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Regulation of Voltage-Gated K^+ Channel Kv1.5 by the Janus Kinase JAK3

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Abstract The tyrosine kinase Janus kinase 3 (JAK3) participates in the regulation of cell proliferation and apoptosis. The kinase further influences ion channels and transport proteins. The present study explored whether JAK3 contributes to the regulation of the voltage-gated K^+ channel Kv1.5, which participates in the regulation of diverse functions including atrial cardiac action potential and tumor cell proliferation. To this end, cRNA encoding Kv1.5 was injected into *Xenopus* oocytes with or without additional injection of cRNA encoding wild-type JAK3, constitutively active ^{A568V}JAK3, or inactive ^{K851A}JAK3. Voltage-gated K^+ channel activity was measured utilizing dual electrode voltage clamp, and Kv1.5 channel protein abundance in the cell membrane was quantified utilizing chemiluminescence of Kv1.5 containing an extracellular hemagglutinin epitope (Kv1.5-HA). As a result, Kv1.5 activity and Kv1.5-HA protein abundance were significantly decreased by wild-type JAK3 and ^{A568V}JAK3, but not by ^{K851A}JAK3. Inhibition of Kv1.5 protein insertion into the cell membrane by brefeldin A (5 μM) resulted in a decline of the voltage-gated current, which was similar in the absence and presence of ^{A568V}JAK3, suggesting that ^{A568V}JAK3 did not accelerate Kv1.5 protein retrieval from the cell membrane. A 24 h treatment with ouabain (100 μM) significantly decreased the voltage-gated current in oocytes expressing Kv1.5 without or with ^{A568V}JAK3 and dissipated the difference between oocytes expressing Kv1.5 alone and oocytes expressing Kv1.5 with ^{A568V}JAK3. In conclusion, JAK3 contributes to the regulation of membrane Kv1.5 protein abundance and activity, an effect sensitive to ouabain and thus possibly involving Na^+/K^+ ATPase activity.

Keywords Oocytes · Voltage clamp · Chemiluminescence · Janus kinase · Ouabain

Introduction

Janus kinase 3 (JAK3), a kinase mainly expressed in hematopoietic cells (Bharadwaj and Agrawal 2007; O'Shea et al. 2004; Vijayakrishnan et al. 2011), contributes to the signaling of hematopoietic cell cytokine receptors (Cornejo et al. 2009; Ghoreschi et al. 2009; Imada and Leonard 2000; O'Shea et al. 2002; Shuai and Liu 2003), stimulating cell proliferation and inhibiting apoptosis of lymphocytes and tumor cells (de Totero et al. 2008; Fainstein et al. 2008; Kim et al. 2010; Nakayama et al. 2009; Uckun et al. 2007). A mutation replacing the alanine at 572 by valine thus yielding the gain of function ^{A572V}JAK3, has been discovered in acute megakaryoblastic leukemia (Malinge et al. 2008; Walters et al. 2006). A mutation replacing ATP coordinating lysine by alanine in the catalytic subunit yields the inactive ^{K855A}JAK3 (Haan et al. 2011). JAK3 expression is up-regulated (Wang et al. 2005) and activated (Wang et al. 2008) upon hypoxia and JAK3 is activated by energy depletion (Bhavsar et al. 2011). JAK3 (Hosseinzadeh et al. 2015) and the related kinase JAK2 are powerful regulators of Na^+/K^+ ATPase (Bhavsar et al. 2014) and JAK3-deficient mice are volume depleted (Umbach et al. 2013).

Ion channels contributing to the stimulation of tumor cell proliferation include the voltage-gated K^+ channel Kv1.5 (Comes et al. 2013; Felipe et al. 2012), a channel

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further involved in the repolarization in cardiac atria (Bilodeau and Trotter 2009; Brendel and Peukert 2003; Gonzalez et al. 2010; Tamargo et al. 2009), and regulation of pulmonary artery smooth muscle cell activity (Archer et al. 2008; Bonnet et al. 2007).

The present study tested whether JAK3 influences Kv1.5 channel activity. To this end, Kv1.5 was expressed in *Xenopus* oocytes without or with additional expression of wild-type JAK3, constitutively active murine ^{A568V}JAK3, or inactive ^{K851A}JAK3. In those oocytes, the voltage-gated K^+ current was determined utilizing dual electrode voltage clamp and the Kv1.5 protein abundance quantified by utilizing chemiluminescence.

