Original Paper

Up-Regulation of Voltage Gated K⁺ Channels Kv1.3 and Kv1.5 by Protein Kinase PKB/Akt

Jamshed Warsi^{a,b} Myriam Fezai^a Mireia Fores^a Bernat Elvira^a Florian Lang^a

^aDepartment of Physiology I, University of Tübingen, Germany; ^bDepartment of Physiology, University of Sindh, Jamshoro, Pakistan

Key Words

Voltage gated K⁺ channels • Protein kinase B • PKB/Akt • Chemiluminescence • Brefeldin

Abstract

Background: The voltage gated K⁺ channels Kv1.3 and Kv1.5 contribute to the orchestration of cell proliferation. Kinases participating in the regulation of cell proliferation include protein kinase B (PKB/Akt). The present study thus explored whether PKB/Akt modifies the abundance and function of Kv1.3 and Kv1.5. **Methods:** Kv1.3 or Kv1.5 was expressed in *Xenopus laevis* oocytes with or without wild-type PKB/Akt, constitutively active ^{T308D/S473D}PKB/Akt or inactive ^{T308A/S473A}PKB/Akt. The channel activity was quantified utilizing dual electrode voltage clamp. Moreover, HA-tagged Kv1.5 protein was determined utilizing chemiluminescence. **Results:** Voltage gated K⁺ currents were observed in Kv1.3 or Kv1.5 expressing oocytes but not in water-injected oocytes or in oocytes expressing PKB/Akt alone. Co-expression of PKB/Akt or ^{T308D/S473D}PKB/Akt, but not co-expression of ^{T308A/S473A}PKB/Akt significantly increased the voltage gated current in both Kv1.3 and Kv1.5 expressing oocytes. As shown for Kv1.5, co-expression of PKB/Akt enhanced the channel protein abundance in the cell membrane. In Kv1.5 expressing oocytes voltage gated current decreased following inhibition of carrier insertion by brefeldin A (5 μM) to similarly low values in the absence and presence of PKB/Akt, suggesting that PKB/Akt stimulated carrier insertion into rather than inhibiting carrier retrieval from the cell membrane. **Conclusion:** PKB/Akt up-regulates both, Kv1.3 and Kv1.5 K⁺ channels.

© 2015 The Author(s)
Published by S. Karger AG, Basel

Introduction

The pleiotropic functions of protein kinase B (PKB/Akt) include the regulation of cell survival and cell proliferation [1-9]. Cell proliferation is further tuned by regulation of ion

J. Warsi and M. Fezai contributed equally thus share first authorship

Prof. Dr. Florian Lang
and Bernat Elvira

Department of Physiology, University of Tübingen, Gmelinstr. 5, D-72076 Tübingen, (Germany)
E-Mail florian.lang@uni-tuebingen.de, E-Mail bernat.elvira@gmail.com

channels [10-14]. As a matter of fact, PKB/Akt has previously been shown to stimulate several ion channels, such as Ca^{2+} channels [15, 16], CFTR Cl^- channels [17], inwardly rectifying K^+ channels [18] as well as delayed rectifying hERG [19] and KCNQ1 [20] K^+ channels.

The present study explored, whether PKB/Akt regulates the voltage gated K^+ channels Kv1.3/Kv1.5, which are both implicated in the regulation of cell proliferation [21-26]. Agonists contributing to the regulation Kv1.3 or Kv1.5 channels include insulin [27-29] and growth factors such as insulin like growth factor IGF1 [30] epidermal growth factor [31] and brain-derived neurotrophic factor (BDNF) [27, 32, 33]. Mechanisms mediating insulin and growth factor dependent regulation of Kv1.3 and Kv1.5 include tyrosine kinases [34, 35] and PI3 kinase signalling with activation of the serum & glucocorticoid inducible kinase and PKB/Akt isoforms [30, 36].

In order to analyse the effect of PKB/Akt on Kv1.3/Kv1.5 channels, voltage gated current was estimated utilizing the dual-electrode voltage-clamp in *Xenopus laevis* oocytes expressing Kv1.3 or Kv1.5 alone or together with wild-type PKB/Akt, constitutively active ^{T308D/S473D}PKB/Akt or inactive ^{T308A/S473A}PKB/Akt.

As a result, co-expression of PKB/Akt or of ^{T308D/S473D}PKB/Akt, but not of ^{T308A/S473A}PKB/Akt enhanced voltage gated current in Kv1.3 or Kv1.5 expressing *Xenopus* oocytes, an effect at least partially due to enhanced insertion of channel protein into the cell membrane.

