK3 β and wild-type PKB (black triangles) or expressing BK with additional co-expression of PKB (white triangles). B, C: Arithmetic means $\pm$ SEM ($n = 12-28$) of the maximal current (B) and the conductance (C) calculated by linear fit of I/V -curves shown in B between +130 mV and +190 mV in *Xenopus* oocytes injected with water (white dotted bar), or expressing BK without (white bar) or with additional co-expression of wild-type GSK3 β (black bar) or with additional expression of both, GSK3 β and PKB (grey bar), or with additional co-expression of PKB (grey dotted bar). **($p < 0.01$) indicates statistically significant difference from oocytes expressing BK alone, # ($p < 0.05$), ## ($p < 0.01$) indicates statistically significant difference from oocytes expressing BK and GSK3 β .

Xenopus laevis oocytes with or without additional injection of cRNA encoding wild-type GSK3 β or, as a negative control, inactive mutant ^{K85R}GSK3 β . The voltage-gated K⁺ current was determined by dual electrode voltage clamp experiments.

As illustrated in Fig. 1, the injection of cRNA encoding BK^{M513I+Δ899-903} 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 GSK3 β was followed by a moderate but significant further increase of the voltage gated current. The injection of cRNA encoding wild-type GSK3 β alone did not significantly modify the voltage gated current (Fig. 1).

In contrast to the injection of cRNA encoding wild-type GSK3 β , the additional injection of cRNA encoding the inactive mutant ^{K85R}GSK3 β did not significantly modify the voltage gated current in BK^{M513I+Δ899-903} expressing *Xenopus* oocytes (Fig. 2).

In order to test whether the effect of GSK3 β could be modified by protein kinase B (PKB/Akt), wild-type GSK3 β was co-expressed with BK^{M513I+Δ899-903} without or with additional co-expression of PKB. As illustrated in Fig. 3, the co-expression of PKB was followed by a significant decline of the voltage gated current which was virtually identical in oocytes expressing BK^{M513I+Δ899-903} together with wild-type GSK3 β + wild-type PKB and in oocytes expressing BK^{M513I+Δ899-903} alone.

A further series of experiments explored whether the effect of GSK3 β could be modified by the antidepressant Lithium. As illustrated in Fig. 4, a 24 hours Lithium-exposure of

Fig. 4. The effect of GSK3 β is blunted by antidepressant Lithium. A: Arithmetic means $\pm$ SEM (n = 12-51) of the current (I) as a function of the potential difference across the cell membrane (V) in *Xenopus* oocytes injected with water (white diamond) or expressing BK alone (white symbols), or expressing BK with additional co-expression of wild-type GSK3 β (black symbols) without (rectangles) or with a 24 hours of 1 mM Lithium exposure (circles) or with 1h/10 mM Lithium exposure (triangle). B,C: Arithmetic means $\pm$ SEM (n = 12-51) of the maximal current (B) and the conductance (C) calculated by linear fit of I/V-curves shown in B between +130 mV and +190 mV in *Xenopus* oocytes injected with water (dotted bar), or expressing BK without (white bar) or with additional co-expression of wild-type GSK3 β (black bar) prior to (left bars) 24 hours of 1mM Lithium exposure (middle bars) or 1 hour of 10mM Lithium exposure (left bar). *** ($p < 0.001$) indicates statistically significant difference from oocytes expressing BK alone, # ($p < 0.05$) indicates statistically significant difference from oocytes expressing BK and GSK3 β without Lithium treatment.

Xenopus oocytes co-expressing BK^{M513I+ Δ 899-903} together with wild-type GSK3 β was followed by a significant decline of the voltage gated current to values not significantly higher than the current in *Xenopus* oocytes expressing BK^{M513I+ Δ 899-903} alone. A 24 hours of Lithium exposure did not significantly modify the current in oocytes expressing BK^{M513I+ Δ 899-903} alone. An 1 hour incubation in 10 mM Lithium did not decrease significantly the K⁺ current in oocytes co-expressing BK^{M513I+ Δ 899-903} with GSK3 β .

