Cellular Physiology and Biochemistry

Cell Physiol Biochem 2016;39:1031-1039
DOI: 10.1159/000447810
Published online: August 19, 2016
Accepted: June 21, 2016

Original Paper

Up-Regulation of the Large-Conductance Ca^{2+}-Activated K^+ Channel by Glycogen Synthase Kinase GSK3β

Myriam Fezai[a] Musaab Ahmed[a] Zohreh Hosseinzadeh[a,b] Florian Lang[a,c]

[a]Department of Cardiology, Vascular Medicine and Physiology, [b]Centre for Ophthalmology, Institute for Ophthalmic Research, University of Tuebingen, Tuebingen, [c]Department 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 pleotropic 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 GSK3ß which is under negative control of protein kinase B (PKB/Akt). GSK3ß is inhibited by the antidepressant Lithium. The present study thus explored whether GSK3ß modifies the activity of BK channels. ***Methods:*** cRNA encoding the Ca^{2+} insensitive BK channel mutant $BK^{M513I+\Delta 899-903}$ was injected into *Xenopus laevis* oocytes without or with additional injection of cRNA encoding wild-type GSK3ß, inactive ^{K85R}GSK3ß, or wild-type GSK3ß with wild-type PKB. K^+ channel activity was measured utilizing dual electrode voltage clamp. ***Results:*** BK channel activity in $BK^{M513I+\Delta 899-903}$ expressing oocytes was significantly increased by co-expression of GSK3ß, but not by co-expression of ^{K85R}GSK3ß. The effect of wild type GSK3ß 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 GSK3ß and in oocytes expressing BK alone. ***Conclusion:*** GSK3ß 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.

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 Tübingen, Gmelinstr. 5, D-72076 Tübingen, (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 GSK3β. The kinase is phosphorylated and down-regulated by protein kinase B (PKB/Akt) [29]. GSK3β is inhibited by the antidepressant Lithium [30].

The present study thus explored, whether GSK3β modifies the activity of BK channels. To this end, the Ca^{2+} insensitive BK channel mutant $BK^{M513I+\Delta899-903}$ was expressed in *Xenopus laevis* oocytes without or with additional expression of wild-type GSK3β, inactive mutant ^{K85R}GSK3ß or wild-type GSK3β 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 Ca^{2+}-insensitive BK channel ($BK^{M513I+\Delta899-903}$) [31, 32] (kindly provided by J Lingle), wild-type human GSK3ß [33], inactive mutant ^{K85R}GSK3ß [34], and wild-type PKB [35] were used for generation of cRNA as described previously [36-40]. The Ca^{2+}-insensitive BK mutant was utilized because the activity of wild-type BK requires an increase in the intracellular Ca^{2+} 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}GSK3ß 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 $MgCl_2$, 1.8 $CaCl_2$, 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 $CaCl_2$, 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 [52, 53].

Statistical analysis

Data are provided as means ± 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 GSK3ß modifies the activity of the large conductance Ca^{2+}-activated K^+ channels (maxi K^+ channel or BK channels). To this end, cRNA encoding Ca^{2+}-insensitive BK channel ($BK^{M513I+\Delta899-903}$) was injected into