Materials and Methods

Constructs

The cDNA constructs encoding wild-type PKB/Akt [17], constitutively active ^{T308D/S473D}PKB/Akt and inactive ^{T308A/S473A}PKB/Akt [37], wild-type Kv1.3 (mouse) [38], and/or wild type Kv1.5 or/and Kv1.5-HA (mouse) [39] were used for generation of cRNA as described previously [40, 41]

Electrophysiological recordings in *Xenopus* oocytes

Xenopus laevis oocytes were prepared as previously described [42]. cRNA encoding PKB/Akt, ^{T308D/S473D}PKB/Akt or ^{T308A/S473A}PKB/Akt (10 ng) and cRNA encoding Kv1.3 or Kv1.5 (2.5 ng) was injected on the same day after the preparation of the *Xenopus* oocytes. All experiments were performed at room temperature (about 22° C) 3 days after the injection [19]. Two-electrode voltage-clamp recordings were performed at a holding potential of -100 mV. The currents were recorded following 2 second depolarizing pulses ranging from -80 to +50 mV in 10 mV and 20 s increments for Kv1.5 or 15 s increments for Kv1.3. The data were filtered at 2 kHz and recorded with a Digidata 1322A A/D-D/A converter and Clampex.9.2 software for data acquisition (Axon Instruments). The analysis of the data was performed with Clampfit 9.2 (Axon Instruments) software [43, 44]. The oocytes were maintained at 17°C in ND96 solution containing: 88.5 mM NaCl, 2 mM KCl, 1 mM MgCl_2 , 1.8 mM CaCl_2 , 5 mM HEPES; Tetracycline (50 mg/l), Ciprofloxacin (1.6 mg/l), Refobacin (100 mg/l) and Theophyllin (90 mg/l) as well as sodium pyruvate (5 mM) were added to the ND96; pH was adjusted to 7.4 by addition of NaOH. The control superfusate (ND96) contained 93.5 mM NaCl, 2 mM KCl, 1.8 mM CaCl_2 , 1 mM MgCl_2 and 5 mM HEPES, pH was adjusted to 7.4 by addition of NaOH (2.5 mM) [45]. The flow rate of the superfusion was 20 ml/min, and a complete exchange of the bath solution was reached within about 10 s [46, 47].

Detection of Kv1.5 cell surface expression by chemiluminescence

To determine HA-Kv1.5 cell surface expression by chemiluminescence [48], oocytes were incubated with mouse monoclonal anti-HA antibody conjugated to horseradish peroxidase (1:1000, Miltenyi Biotec Inc, CA, USA). Individual oocytes were placed in 96 well plates with 20 µl of SuperSignal ELISA Femto Maximum Sensitivity Substrate (Pierce, Rockford, IL, USA) and chemiluminescence of single oocytes was quantified in a luminometer (Walter Wallac 2 plate reader, Perkin Elmer, Juegesheim, Germany) by integrating the signal over a period of 1 sec. Results display normalized relative light units.

Statistical analysis

Data are provided as arithmetic means $\pm$ SEM; n represents the number of oocytes investigated. All oocyte experiments were repeated with at least three batches of oocytes and in all repetitions qualitatively

similar data were obtained. All data were tested for significance by using ANOVA (Tukey or Kruskal-Wallis test) or t-test, as appropriate. Results with $p < 0.05$ were considered statistically significant.

Results

In order to define the impact of protein kinase B (PKB/Akt) on the voltage gated K^+ channels Kv1.3 and Kv1.5, cRNA encoding the channels was injected into *Xenopus laevis* oocytes with or without additional injection of cRNA encoding PKB/Akt. Voltage gated K^+ channel current was then determined with the dual-electrode voltage-clamp technique. As illustrated in Fig. 1, no appreciable currents were observed in water-injected *Xenopus* oocytes, indicating that expression of endogenous voltage gated K^+ channels was minimal. Injection of cRNA encoding Kv1.3 was, however, followed by the appearance of robust voltage gated current (Fig. 1). Co-expression of wild type PKB/Akt was followed by a marked increase of the voltage gated current by $17 \pm 2\%$ (Fig. 1).

The effect of wild type PKB/Akt was mimicked by the constitutively active $T308D/S473D$ PKB/Akt mutant, which increased the current by $17 \pm 2\%$. In contrast, the inactive mutant $T308A/S473A$ PKB/Akt did not significantly modify the voltage gated current in Kv1.3 expressing *Xenopus* oocytes (Fig. 2).