In order to test whether the co-expression of wild-type GSK3 β stabilizes BK^{M513I+ Δ 899-903} in the cell membrane, the insertion of new channel protein into the cell membrane was prevented by brefeldin A (5 μ M). As illustrated in Fig. 5, the brefeldin A treatment was followed by a decay of the voltage gated current. The decay was similar in *Xenopus* oocytes expressing BK^{M513I+ Δ 899-903} alone and in *Xenopus* oocytes expressing BK^{M513I+ Δ 899-903} with additional co-expression of wild-type GSK3 β . Thus, GSK3 β did not appreciably modify the channel stability in the cell membrane.

Discussion

The present study uncovers a novel function of glycogen synthase kinase GSK3 β , i.e. the up-regulation of large conductance Ca²⁺-activated K⁺ channels (maxi K⁺ channel or BK channels). Co-expression of the wild-type GSK3 β but not of the inactive mutant K85R GSK3 β

Fig. 5. Decay of current following brefeldin A treatment in *Xenopus laevis* oocytes expressing BK alone or BK together with GSK3 β . A: Arithmetic means $\pm$ SEM ($n = 20-43$) of the current (I) as a function of the potential difference across the cell membrane (V) in *Xenopus* oocytes expressing BK alone (white rectangles) or expressing BK with additional co-expression of wild-type GSK3 β (black rectangles) prior to 24 hours (triangles) or 48 hours (circles) exposure to brefeldin A (5 μ M). B,C: Arithmetic means $\pm$ SEM ($n = 20-43$) of the maximal current (B) and the conductance (C) calculated by linear fit of I/V -curves shown in A between +130 mV and +190 mV in *Xenopus* oocytes expressing BK without (white bars) or with (black bars) additional co-expression of wild-type GSK3 β prior to (left bars), 24 hours (middle bars) or 48 hours (right bars) exposure to brefeldin A (5 μ M). *** ($p < 0.001$) indicates statistically significant difference from oocytes expressing BK alone, # ($p < 0.05$), ### ($p < 0.001$) indicates statistically significant difference from absence of brefeldin A.

was followed by a significant increase of the voltage gated current in *Xenopus* oocytes expressing the Ca²⁺-insensitive BK channel BK^{M513I+Δ899-903}. Expression of GSK3 β alone did not appreciably modify the voltage gated current, indicating that GSK3 β was not effective by modifying an endogenous channel with properties similar to BK^{M513I+Δ899-903}.

The present observations did not define the cellular mechanisms involved in the up-regulation of BK channel activity by GSK3 β . In theory the kinase could be effective by direct phosphorylation of the channel protein or by phosphorylation of proteins involved in the regulation of trafficking or function of the channels. The experiments with brefeldin A suggest that GSK3 β does not affect channel retrieval from the cell membrane.

The effect of GSK3 β was virtually abrogated by the additional co-expression of protein kinase B which is a known negative regulator of GSK3 β activity [29]. Moreover, the effect of GSK3 β was blunted by the antidepressant Lithium, a known inhibitor of GSK3 β [30]. It is tempting to speculate that effects and/or side effects of Lithium may, in part, result from disruption of GSK3 β -induced up-regulation of BK channel activity. However, the present observations do not allow safe conclusions as to the *in vivo* sensitivity of BK channels to GSK3 β or Lithium.

In theory, the observed effect of GSK3 β on BK channels could contribute to the known effect of the kinase on neuronal excitability [27]. The pleiotropic effects of large conductance Ca²⁺-activated K⁺ channels include regulation of neuronal excitability [5]. Activation of K⁺ channels is expected to counteract depolarization and thus excitation. Moreover, the observed GSK3 β sensitivity of BK channels could contribute to the impact of the kinase on cell size

[28]. K⁺ channels are pivotal molecules in the regulation of cell volume [54-56]. Stimulation of BK channel activity is expected to trigger cellular K⁺ loss and secondary Cl⁻ exit due to hyperpolarization of the cell membrane [54-56].

Conclusion

GSK3 β up-regulates BK channel activity, an effect possibly modifying cell volume and neuroexcitation.

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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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