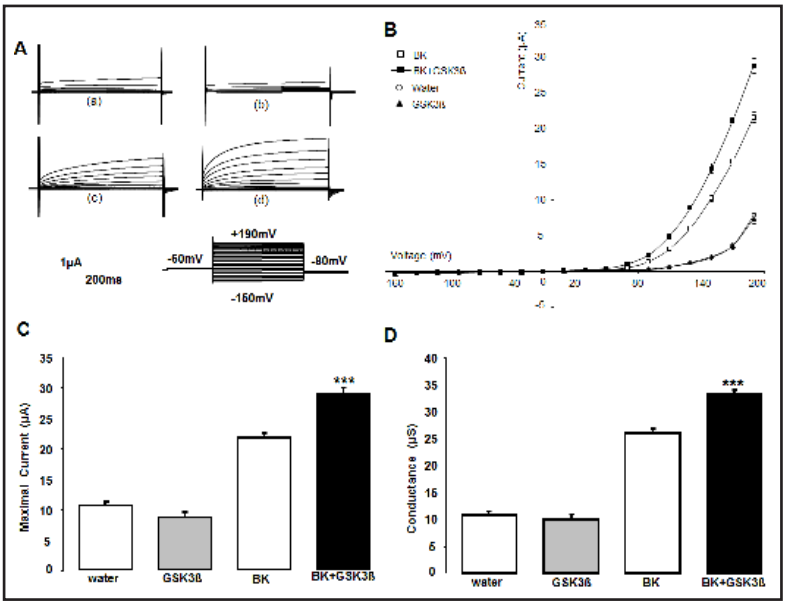

Fig. 1. Co-expression of wild-type GSK3β increases the K^+ current in BK expressing *Xenopus laevis* oocytes. A: Representative original tracings showing currents in *Xenopus* oocytes injected with water (a), expressing GSK3 alone (b) expressing BK alone (c) or expressing BK with additional co-expression of wild-type GSK3β (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 ± 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 GSK3β alone (black triangles) or expressing BK without (white rectangles) or with (black rectangles) additional co-expression of wild-type GSK3β. C,D: Arithmetic means ± 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 GSK3ß alone (grey bar) or expressing BK without (white bar) or with (black bar) additional co-expression of wild-type GSK3ß. *** (p<0.001) indicates statistically significant difference from oocytes expressing BK alone.

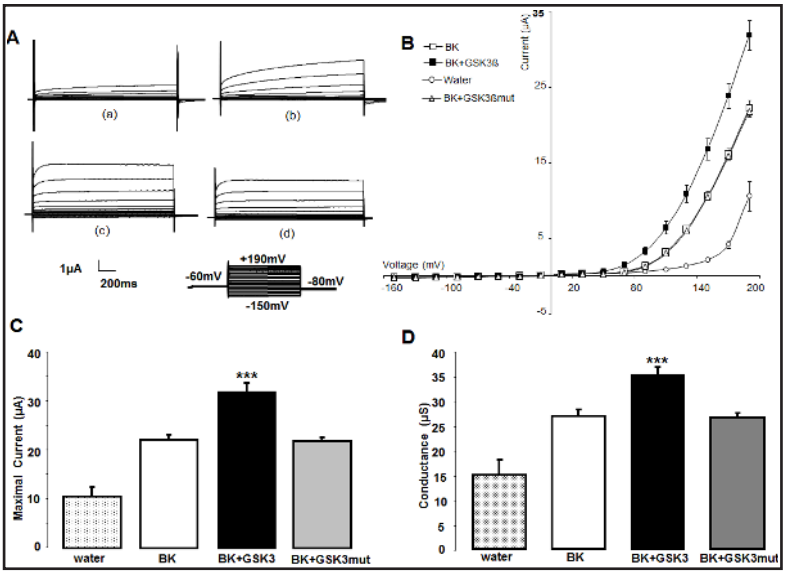

Fig. 2. The effect of GSK3b is disrupted by the inactivating mutation ^{K85R}GSK3β. A: Representative original tracings showing currents in *Xenopus* oocytes injected with water (a), expressing BK alone (b) or with additional co-expression of wild-type GSK3ß (c) or inactive ^{K85R}GSK3ß (d). The voltage 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 ± 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 GSK3ß (black rectangles), or inactive ^{K85R}GSK3β (white triangles). C,D: Arithmetic means ± 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 GSK3ß (black bar) or inactive ^{K85R}GSK3ß (grey bar). *** (p<0.001) indicates statistically significant difference from oocytes expressing BK alone.