Fig. 1. Co-expression of wild type PKB/Akt increased the K^+ current in Kv1.3-expressing *Xenopus laevis* oocytes. A: Representative original tracings showing currents in *Xenopus* oocytes injected with water (a), expressing Kv1.3 alone (b) or expressing Kv1.3 with additional co-expression of wild-type PKB/Akt (c). The voltage protocol is shown (not to scale). Currents were activated by depolarization from -80 to +50 mV in 15 second increments of 10 mV steps from a holding potential of -100 mV. B: Arithmetic means $\pm$ SEM ($n = 15 - 30$) 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 Kv1.3 without (white squares) or with (white triangles) additional co-expression of wild-type PKB/Akt. C: Arithmetic means $\pm$ SEM ($n = 15 - 30$) of the conductance calculated by linear fit of I/V -curves shown in B between 20 mV and 50 mV in *Xenopus* oocytes injected with water (dotted bar), or expressing Kv1.3 without (white bar) or with (black bar) additional co-expression of wild-type PKB/Akt. **($p < 0.01$) indicates statistically significant difference from oocytes expressing Kv1.3 alone.

Fig. 2. Co-expression of constitutively active $T308D/S473D$ PKB/Akt but not of inactive $T308A/S473A$ PKB/Akt increased the K^+ current in Kv1.3-expressing *Xenopus laevis* oocytes. **A:** Representative original tracings showing currents in *Xenopus* oocytes injected with water (a), expressing Kv1.3 alone (b), expressing Kv1.3 with additional co-expression of constitutively active $T308D/S473D$ PKB/Akt (c) or expressing Kv1.3 with additional co-expression of inactive $T308A/S473A$ PKB/Akt (d). The voltage protocol is shown (not to scale). Currents were activated by depolarization from -80 to +50 mV in 15 second increments of 10 mV steps from a holding potential of -100 mV. **B:** Arithmetic means $\pm$ SEM ($n = 12 - 19$) of the current (I) as a function of the potential difference across the cell membrane (V) in *Xenopus* oocytes injected with water (black circles) or expressing Kv1.3 without (white squares) or with additional co-expression of constitutively active $T308D/S473D$ PKB/Akt (white triangles) or of inactive $T308A/S473A$ PKB/Akt (white circles). **C:** Arithmetic means $\pm$ SEM ($n = 12 - 19$) of the conductance calculated by linear fit of I/V-curves shown in B between 20 mV and 50 mV in *Xenopus* oocytes injected with water (dotted bar), or expressing Kv1.3 without (white bar) or with additional co-expression of constitutively active $T308D/S473D$ PKB/Akt (black bar) or of inactive $T308A/S473A$ PKB/Akt (grey bar). * ($p < 0.05$) indicates statistically significant difference from oocytes expressing Kv1.3 alone.

Similar observations were made in *Xenopus* oocytes expressing Kv1.5. Injection of cRNA encoding Kv1.5 was again followed by the appearance of robust voltage gated currents (Fig. 3), which were again markedly increased (by $11 \pm 1\%$) following co-expression of wild type PKB/Akt (Fig. 3). The expression of PKB/Akt alone, however, failed to generate a voltage gated current.

As illustrated in Fig. 4, the effect of wild type PKB/Akt was mimicked by the constitutively active $T308D/S473D$ PKB/Akt mutant, which increased the current by $27 \pm 1\%$. In contrast, the current was not significantly modified by the inactive mutant $T308A/S473A$ PKB/Akt (Fig. 4).

The enhanced channel activity could have resulted from increased channel protein abundance in the plasma membrane. To explore this possibility, chemiluminescence experiments were performed. As illustrated in Fig. 5, the co-expression of PKB/Akt resulted in a significant increase (by $32 \pm 10\%$) of the Kv1.5-HA channel protein abundance within the oocyte membrane.

In theory, PKB/Akt could up-regulate Kv1.5 by fostering insertion of channel protein into the cell membrane or by delaying retrieval of channel protein from the cell membrane.