Materials and Methods

Ethical Statement

All experiments conform to 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. All surgical procedures on the adult *Xenopus laevis* 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).

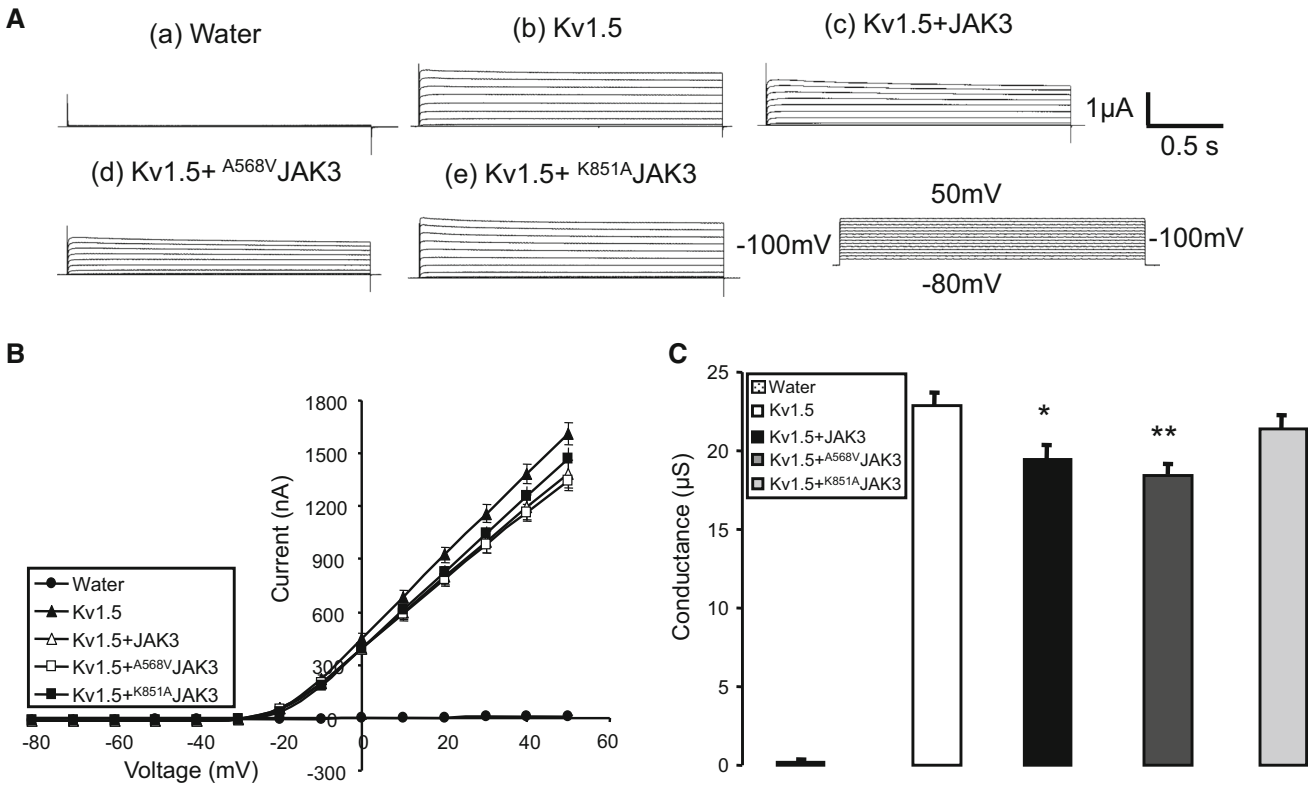

Fig. 1 Co-expression of wild-type JAK3 and constitutively active ^{A568V}JAK3 but not inactive ^{K851A}JAK3 decreased the K^+ current in Kv1.5 expressing *Xenopus* oocytes. **a** Representative original tracings showing currents in *Xenopus* oocytes injected with water (*a*), expressing Kv1.5 alone (*b*) or expressing Kv1.5 with additional co-expression of wild-type JAK3 (*c*) or with constitutively active ^{A568V}JAK3 (*d*), or with inactive ^{K851A}JAK3 (*e*). The voltage protocol is shown (not to scale). Currents were recorded following steps to voltages between −80 and +50 mV for 20 s from a holding potential of −100 mV. **b** Arithmetic means ± SEM (n = 12–17) 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 (*black triangles*) or with (*white triangles*) additional co-expression of wild-type JAK3, or with constitutively active ^{A568V}JAK3 (*white squares*) or with inactive ^{K851A}JAK3 (*black squares*). **c** Arithmetic means ± SEM (n = 12–17) of the conductance calculated by linear fit of *I*/*V*-curves shown in **b** between 20 and 50 mV in *Xenopus* oocytes injected with water (*dotted bar*), or expressing Kv1.5 without (*white bar*) or with (*black bar*) additional co-expression of wild-type JAK3 or with constitutively active ^{A568V}JAK3 (*dark gray bar*), or with inactive ^{K851A}JAK3 (*gray bar*). *($p < 0.05$) and **($p < 0.01$) indicate statistically significant difference from oocytes expressing Kv1.5 alone