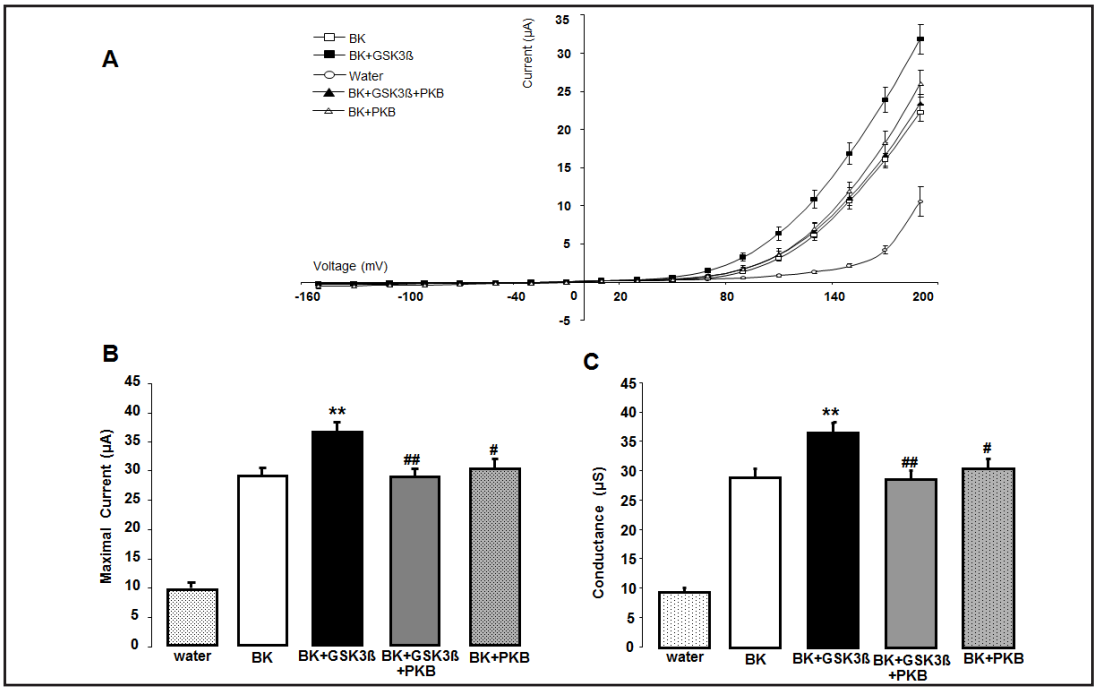

Fig. 3. The effect of GSK3ß is disrupted by additional co-expression of PKB. A: Arithmetic means ± SEM (n = 12-28) of the current (I) as a function of the potential difference across the cell membrane (V) in *Xenopus* oocytes injected with water (white circles) or expressing BK alone (white rectangles), expressing BK with additional co-expression of wild-type GSK3ß (black rectangles) or expressing BK with additional co-expression of wild-type GSK3ß and wild-type PKB (black triangles) or expressing BK with additional co-expression of PKB (white trianlges). B,C: Arithmetic means ± 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 GSK3ß (black bar) or with additional expression of both, GSK3ß 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 GSK3ß.

Xenopus laevis oocytes with or without additional injection of cRNA encoding wild-type GSK3ß or, as a negative control, inactive mutant ^{K85R}GSK3ß. The voltage-gated K^+ current was determined by dual electrode voltage clamp experiments.

As illustrated in Fig. 1, the injection of cRNA encoding $BK^{M513I+\Delta899-903}$ 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 GSK3ß was followed by a moderate but significant further increase of the voltage gated current. The injection of cRNA encoding wild-type GSK3ß alone did not significantly modify the voltage gated current (Fig. 1).

In contrast to the injection of cRNA encoding wild-type GSK3ß, the additional injection of cRNA encoding the inactive mutant ^{K85R}GSK3ß did not significantly modify the voltage gated current in $BK^{M513I+\Delta899-903}$ expressing *Xenopus* oocytes (Fig. 2).

In order to test whether the effect of GSK3ß could be modified by protein kinase B (PKB/Akt), wild-type GSK3ß was co-expressed with $BK^{M513I+\Delta899-903}$ 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 $BK^{M513I+\Delta899-903}$ together with wild-type GSK3β + wild-type PKB and in oocytes expressing $BK^{M513I+\Delta899-903}$ alone.

A further series of experiments explored whether the effect of GSK3β could be modified by the antidepressant Lithium. As illustrated in Fig. 4, a 24 hours Lithium-exposure of