Fig. 3. Co-expression of wild type PKB/Akt increased the K^+ current in Kv1.5-expressing *Xenopus laevis* oocytes. A: Representative original tracings showing currents in *Xenopus* oocytes injected with water (a), expressing PKB/Akt alone (b), expressing Kv1.5 alone (c) or expressing Kv1.5 with additional co-expression of wild-type PKB/Akt (d). The voltage protocol is shown (not to scale). Currents were activated by depolarization from -80 to +50 mV in 20 second increments of 10 mV steps from a holding potential of -100 mV. B: Arithmetic means $\pm$ SEM ($n = 23 - 47$) of the current (I) as a function of the potential difference across the cell membrane (V) in *Xenopus* oocytes injected with water (black circles), or expressing PKB/Akt alone (white circles) or expressing Kv1.5 without (white squares) or with (white triangles) additional co-expression of wild-type PKB/Akt. C: Arithmetic means $\pm$ SEM ($n = 23 - 47$) of the conductance calculated by linear fit of I/V -curves shown in B between 20 mV and 50 mV in *Xenopus* oocytes injected with water (dotted bar), or expressing PKB/Akt alone (striped bar), or expressing Kv1.5 without (white bar) or with (black bar) additional co-expression of wild-type PKB/Akt. * ($p < 0.05$) indicates statistically significant difference from oocytes expressing Kv1.5 alone.

To discriminate between these two possibilities, Kv1.5-expressing *Xenopus* oocytes were treated with 5 μ M brefeldin A, an inhibitor of protein insertion into the cell membrane. As shown in Fig. 6, brefeldin A decreased the voltage gated current to similar low values in oocytes expressing Kv1.5 alone and in oocytes expressing Kv1.5 together with PKB/Akt. Apparently, retrieval of carrier protein from the cell membrane was not significantly different between *Xenopus* oocytes expressing Kv1.5 alone and *Xenopus* oocytes expressing Kv1.5 together with PKB/Akt. Therefore, PKB/Akt up-regulated Kv1.5 channels most likely by stimulating channel insertion into the cell membrane.

Discussion

The present study reveals a novel function of PKB/Akt, i.e. the up-regulation of the voltage gated K^+ channels Kv1.3 and Kv1.5. The effect was mimicked by the constitutively active T308D/S473D PKB/Akt, but not by the inactive mutant T308A/S473A PKB/Akt. The experiments utilizing brefeldin A suggest that PKB/Akt stimulates insertion of channel protein into the cell membrane rather than delaying retrieval of channel protein from the cell membrane.

The regulation of voltage gated K^+ channels could, at least in theory, impact on cell volume [49]. K^+ exit through K^+ channels generates a cell-negative potential difference across the cell membrane driving Cl^- exit. The up-regulation of K^+ channels is expected to hyperpolarize the

Fig. 4. Co-expression of constitutively active $T308D/S473D$ PKB/Akt but not of inactive $T308A/S473A$ PKB/Akt increased the K^+ current in Kv1.5-expressing *Xenopus laevis* oocytes. A: Representative original tracings showing currents in *Xenopus* oocytes injected with water (a), expressing Kv1.5 alone (b) or expressing Kv1.5 with additional co-expression of constitutively active $T308D/S473D$ PKB/Akt (c) or expressing Kv1.5 with additional co-expression of inactive $T308A/S473A$ PKB/Akt (d). The voltage protocol is shown (not to scale). Currents were activated by depolarization from -80 to +50 mV in 20 second increments of 10 mV steps from a holding potential of -100 mV. B: Arithmetic means $\pm$ SEM ($n = 15 - 25$) of the current (I) as a function of the potential difference across the cell membrane (V) in *Xenopus* oocytes injected with water (black circles) or expressing Kv1.5 without (white squares) or with additional co-expression of constitutively active $T308D/S473D$ PKB/Akt (white triangles) or of inactive $T308A/S473A$ PKB/Akt (white circles). C: Arithmetic means $\pm$ SEM ($n = 15 - 25$) of the conductance calculated by linear fit of I/V -curves shown in B between 20 mV and 50 mV in *Xenopus* oocytes injected with water (dotted bar), or expressing Kv1.5 without (white bar) or with additional co-expression of constitutively active $T308D/S473D$ PKB/Akt (black bar) or of inactive $T308A/S473A$ PKB/Akt (grey bar). * ($p < 0.05$) indicates statistically significant difference from oocytes expressing Kv1.5 alone.