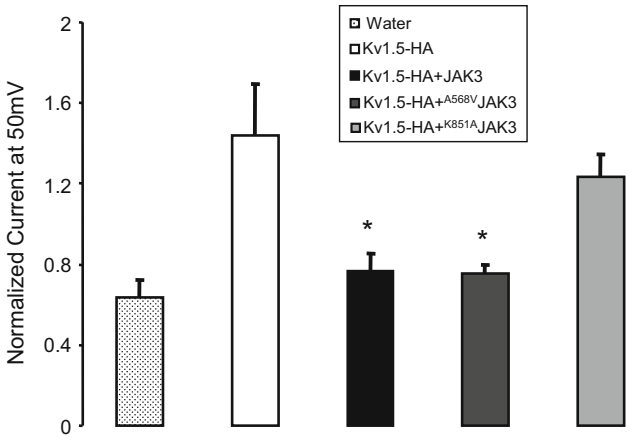

Fig. 2 Co-expression of wild-type JAK3 and constitutively active ^{A568V}JAK3 but not inactive ^{K851A}JAK3 decreased the K^+ current in Kv1.5-HA expressing *Xenopus* oocytes. Arithmetic means ± SEM ($n = 9$–17) of the normalized voltage-gated K^+ current at +50 mV in *Xenopus* oocytes injected with water (*dotted bar*), expressing Kv1.5-HA alone (*white bar*) or expressing Kv1.5-HA together with wild-type JAK3 (*black bar*) or with constitutively active ^{A568V}JAK3 (*dark gray*) or inactive ^{K851A}JAK3 (*gray bar*). *($p < 0.05$) indicates statistically significant difference from oocytes expressing Kv1.5-HA alone

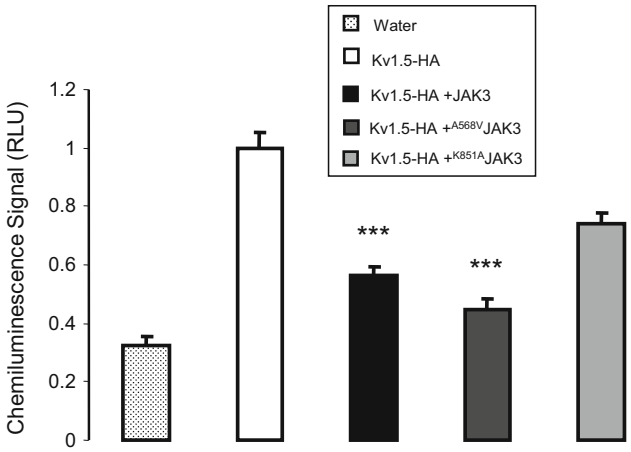

Fig. 3 Wild-type JAK3 and ^{A568V}JAK3 but not inactive ^{K851A}JAK3 decreased Kv1.5-HA protein abundance within the oocyte membrane. Arithmetic means ± SEM ($n = 57$–77) of normalized Kv1.5-HA chemiluminescence in *Xenopus* oocytes injected with water (*dotted bar*), expressing Kv1.5-HA alone (*white bar*) or expressing Kv1.5-HA together with wild-type JAK3 (*black bar*) or with constitutively active ^{A568V}JAK3 (*dark gray bar*) or with catalytically inactive ^{K851A}JAK3 (*gray bar*). For normalization, the chemiluminescence of each oocyte was divided by the mean chemiluminescence of oocytes expressing Kv1.5-HA alone. ***($p < 0.001$) indicates statistically significant difference from oocytes expressing Kv1.5-HA alone