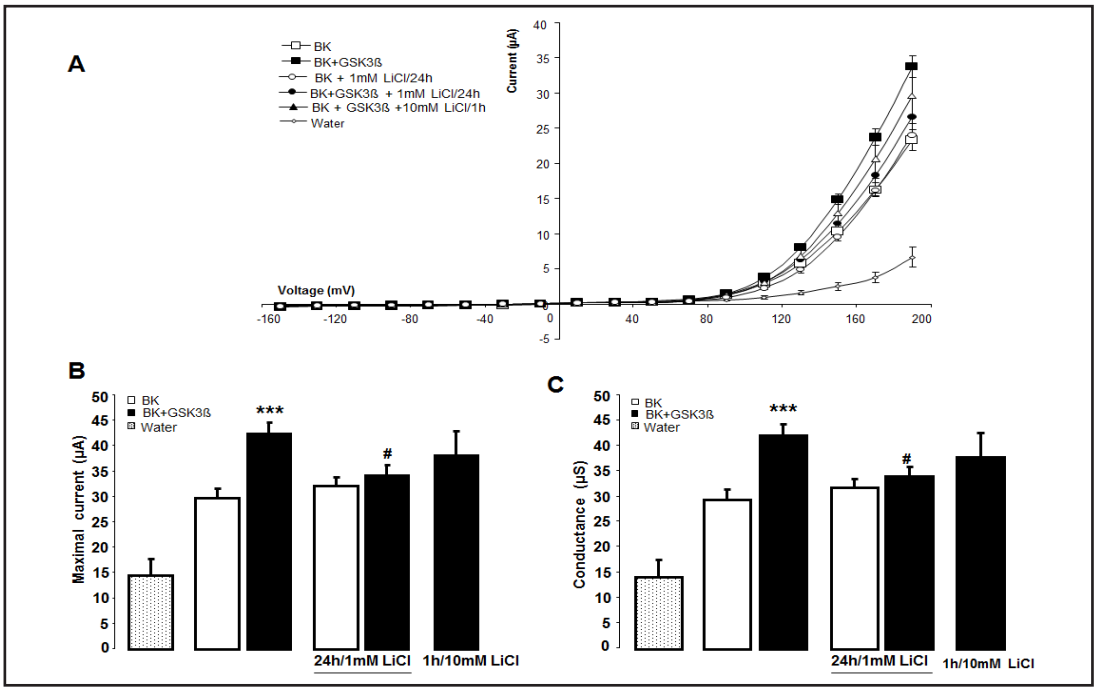

Fig. 4. The effect of GSK3ß is blunted by antidepressant Lithium. A: Arithmetic means ± SEM (n = 12-51) of the current (I) as a function of the potential difference across the cell membrane (V) in *Xenopus* oocytes injected with water (white diamond) or expressing BK alone (white symboles), or expressing BK with additional co-expression of wild-type GSK3ß (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 ± 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 GSK3ß (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 GSK3ß without Lithium treatment.

Xenopus oocytes co-expressing $BK^{M513I+\Delta899-903}$ together with wild-type GSK3β was followed by a significant decline of the voltage gated current to values not significantly higher than the current in *Xenopus* oocytes expressing $BK^{M513I+\Delta899-903}$ alone. A 24 hours of Lithium exposure did not significantly modify the current in oocytes expressing $BK^{M513I+\Delta899-903}$ alone. An 1 hour incubation in 10 mM Lithium did not decrease significantly the K^+ current in oocytes co-expressing $BK^{M513I+\Delta899-903}$ with GSK3β.

In order to test whether the co-expression of wild-type GSK3β stabilizes $BK^{M513I+\Delta899-903}$ in the cell membrane, the insertion of new channel protein into the cell membrane was prevented by brefeldin A (5 µM). 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 $BK^{M513I+\Delta899-903}$ alone and in *Xenopus* oocytes expressing $BK^{M513I+\Delta899-903}$ with additional co-expression of wild-type GSK3β. Thus, GSK3β did not appreciably modify the channel stability in the cell membrane.

Discussion

The present study uncovers a novel function of glycogen synthase kinase GSK3ß, i.e. the up-regulation of large conductance Ca^{2+}-activated K^+ channels (maxi K^+ channel or BK channels). Co-expression of the wild-type GSK3ß but not of the inactive mutant ^{K85R}GSK3ß