Fig. 5. PKB/Akt increased Kv1.5-HA protein abundance within the oocyte membrane. Arithmetic means $\pm$ SEM ($n = 94 - 98$) of normalized Kv1.5-HA chemiluminescence in *Xenopus* oocytes injected with water (dotted bar), expressing Kv1.5-HA alone (white bar) or expressing Kv1.5-HA together with wild-type PKB/Akt (black bar). For normalization, the chemiluminescence was divided by the chemiluminescence of oocytes expressing Kv1.5-HA alone. * ($p < 0.05$) indicates statistically significant difference from oocytes expressing Kv1.5-HA alone.

cell membrane thus driving Cl^- exit. PKB/Akt activation has, however, been shown to increase cell size [50] and T-cells overexpressing active Akt1 are enlarged [51]. The swelling effect of PKB/Akt may result from cellular ion accumulation by the Na^+/H^+ exchanger [52].

Fig. 6. Effect of brefeldin A on *Xenopus laevis* oocytes expressing either Kv1.5 alone or with PKB/Akt. Arithmetic means $\pm$ SEM (n = 16 - 19) of the conductance calculated by respective linear fit of I/V-curves between 20 mV and 50 mV in *Xenopus* oocytes injected with cRNA encoding Kv1.5 without (white bars) or with (black bars) PKB/Akt and incubated without (left bars) or with 5 μ M brefeldin A for 16 hours (middle bars) or 24 hours (right bars) prior to the measurement. ** (p<0.01) indicates significant difference from the absence of PKB/Akt. ### (p<0.001) indicates significant difference from the absence of brefeldin A.

Up-regulation of Kv1.3 or Kv1.5 K⁺ channels may further affect cell proliferation, which, at least in some cell types, requires activity of Kv1.3 channels [21-25] and/or Kv1.5 channels [22, 26]. Accordingly, up-regulation of Kv1.3 and/or Kv1.5 may participate in the stimulation of cell proliferation and tumor-formation by PKB/Akt [1-9].

Kv1.3 and Kv1.5 channels serve a number of further functions including Kv1.3 sensitive immune response & inflammation [23, 53-55] or diabetes [56], as well as Kv1.5 sensitive cardiac atrial repolarization [57-60], inhibition of insulin release [61], and regulation of pulmonary artery smooth muscle cell activity [62, 63].

In conclusion, PKB/Akt up-regulates the voltage gated K⁺ channels Kv1.3 and Kv1.5, an effect possibly participating in the orchestration of cell proliferation and further Kv1.3 and Kv1.5 sensitive functions.

Acknowledgements

The authors acknowledge the meticulous preparation of the manuscript by Lejla Subasic and Tanja Loch and the technical support by Elfriede Faber. This study was supported by the Deutsche Forschungsgemeinschaft, GRK 1302, SFB 773 B4/A1, La 315/13-3, and Open Access Publishing Fund of Tuebingen University.

Disclosure Statement

The authors declare that they have nothing to disclose.

References

- 1 Carnero A: The PKB/AKT pathway in cancer. *Curr. Pharm. Des* 2010;16:34-44.
- 2 Chin YR, Toker A: Akt isoform-specific signaling in breast cancer: uncovering an anti-migratory role for palladin. *Cell Adh. Migr* 2011;5:211-214.
- 3 Ching CB, Hansel DE: Expanding therapeutic targets in bladder cancer: the PI3K/Akt/mTOR pathway. *Lab Invest* 2010;90:1406-1414.
- 4 Dienstmann R, Rodon J, Markman B, Tabernero J: Recent developments in anti-cancer agents targeting PI3K, Akt and mTORC1/2. *Recent Pat Anticancer Drug Discov* 2011;6:210-236.
- 5 Dillon RL, Muller WJ: Distinct biological roles for the akt family in mammary tumor progression. *Cancer Res* 2010;70:4260-4264.