Constructs

Constructs encoding mouse wild-type Kv1.5 or Kv1.5 containing an extracellular hemagglutinin epitope (KV1.5-HA) (Mia et al. 2012), and rat wild-type Kv1.2 (Suessbrich et al. 1997) and/or mouse wild-type JAK3, inactive ^{K851A}JAK3 mutant and gain of function ^{A568V}JAK3 mutant (Warsi et al. 2013), were used for generation of cRNA as described previously (Hosseinzadeh et al. 2013a; Warsi et al. 2013).

Voltage Clamp in Xenopus Oocytes

Xenopus oocytes were prepared as previously described (Almilaji et al. 2013a; Fezai et al. 2014). cRNA encoding Kv1.5 (2.5 ng) or cRNA encoding Kv1.5 (1.25 ng) + Kv1.2 (1.25 ng) or cRNA encoding wild-type JAK3 (10 ng or, where indicated 1 ng, 2.5 ng, or 5 ng), constitutively active ^{A568V}JAK3 (10 ng) or inactive ^{K851A}JAK3 kinase (10 ng), were injected on the same day after preparation of the oocytes. The oocytes were maintained at 17 °C in ND96, a solution containing (in mM): 88.5 NaCl, 2 KCl, 1 $MgC1_2$, 1.8 $CaC1_2$, 5 HEPES, and 5 sodium pyruvate ($C_3H_3NaO_3$). Gentamycin (100 mg/l), Tetracycline (50 mg/l), Ciprofloxacin (1.6 mg/l), and Theophylline (90 mg/l) were added and pH adjusted to 7.4 (Almilaji et al. 2014, 2013b). Where indicated, brefeldin A (5 µM final concentration) or ouabain octahydrate (100 µM final concentration) was added to the respective solutions. The

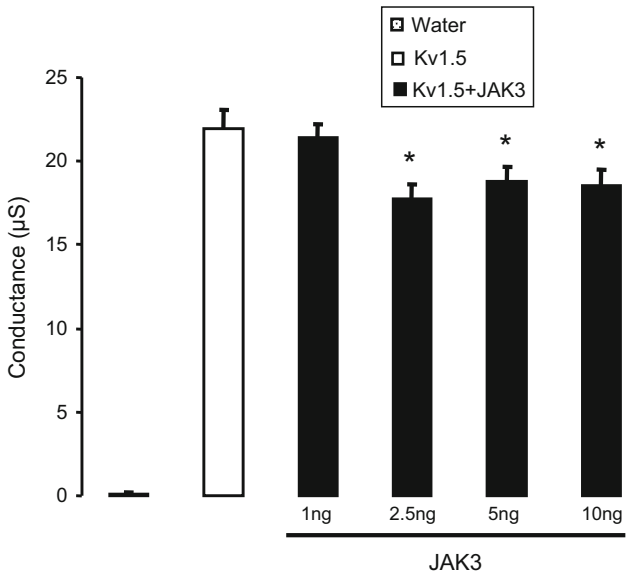

Fig. 4 The effect of different amounts of JAK3 cRNA on voltage-gated current in Kv1.5 expressing oocytes. Arithmetic means ± SEM ($n = 11$–35) of the conductance calculated by linear fit of respective *I/V*-curves between 20 and 50 mV in *Xenopus* oocytes injected with water (*dotted bar*), injected with cRNA (2.5 ng) encoding Kv1.5 alone (*white bar*), or injected with cRNA encoding Kv1.5 (2.5 ng) and wild-type JAK3 (1–10 ng) (*black bars*). *($p < 0.05$) indicates statistically significant difference from oocytes expressing Kv1.5 alone