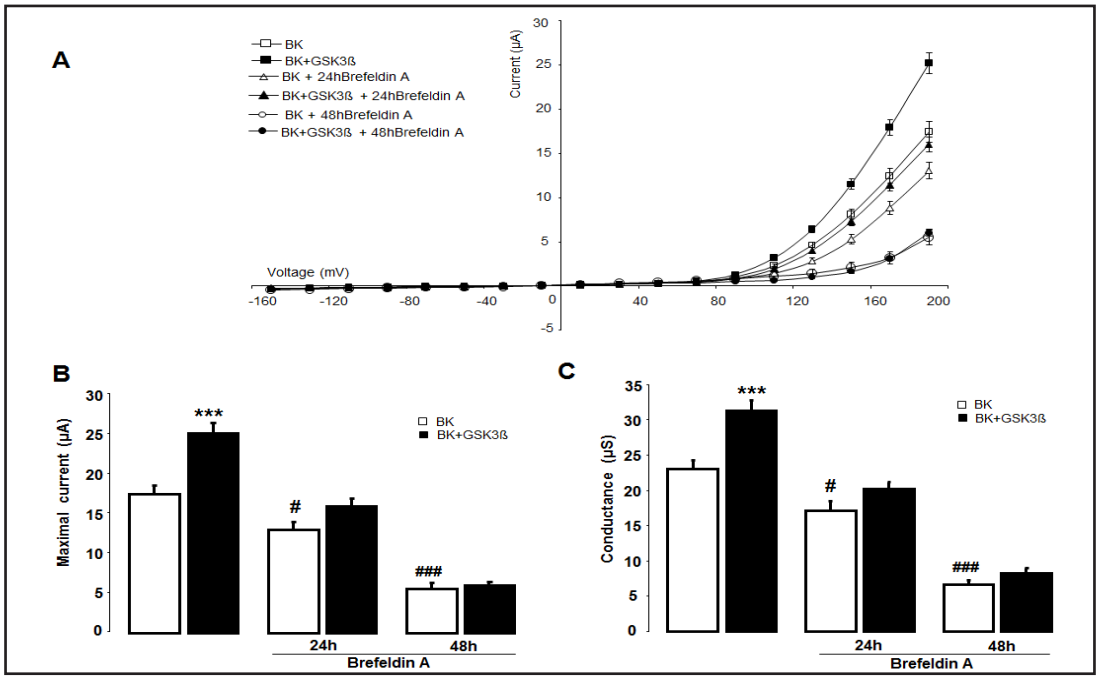

Fig. 5. Decay of current following brefeldin A treatment in *Xenopus laevis* oocytes expressing BK alone or BK together with GSK3ß. A: Arithmetic means ± SEM (n = 20-43) of the current (I) as a function of the potential difference across the cell membrane (V) in *Xenopus* oocytes expressing BK alone (white rectangles) or expressing BK with additional co-expression of wild-type GSK3ß (black rectangles) prior to 24 hours (triangles) or 48 hours (circles) exposure to brefeldin A (5 µM). B,C: Arithmetic means ± 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 GSK3ß prior to (left bars), 24 hours (middle bars) or 48 hours (right bars) exposure to brefeldin A (5 µM). *** (p<0.001) indicates statistically significant difference from oocytes expressing BK alone, #(p<0.05), ###(p<0.001) indicates statistically significant difference from absencence of brefeldin A.

was followed by a significant increase of the voltage gated current in *Xenopus* oocytes expressing the Ca^{2+}-insensitive BK channel $BK^{M513I+\Delta899-903}$. Expression of GSK3ß alone did not appreciably modify the voltage gated current, indicating that GSK3ß was not effective by modifying an endogenous channel with properties similar to $BK^{M513I+\Delta899-903}$.

The present observations did not define the cellular mechanisms involved in the up-regulation of BK channel activity by GSK3ß. 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 GSK3ß does not affect channel retrieval from the cell membrane.

The effect of GSK3ß was virtually abrogated by the additional co-expression of protein kinase B which is a known negative regulator of GSK3ß activity [29]. Moreover, the effect of GSK3ß was blunted by the antidepressant Lithium, a known inhibitor of GSK3ß [30]. It is tempting to speculate that effects and/or side effects of Lithium may, in part, result from disruption of GSK3ß-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 GSK3ß or Lithium.

In theory, the observed effect of GSK3ß on BK channels could contribute to the known effect of the kinase on neuronal excitability [27]. The pleotropic effects of large conductance Ca^{2+}-activated K^+ channels include regulation of neuronal excitability [5]. Activation of K^+ channels is expected to counteract depolarization and thus excitation. Moreover, the observed GSK3β 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

GSK3β up-regulates BK channel activity, an effect possibly modifying cell volume and neuroexcitation.

Acknowledgements

The authors acknowledge the meticulous preparation of the manuscript by Lejla Subasic and Tanja Loch, and technical support by Elfriede Faber. This study was supported by the Deutsche Forschungsgemeinschaft and Open Access Publishing Fund of Tuebingen University.