- 6 Georgescu MM: PTEN Tumor Suppressor Network in PI3K-Akt Pathway Control. *Genes Cancer* 2010;1:1170-1177.
- 7 Martelli AM, Evangelisti C, Follo MY, Ramazzotti G, Fini M, Giardino R, Manzoli L, McCubrey JA, Cocco L: Targeting the Phosphatidylinositol 3-Kinase/Akt/Mammalian Target of Rapamycin Signaling Network in Cancer Stem Cells. *Curr. Med Chem* 2011;
- 8 Steelman LS, Chappell WH, Abrams SL, Kempf RC, Long J, Laidler P, Mijatovic S, Maksimovic-Ivanic D, Stivala F, Mazzarino MC, Donia M, Fagone P, Malaponte G, Nicoletti F, Libra M, Milella M, Tafuri A, Bonati A, Basecke J, Cocco L, Evangelisti C, Martelli AM, Montalto G, Cervello M, McCubrey JA: Roles of the Raf/MEK/ERK and PI3K/PTEN/Akt/mTOR pathways in controlling growth and sensitivity to therapy-implications for cancer and aging. *Aging (Albany. NY)* 2011;3:192-222.
- 9 Viglietto G, Amodio N, Malanga D, Scrima M, De Marco C: Contribution of PKB/AKT signaling to thyroid cancer. *Front Biosci* 2011;16:1461-1487.
- 10 Bortner CD, Cidlowski JA: Ion channels and apoptosis in cancer. *Philos Trans R Soc Lond B Biol Sci* 2014;369:20130104.
- 11 Dobrovinskaya O, Delgado-Enciso I, Quintero-Castro LJ, Best-Aguilera C, Rojas-Sotelo RM, Pottosin I: Placing ion channels into a signaling network of T cells: from maturing thymocytes to healthy T lymphocytes or leukemic T lymphoblasts. *Biomed Res Int* 2015;2015:750203.
- 12 Lang F, Stournaras C: Ion channels in cancer: future perspectives and clinical potential. *Philos Trans R Soc Lond B Biol Sci* 2014;369:20130108.
- 13 Litan A, Langhans SA: Cancer as a channelopathy: ion channels and pumps in tumor development and progression. *Front Cell Neurosci* 2015;9:86.
- 14 Rao VR, Perez-Neut M, Kaja S, Gentile S: Voltage-gated ion channels in cancer cell proliferation. *Cancers (Basel)* 2015;7:849-875.
- 15 Embark HM, Setiawan I, Poppendieck S, van de Graaf SF, Boehmer C, Palmada M, Wieder T, Gerstberger R, Cohen P, Yun CC, Bindels RJ, Lang F: Regulation of the epithelial Ca²⁺ channel TRPV5 by the NHE regulating factor NHERF2 and the serum and glucocorticoid inducible kinase isoforms SGK1 and SGK3 expressed in *Xenopus* oocytes. *Cell Physiol Biochem* 2004;14:203-212.
- 16 Sopjani M, Kunert A, Czarkowski K, Klaus F, Laufer J, Foller M, Lang F: Regulation of the Ca²⁺ channel TRPV6 by the kinases SGK1, PKB/Akt, and PIKfyve. *J Membr. Biol* 2010;233:35-41.
- 17 Gehring EM, Lam RS, Siraskar G, Koutsouki E, Seeböhm G, Ureche ON, Ureche L, Baltaev R, Tavares JM, Lang F: PIKfyve upregulates CFTR activity. *Biochem. Biophys. Res. Commun* 2009;390:952-957.
- 18 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.
- 19 Pakladok T, Almilaji A, Munoz C, Alesutan I, Lang F: PIKfyve sensitivity of hERG channels. *Cell Physiol Biochem* 2013;31:785-794.
- 20 Embark HM, Bohmer C, Vallon V, Luft F, Lang F: Regulation of KCNE1-dependent K⁽⁺⁾ current by the serum and glucocorticoid-inducible kinase (SGK) isoforms. *Pflugers Arch* 2003;445:601-606.
- 21 Bradding P, Wulff H: The K⁺ channels K(Ca)_{3.1} and K(v)_{1.3} as novel targets for asthma therapy. *Br J Pharmacol* 2009;157:1330-1339.
- 22 Felipe A, Bielanska J, Comes N, Vallejo A, Roig S, Ramon YCS, Condom E, Hernandez-Losa J, Ferreres JC: Targeting the voltage-dependent K⁽⁺⁾ channels Kv1.3 and Kv1.5 as tumor biomarkers for cancer detection and prevention. *Curr Med Chem* 2012;19:661-674.
- 23 Rangaraju S, Chi V, Pennington MW, Chandy KG: Kv1.3 potassium channels as a therapeutic target in multiple sclerosis. *Expert Opin Ther Targets* 2009;13:909-924.
- 24 Vianna JB: [Treatment of Cancer of the Bladder]. *Rev Bras Cir* 1963;46:329-348.
- 25 Wickenden A: K⁽⁺⁾ channels as therapeutic drug targets. *Pharmacol Ther* 2002;94:157-182.
- 26 Comes N, Bielanska J, Vallejo-Gracia A, Serrano-Albarras A, Marruecos L, Gomez D, Soler C, Condom E, Ramon YCS, Hernandez-Losa J, Ferreres JC, Felipe A: The voltage-dependent K⁽⁺⁾ channels Kv1.3 and Kv1.5 in human cancer. *Front Physiol* 2013;4:283.
- 27 Colley B, Tucker K, Fadool DA: Comparison of modulation of Kv1.3 channel by two receptor tyrosine kinases in olfactory bulb neurons of rodents. *Receptors Channels* 2004;10:25-36.
- 28 Fadool DA, Tucker K, Phillips JJ, Simmen JA: Brain insulin receptor causes activity-dependent current suppression in the olfactory bulb through multiple phosphorylation of Kv1.3. *J Neurophysiol* 2000;83:2332-2348.