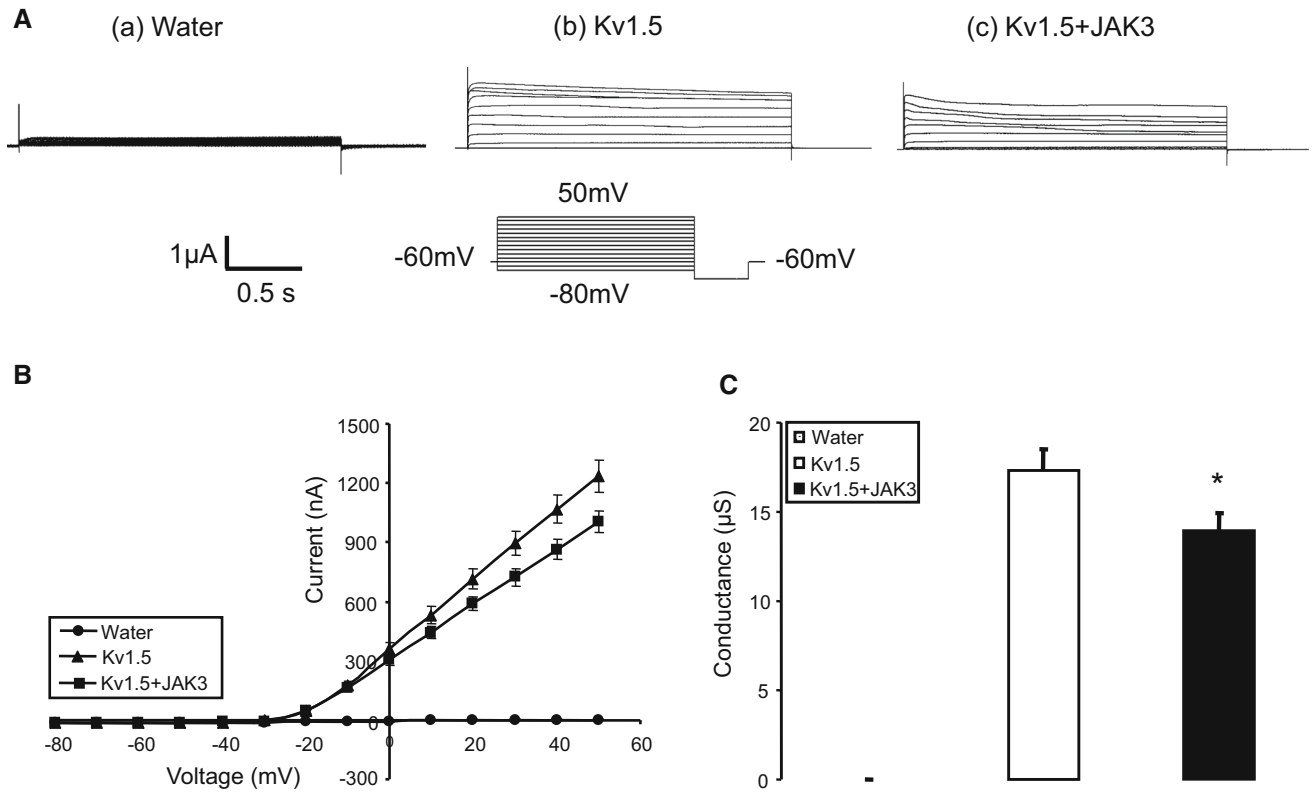

Fig. 5 Co-expression of wild-type JAK3 decreased the K^+ current in Kv1.5 expressing *Xenopus* oocytes at holding potential of −60 mV. **a** Representative original tracings showing currents in *Xenopus* oocytes injected with water (*a*), expressing Kv1.5 alone (*b*) or expressing Kv1.5 with additional co-expression of wild-type JAK3 (*c*). The voltage protocol is shown (not to scale). Currents were recorded following steps to voltages between −80 and +50 mV for 20 s from a holding potential of −60 mV. **b** Arithmetic means ± SEM (n = 12–20) 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 (*black triangles*) or with (*black squares*) additional co-expression of wild-type JAK3. **c** Arithmetic means ± SEM (n = 12–20) of the conductance calculated by linear fit of I/V-curves shown in **b** between 20 and 50 mV in *Xenopus* oocytes injected with water (*dotted bar*), or expressing Kv1.5 without (*white bar*) or with (*black bar*) additional co-expression of wild-type JAK3. *($p < 0.05$) indicates statistically significant difference from oocytes expressing Kv1.5 alone

voltage clamp experiments were performed at room temperature 3 days after the first injection. Kv1.5 channel currents were elicited every 20 s with 3 s pulses from −80 to +50 mV in 20 s increments of 10 mV steps from a holding potential of −100 mV (or, where indicated of −60 mV). The data were filtered at 2 kHz and recorded with a Digidata A/D–D/A converter (1322A Axon Instruments) and Clampex 9.2 software for data acquisition and analysis (Axon Instruments) (Hosseinzadeh et al. 2014, 2013b; Munoz et al. 2013). 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 (Pakladok et al. 2014; Warsi et al. 2014c).