Disclosure Statement

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

References

1 Donnelier J, Braun ST, Dolzhanskaya N, Ahrendt E, Braun AP, Velinov M, Braun JE: Increased Expression of the Large Conductance, Calcium-Activated K+ (BK) Channel in Adult-Onset Neuronal Ceroid Lipofuscinosis. PLoS One 2015;10:e0125205.
2 Dopico AM, Bukiya AN, Martin GE: Ethanol modulation of mammalian BK channels in excitable tissues: molecular targets and their possible contribution to alcohol-induced altered behavior. Front Physiol 2014;5:466.
3 Weiss N: Should they team up to make your brain clock? Neurobiol Aging 2015;36:2184-2185.
4 Yan XH, Guo XY, Jiao FY, Liu X, Liu Y: Activation of large-conductance Ca-activated K channels inhibits glutamate-induced oxidative stress through attenuating ER stress and mitochondrial dysfunction. Neurochem Int 2015;10.1016/j.neuint.2015.07.004
5 Bentzen BH, Olesen SP, Ronn LC, Grunnet M: BK channel activators and their therapeutic perspectives. Front Physiol 2014;5:389.
6 Moss CR, 2nd, Gilbert CA, Gabriel SA, Gu Q: Protease-activated receptor-2 inhibits BK channel activity in bronchopulmonary sensory neurons. Neurosci Lett 2015;589:13-18.
7 Springer SJ, Burkett BJ, Schrader LA: Modulation of BK channels contributes to activity-dependent increase of excitability through MTORC1 activity in CA1 pyramidal cells of mouse hippocampus. Front Cell Neurosci 2014;8:451.
8 Wang L, Kang H, Li Y, Shui Y, Yamamoto R, Sugai T, Kato N: Cognitive recovery by chronic activation of the large-conductance calcium-activated potassium channel in a mouse model of Alzheimer's disease. Neuropharmacology 2015;92:8-15.
9 N'Gouemo P: Targeting BK (big potassium) channels in epilepsy. Expert Opin Ther Targets 2011;15:1283-1295.
10 Peers C, Boyle JP: Oxidative modulation of K+ channels in the central nervous system in neurodegenerative diseases and aging. Antioxid Redox Signal 2015;22:505-521.
11 Sun P, Zhang Q, Zhang Y, Wang F, Wang L, Yamamoto R, Sugai T, Kato N: Fear conditioning suppresses large-conductance calcium-activated potassium channels in lateral amygdala neurons. Physiol Behav

2015;138:279-284.
12 Vandael DH, Marcantoni A, Mahapatra S, Caro A, Ruth P, Zuccotti A, Knipper M, Carbone E: Ca(v)1.3 and BK channels for timing and regulating cell firing. Mol Neurobiol 2010;42:185-198.
13 Lee US, Cui J: BK channel activation: structural and functional insights. Trends Neurosci 2010;33:415-423.
14 Liu J, Ye J, Zou X, Xu Z, Feng Y, Zou X, Chen Z, Li Y, Cang Y: CRL4A(CRBN) E3 ubiquitin ligase restricts BK channel activity and prevents epileptogenesis. Nat Commun 2014;5:3924.
15 Lorca RA, Stamnes SJ, Pillai MK, Hsiao JJ, Wright ME, England SK: N-terminal isoforms of the large-conductance Ca(2)(+)-activated K(+) channel are differentially modulated by the auxiliary beta1-subunit. J Biol Chem 2014;289:10095-10103.
16 Mulholland PJ, Hopf FW, Bukiya AN, Martin GE, Liu J, Dopico AM, Bonci A, Treistman SN, Chandler LJ: Sizing up ethanol-induced plasticity: the role of small and large conductance calcium-activated potassium channels. Alcohol Clin Exp Res 2009;33:1125-1135.