- 29 Marks DR, Fadool DA: Post-synaptic density perturbs insulin-induced Kv1.3 channel modulation via a clustering mechanism involving the SH3 domain. *J Neurochem* 2007;103:1608-1627.
- 30 Gamper N, Fillon S, Huber SM, Feng Y, Kobayashi T, Cohen P, Lang F: IGF-1 up-regulates K⁺ channels via PI3-kinase, PDK1 and SGK1. *Pflugers Arch* 2002;443:625-634.
- 31 Bowlby MR, Fadool DA, Holmes TC, Levitan IB: Modulation of the Kv1.3 potassium channel by receptor tyrosine kinases. *J Gen Physiol* 1997;110:601-610.
- 32 Colley BS, Biju KC, Visegrady A, Campbell S, Fadool DA: Neurotrophin B receptor kinase increases Kv subfamily member 1.3 (Kv1.3) ion channel half-life and surface expression. *Neuroscience* 2007;144:531-546.
- 33 Colley BS, Cavallin MA, Biju K, Marks DR, Fadool DA: Brain-derived neurotrophic factor modulation of Kv1.3 channel is disregulated by adaptor proteins Grb10 and nShc. *BMC Neurosci* 2009;10:8.
- 34 Fadool DA: Tyrosine phosphorylation downregulates a potassium current in rat olfactory bulb neurons and a cloned Kv1.3 channel. *Ann N Y Acad Sci* 1998;855:529-532.
- 35 Fadool DA, Levitan IB: Modulation of olfactory bulb neuron potassium current by tyrosine phosphorylation. *J Neurosci* 1998;18:6126-6137.
- 36 Lang F, Shumilina E: Regulation of ion channels by the serum- and glucocorticoid-inducible kinase SGK1. *FASEB J* 2013;27:3-12.
- 37 Palmada M, Speil A, Jeyaraj S, Bohmer C, Lang F: The serine/threonine kinases SGK1, 3 and PKB stimulate the amino acid transporter ASCT2. *Biochem. Biophys. Res. Commun* 2005;331:272-277.
- 38 Tyan L, Sopjani M, Dermaku-Sopjani M, Schmid E, Yang W, Xuan NT, Shumilina E, Lang F: Inhibition of voltage-gated K⁺ channels in dendritic cells by rapamycin. *Am J Physiol Cell Physiol* 2010;299:C1379-1385.
- 39 Munoz C, Tovolli RH, Sopjani M, Alesutan I, Lam RS, Seebohm G, Foller M, Lang F: Activation of voltage gated K(+) channel Kv1.5 by beta-catenin. *Biochem Biophys Res Commun* 2012;417:692-696.
- 40 Almilaji A, Munoz C, Hosseinzadeh Z, Lang F: Upregulation of Na⁺,Cl⁻-coupled betaine/gamma-amino-butyric acid transporter BGT1 by Tau tubulin kinase 2. *Cell Physiol Biochem* 2013;32:334-343.
- 41 Almilaji A, Sopjani M, Elvira B, Borrás 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.
- 42 Hosseinzadeh Z, Dong L, Bhavsar SK, Warsi J, Almilaji A, Lang F: Upregulation of peptide transporters PEPT1 and PEPT2 by Janus kinase JAK2. *Cell Physiol Biochem* 2013;31:673-682.
- 43 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.
- 44 Almilaji A, Szteyn K, Fein E, Pakladok T, Munoz C, Elvira B, Towhid ST, Alesutan I, Shumilina E, Bock CT, Kandolf R, Lang F: Down-regulation of Na/K⁺ atpase activity by human parvovirus B19 capsid protein VP1. *Cell Physiol Biochem* 2013;31:638-648.
- 45 Fezai M, Elvira B, Borrás 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 Dermaku-Sopjani M, Almilaji A, Pakladok T, Munoz C, Hosseinzadeh Z, Blecua M, Sopjani M, Lang F: Down-regulation of the Na⁺-coupled phosphate transporter NaPi-IIa by AMP-activated protein kinase. *Kidney Blood Press Res* 2013;37:547-556.
- 47 Elvira B, Munoz C, Borrás J, Chen H, Warsi J, Ajay SS, Shumilina E, Lang F: SPAK and OSR1 dependent down-regulation of murine renal outer medullary K channel ROMK1. *Kidney Blood Press Res* 2014;39:353-360.
- 48 Alesutan IS, Ureche ON, Laufer J, Klaus F, Zurn A, Lindner R, Strutz-Seebohm N, Tavare JM, Boehmer C, Palmada M, Lang UE, Seebohm G, Lang F: Regulation of the glutamate transporter EAAT4 by PIKfyve. *Cell Physiol Biochem* 2010;25:187-194.
- 49 Lang F, Busch GL, Ritter M, Volkl H, Waldegger S, Gulbins E, Haussinger D: Functional significance of cell volume regulatory mechanisms. *Physiol Rev* 1998;78:247-306.
- 50 Rodriguez J, Vernus B, Toubiana M, Jublanc E, Tintignac L, Leibovitch S, Bonniieu A: Myostatin inactivation increases myotube size through regulation of translational initiation machinery. *J Cell Biochem* 2011;
- 51 Malstrom S, Tili E, Kappes D, Ceci JD, Tschlis PN: Tumor induction by an Lck-MyrAkt transgene is delayed by mechanisms controlling the size of the thymus. *Proc Natl Acad Sci U S A* 2001;98:14967-14972.
- 52 Bhandaru M, Yang W, Rotte A, Pasham V, Lang F: Regulation of Na⁽⁺⁾/H⁽⁺⁾ exchanger in dendritic cells by Akt2. *Pflugers Arch* 2012;463:355-363.