Detection of Kv1.5-HA Cell Surface Expression by Chemiluminescence

To determine Kv1.5-HA cell surface expression by chemiluminescence, the oocytes were incubated with mouse monoclonal anti-HA antibody conjugated to horseradish peroxidase (1:1000, Miltenyi Biotec Inc, CA, USA) (Warsi et al. 2014a, b). Individual oocytes were placed in 96-well plates with 20 μl of Super Signal 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 s (Elvira et al. 2014). Results display normalized arbitrary light units. Integrity of the

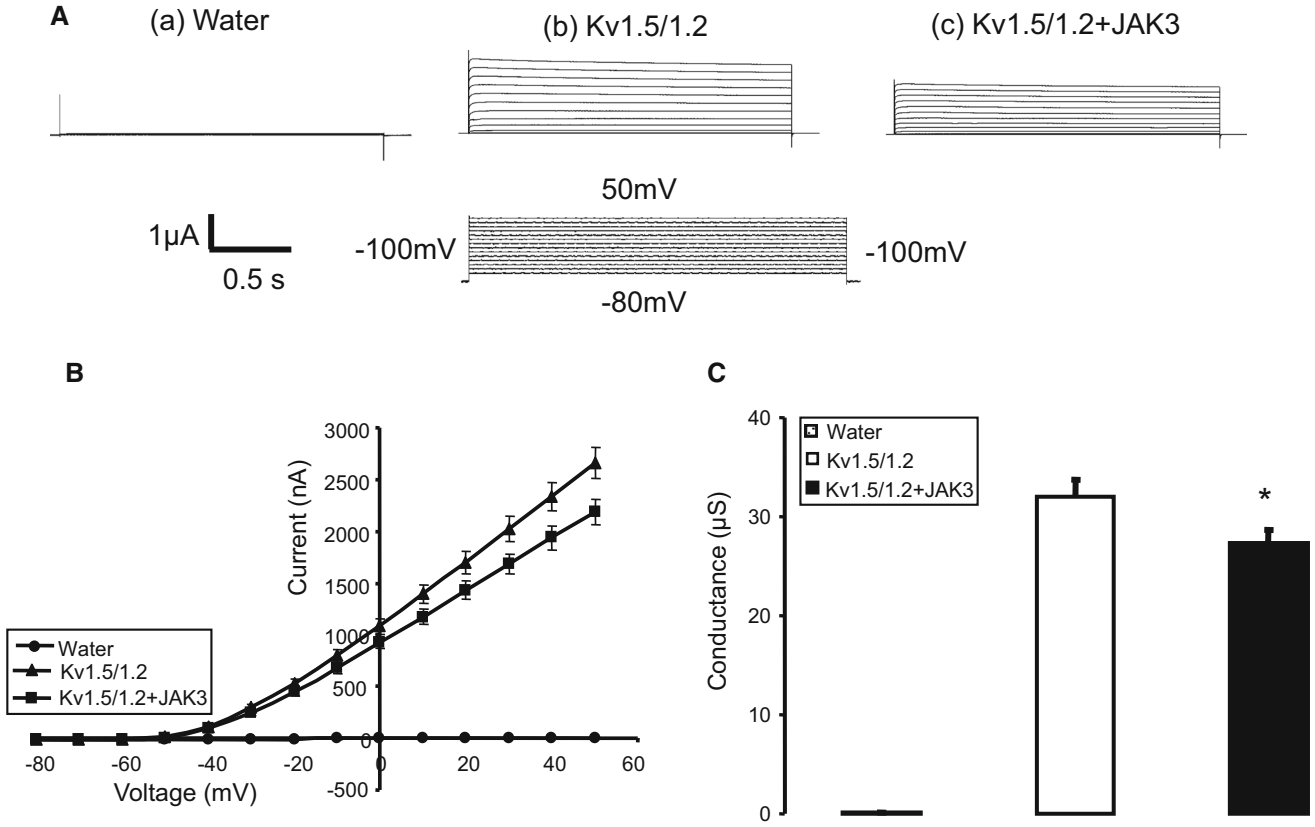

Fig. 6 Co-expression of wild-type JAK3 decreased the K^+ current in Kv1.5/Kv1.2 expressing *Xenopus* oocytes. **a** Representative original tracings showing currents in *Xenopus* oocytes injected with water (*a*), expressing Kv1.5/Kv1.2 alone (*b*) or expressing Kv1.5/Kv1.2 with additional co-expression of wild-type JAK3 (*c*). The voltage protocol is shown (not to scale). Currents were recorded following steps to voltages between −80 and +50 mV for 20 s from a holding potential of −100 mV. **b** Arithmetic means ± SEM ($n = 12$–20) 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/Kv1.2 without (*black triangles*) or with (*black squares*) additional co-expression of wild-type JAK3. **c** Arithmetic means ± SEM ($n = 12$–20) of the conductance calculated by linear fit of I/V-curves shown in **b** between 20 and 50 mV in *Xenopus* oocytes injected with water (*dotted bar*), or expressing Kv1.5/Kv1.2 without (*white bar*) or with (*black bar*) additional co-expression of wild-type JAK3. *($p < 0.05$) indicates statistically significant difference from oocytes expressing Kv1.5/Kv1.2 without JAK3

measured oocytes was assessed by visual control after the measurement to avoid unspecific light signals from the cytosol (Dermaku-Sopjani et al. 2013; Pakladok et al. 2013).

Statistical Analysis

Data are provided as mean ± SEM, and 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 2–3 batches of oocytes; in all repetitions, qualitatively similar data were obtained. Data were tested for significance using ANOVA (Tukey's test or Kruskal–Wallis test) as appropriate. Results with $p < 0.05$ were considered statistically significant.