17 Ahrendt E, Kyle B, Braun AP, Braun JE: Cysteine string protein limits expression of the large conductance, calcium-activated K(+) (BK) channel. PLoS One 2014;9:e86586.
18 Cox N, Toro B, Pacheco-Otalora LF, Garrido-Sanabria ER, Zarei MM: An endoplasmic reticulum trafficking signal regulates surface expression of beta4 subunit of a voltage- and Ca(2)(+)-activated K(+) channel. Brain Res 2014;1553:12-23.
19 Hou S, Heinemann SH, Hoshi T: Modulation of BKCa channel gating by endogenous signaling molecules. Physiology (Bethesda) 2009;24:26-35.
20 Ksiazek A, Ladno W, Szulczyk B, Grzelka K, Szulczyk P: Properties of BK-type Ca(+) (+)-dependent K(+) channel currents in medial prefrontal cortex pyramidal neurons in rats of different ages. Front Cell Neurosci 2013;7:185.
21 Ghatta S, Nimmagadda D, Xu X, O'Rourke ST: Large-conductance, calcium-activated potassium channels: structural and functional implications. Pharmacol Ther 2006;110:103-116.
22 Lawson K: Is there a role for potassium channel openers in neuronal ion channel disorders? Expert Opin Investig Drugs 2000;9:2269-2280.
23 Vergara C, Latorre R, Marrion NV, Adelman JP: Calcium-activated potassium channels. Curr Opin Neurobiol 1998;8:321-329.
24 Fernandez-Fernandez JM, Andrade YN, Arniges M, Fernandes J, Plata C, Rubio-Moscardo F, Vazquez E, Valverde MA: Functional coupling of TRPV4 cationic channel and large conductance, calcium-dependent potassium channel in human bronchial epithelial cell lines. Pflugers Arch 2008;457:149-159.
25 Lionetto MG, Rizzello A, Giordano ME, Maffia M, De Nuccio F, Nicolardi G, Hoffmann EK, Schettino T: Molecular and functional expression of high conductance Ca 2+ activated K+ channels in the eel intestinal epithelium. Cell Physiol Biochem 2008;21:373-384.
26 Soto D, Comes N, Ferrer E, Morales M, Escalada A, Pales J, Solsona C, Gual A, Gasull X: Modulation of aqueous humor outflow by ionic mechanisms involved in trabecular meshwork cell volume regulation. Invest Ophthalmol Vis Sci 2004;45:3650-3661.
27 Kim MS, Shutov LP, Gnanasekaran A, Lin Z, Rysted JE, Ulrich JD, Usachev YM: Nerve growth factor (NGF) regulates activity of nuclear factor of activated T-cells (NFAT) in neurons via the phosphatidylinositol 3-kinase (PI3K)-Akt-glycogen synthase kinase 3beta (GSK3beta) pathway. J Biol Chem 2014;289:31349-31360.
28 Gruson D, Ginion A, Decroly N, Lause P, Vanoverschelde JL, Ketelslegers JM, Bertrand L, Thissen JP: Urocortin-induced cardiomyocytes hypertrophy is associated with regulation of the GSK-3beta pathway. Heart Vessels 2012;27:202-207.
29 Valvezan AJ, Klein PS: GSK-3 and Wnt Signaling in Neurogenesis and Bipolar Disorder. Front Mol Neurosci 2012;5:1.
30 O'Brien WT, Klein PS: Validating GSK3 as an in vivo target of lithium action. Biochem Soc Trans 2009;37:1133-1138.
31 Sausbier M, Hu H, Arntz C, Feil S, Kamm S, Adelsberger H, Sausbier U, Sailer CA, Feil R, Hofmann F, Korth M, Shipston MJ, Knaus HG, Wolfer DP, Pedroarena CM, Storm JF, Ruth P: Cerebellar ataxia and Purkinje cell dysfunction caused by Ca2+-activated K+ channel deficiency. Proc Natl Acad Sci U S A 2004;101:9474-9478.
32 Xia XM, Zeng X, Lingle CJ: Multiple regulatory sites in large-conductance calcium-activated potassium channels. Nature 2002;418:880-884.