- 53 Nguyen W: Novel Kv1.3 blockers for immunosuppression: W02012155199. *Expert Opin Ther Pat* 2013;23:1511-1516.
- 54 Wang J, Xiang M: Targeting potassium channels Kv1.3 and KC a 3.1: routes to selective immunomodulators in autoimmune disorder treatment? *Pharmacotherapy* 2013;33:515-528.
- 55 Wulff H, Pennington M: Targeting effector memory T-cells with Kv1.3 blockers. *Curr Opin Drug Discov Devel* 2007;10:438-445.
- 56 Choi BH, Hahn SJ: Kv1.3: a potential pharmacological target for diabetes. *Acta Pharmacol Sin* 2010;31:1031-1035.
- 57 Bilodeau MT, Trotter BW: Kv1.5 blockers for the treatment of atrial fibrillation: approaches to optimization of potency and selectivity and translation to in vivo pharmacology. *Curr Top Med Chem* 2009;9:436-451.
- 58 Brendel J, Peukert S: Blockers of the Kv1.5 channel for the treatment of atrial arrhythmias. *Curr Med Chem Cardiovasc Hematol Agents* 2003;1:273-287.
- 59 Gonzalez T, David M, Moreno C, Macias A, Valenzuela C: Kv1.5-Kv beta interactions: molecular determinants and pharmacological consequences. *Mini Rev Med Chem* 2010;10:635-642.
- 60 Tamargo J, Caballero R, Gomez R, Delpon E: I(Kur)/Kv1.5 channel blockers for the treatment of atrial fibrillation. *Expert Opin Investig Drugs* 2009;18:399-416.
- 61 Ullrich S, Berchtold S, Ranta F, Seebohm G, Henke G, Lupescu A, Mack AF, Chao CM, Su J, Nitschke R, Alexander D, Friedrich B, Wulff P, Kuhl D, Lang F: Serum- and glucocorticoid-inducible kinase 1 (SGK1) mediates glucocorticoid-induced inhibition of insulin secretion. *Diabetes* 2005;54:1090-1099.
- 62 Archer SL, Gomberg-Maitland M, Maitland ML, Rich S, Garcia JG, Weir EK: Mitochondrial metabolism, redox signaling, and fusion: a mitochondria-ROS-HIF-1alpha-Kv1.5 O2-sensing pathway at the intersection of pulmonary hypertension and cancer. *Am J Physiol Heart Circ Physiol* 2008;294:H570-578.
- 63 Bonnet S, Rochefort G, Sutendra G, Archer SL, Haromy A, Webster L, Hashimoto K, Bonnet SN, Michelakis ED: The nuclear factor of activated T cells in pulmonary arterial hypertension can be therapeutically targeted. *Proc Natl Acad Sci U S A* 2007;104:11418-11423.