Results

The present study explored whether Janus-activated kinase JAK3 participates in the regulation of the voltage-gated K^+ channel Kv1.5. To this end, cRNA encoding Kv1.5 was injected into *Xenopus* oocytes with or without additional injection of cRNA encoding JAK3 or constitutively active ^{A568V}JAK3 or catalytically inactive mutant ^{K851A}JAK3. The voltage-gated K^+ current was determined by dual electrode voltage clamp experiments, thus allowing the calculation of the respective K^+ conductance (G_K). As illustrated in Fig. 1, G_K was negligible in water injected oocytes. Thus, oocytes did not express endogenous voltage-gated K^+ channels. In contrast, large voltage-gated K^+ currents were observed in oocytes injected with cRNA encoding Kv1.5. The additional injection of cRNA encoding wild-type JAK3 was followed by a significant

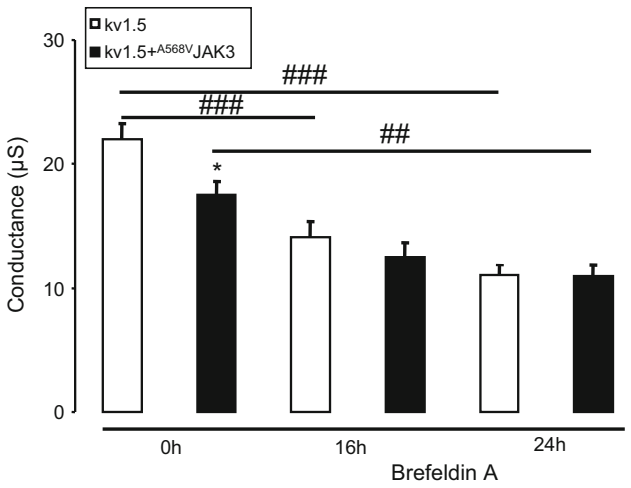

Fig. 7 Effect of brefeldin A on voltage-gated current in Kv1.5 and ^{A568V}JAK3 expressing oocytes. Arithmetic means ± SEM ($n = 22$–25) of conductance calculated by linear fit of respective *I/V*-curves between 20 and 50 mV in *Xenopus* oocytes injected with Kv1.5 alone (Kv1.5, *white bars*) or expressing Kv1.5 together with constitutively active ^{A568V}JAK3 (*black bars*) prior to (*left bars*, 0 h) and following incubation with brefeldin A (5 µM) for 16 h (16 h) or 24 h (24 h). *($p < 0.05$) indicates statistically significant difference from oocytes expressing Kv1.5 alone. ##($p < 0.01$) and ###($p < 0.001$) indicate statistically significant difference from the respective oocytes prior to brefeldin A treatment

decrease of G_K. As shown in Fig. 1, the effect of wild-type JAK3 was mimicked by the constitutively active ^{A568V}JAK3. In Kv1.5 expressing oocytes, the additional injection of cRNA encoding ^{A568V}JAK3 was followed by a significant decrease of G_K. In contrast, co-expression of catalytically inactive mutant ^{K851A}JAK3 did not significantly modify G_K in Kv1.5 expressing oocytes.