33 Rexhepaj R, Dermaku-Sopjani M, Gehring EM, Sopjani M, Kempe DS, Foller M, Lang F: Stimulation of electrogenic glucose transport by glycogen synthase kinase 3. Cell Physiol Biochem 2010;26:641-646.
34 Sun H, Jiang YJ, Yu QS, Luo CC, Zou JW: Effect of mutation K85R on GSK-3beta: Molecular dynamics simulation. Biochem Biophys Res Commun 2008;377:962-965.
35 Munoz C, Almilaji A, Setiawan I, Foller M, Lang F: Up-regulation of the inwardly rectifying K(+) channel Kir2.1 (KCNJ2) by protein kinase B (PKB/Akt) and PIKfyve. J Membr Biol 2013;246:189-197.
36 Ahmed M, Salker MS, Elvira B, Umbach AT, Fakhri H, Saeed AM, Shumilina E, Hosseinzadeh Z, Lang F: SPAK Sensitive Regulation of the Epithelial Na Channel ENaC. Kidney Blood Press Res 2015;40:335-343.
37 Warsi J, Elvira B, Bissinger R, Shumilina E, Hosseinzadeh Z, Lang F: Downregulation of peptide transporters PEPT1 and PEPT2 by oxidative stress responsive kinase OSR1. Kidney Blood Press Res 2014;39:591-599.
38 Fezai M, Ahmed M, Hosseinzadeh Z, Elvira B, Lang F: SPAK and OSR1 Sensitive Kir2.1 K+ Channels. Neurosignals 2015;23:20-33.
39 Warsi J, Hosseinzadeh Z, Elvira B, Bissinger R, Shumilina E, Lang F: Regulation of ClC-2 activity by SPAK and OSR1. Kidney Blood Press Res 2014;39:378-387.
40 Elvira B, Warsi J, Fezai M, Munoz C, Lang F: SPAK and OSR1 Sensitive Cell Membrane Protein Abundance and Activity of KCNQ1/E1 K+ Channels. Cell Physiol Biochem 2015;37:2032-2042.
41 Foller M, Jaumann M, Dettling J, Saxena A, Pakladok T, Munoz C, Ruth P, Sopjani M, Seebohm G, Ruttiger L, Knipper M, Lang F: AMP-activated protein kinase in BK-channel regulation and protection against hearing loss following acoustic overstimulation. FASEB J 2012;26:4243-4253.
42 Hosseinzadeh Z, Almilaji A, Honisch S, Pakladok T, Liu G, Bhavsar SK, Ruth P, Shumilina E, Lang F: Upregulation of the large conductance voltage- and Ca2+-activated K+ channels by Janus kinase 2. Am J Physiol Cell Physiol 2014;306:C1041-1049.
43 Almilaji A, Sopjani M, Elvira B, Borras J, Dermaku-Sopjani M, Munoz C, Warsi J, Lang UE, Lang F: Upregulation of the creatine transporter Slc6A8 by Klotho. Kidney Blood Press Res 2014;39:516-525.
44 Hosseinzadeh Z, Honisch S, Schmid E, Jilani K, Szteyn K, Bhavsar S, Singh Y, Palmada M, Umbach AT, Shumilina E, Lang F: The Role of Janus Kinase 3 in the Regulation of Na(+)/K(+) ATPase under Energy Depletion. Cell Physiol Biochem 2015;36:727-740.
45 Fezai M, Elvira B, Borras J, Ben-Attia M, Hoseinzadeh Z, Lang F: Negative regulation of the creatine transporter SLC6A8 by SPAK and OSR1. Kidney Blood Press Res 2014;39:546-554.
46 Fezai M, Elvira B, Warsi J, Ben-Attia M, Hosseinzadeh Z, Lang F: Up-Regulation of Intestinal Phosphate Transporter NaPi-IIb (SLC34A2) by the Kinases SPAK and OSR1. Kidney Blood Press Res 2015;40:555-564.
47 Warsi J, Dong L, Elvira B, Salker MS, Shumilina E, Hosseinzadeh Z, Lang F: SPAK dependent regulation of peptide transporters PEPT1 and PEPT2. Kidney Blood Press Res 2014;39:388-398.
48 Warsi J, Singh Y, Elvira B, Hosseinzadeh Z, Lang F: Regulation of Large Conductance Voltage-and Ca2+-Activated K+ Channels by the Janus Kinase JAK3. Cell Physiol Biochem 2015;37:297-305.
49 Alesutan I, Voelkl J, Stockigt F, Mia S, Feger M, Primessnig U, Sopjani M, Munoz C, Borst O, Gawaz M, Pieske B, Metzler B, Heinzel F, Schrickel JW, Lang F: AMP-activated protein kinase alpha1 regulates cardiac gap junction protein connexin 43 and electrical remodeling following pressure overload. Cell Physiol Biochem 2015;35:406-418.
50 Hosseinzadeh Z, Sopjani M, Pakladok T, Bhavsar SK, Lang F: Downregulation of KCNQ4 by Janus kinase 2. J Membr Biol 2013;246:335-341.
51 Almilaji A, Honisch S, Liu G, Elvira B, Ajay SS, Hosseinzadeh Z, Ahmed M, Munoz C, Sopjani M, Lang F: Regulation of the voltage gated K channel Kv1.3 by recombinant human klotho protein. Kidney Blood Press Res 2014;39:609-622.
52 Fezai M, Warsi J, Lang F: Regulation of the Na+,Cl- coupled Creatine Transporter CreaT (SLC6A8) by the Janus Kinase JAK3. Neurosignals 2015;23:11-19.
53 Warsi J, Abousaab A, Lang F: Up-Regulation of Excitatory Amino Acid Transporters EAAT1 and EAAT2 by ß-Klotho. Neurosignals 2015;23:59-70.
54 Hoffmann EK: Ion channels involved in cell volume regulation: effects on migration, proliferation, and programmed cell death in non adherent EAT cells and adherent ELA cells. Cell Physiol Biochem 2011;28:1061-1078.
55 Hoffmann EK, Lambert IH, Pedersen SF: Physiology of cell volume regulation in vertebrates. Physiol Rev 2009;89:193-277.
56 Lang F: Mechanisms and significance of cell volume regulation. J Am Coll Nutr 2007;26:S613-S623.