An additional series of experiments was carried out to determine the Kv1.5-HA current. As illustrated in Fig. 2, sizeable currents were recorded in Kv1.5-HA expressing oocytes. The currents were significantly decreased when co-expressed with wild-type JAK3 or with constitutively active ^{A568V}JAK3, but not with the catalytically inactive mutant ^{K851A}JAK3 (Fig. 2).

JAK3 and ^{A568V}JAK3 could have modified Kv1.5 channel activity by inactivating the Kv1.5 channels or by decreasing Kv1.5 channel protein abundance in the cell membrane. Chemiluminescence was employed in order to explore whether JAK3 and ^{A568V}JAK3 co-expression impacts on Kv1.5 channel protein abundance in the cell membrane. In order to identify Kv1.5 protein in the membrane, Kv1.5 was tagged with an extracellular hemagglutinin epitope (Kv1.5-HA) allowing protein detection with an antibody directed against this epitope. As illustrated in Fig. 3, co-expression of JAK3 and ^{A568V}JAK3 significantly decreased the Kv1.5 protein abundance in the cell membrane of *Xenopus* oocytes. Co-expression of Kv1.5-HA with catalytically inactive ^{K851A}JAK3 did not significantly alter the cell membrane Kv1.5 protein abundance.

A further series of experiments explored, whether the effect of JAK3 was dependent on the amount of cRNA JAK3 injected. As shown in Fig. 4, significant down-regulation of Kv1.5-induced K^+ current was observed following additional injection of ≥2 ng cRNA encoding JAK3.

As illustrated in Fig. 5, Kv1.5 channel currents were similarly down-regulated following co-expression with wild-type JAK3, when measured at a holding potential of −60 mV instead of −100 mV.

Additional experiments were performed on oocytes expressing both, Kv1.5 and Kv1.2. As illustrated by Fig. 6, the voltage-gated K^+ currents of Kv1.5 and Kv1.2 expressing oocytes were again significantly down-regulated following co-expression of wild-type JAK3 (Fig. 6).

The influence of ^{A568V}JAK3 on Kv1.5 protein abundance and activity could have resulted from either decreased insertion of channel protein into the cell membrane or accelerated retrieval of channel protein from the cell membrane. In order to discriminate between those two possibilities, the insertion of new channel protein into the cell membrane was inhibited by incubation of the oocytes with brefeldin A (5 µM). In the presence of brefeldin A, the retrieval of channel protein from the cell membrane is apparent from the decay of voltage-gated K^+ current. As illustrated in Fig. 7, the decay of voltage-gated K^+ current was similar in oocytes expressing Kv1.5 alone and in oocytes expressing both, Kv1.5 and ^{A568V}JAK3. Thus, co-expression of ^{A568V}JAK3 did not significantly accelerate the retrieval of channel protein from the cell membrane.

A further series of experiments addressed the theoretical possibility that JAK3 modified the Kv1.5 channels indirectly by down-regulating the Na^+/K^+ ATPase with subsequent decrease of Na^+/K^+ ATPase-sensitive Kv1.5 channel activity. In order to test this possibility, experiments were performed in oocytes without or with prior treatment with the Na^+/K^+ ATPase inhibitor ouabain (100 µM). To this end, the voltage-gated current in oocytes expressing Kv1.5 alone and in oocytes expressing both, Kv1.5 and JAK3, was determined in the absence and presence of ouabain (100 µM, added 24 h prior to the electrophysiological experiments). As illustrated in Fig. 8, ouabain treatment significantly decreased the voltage-gated current in oocytes expressing Kv1.5 with or without JAK3, and dissipated the differences between the voltage-gated current in oocytes expressing Kv1.5 alone and oocytes expressing both, Kv1.5 and JAK3. In contrast to a 24 h treatment with ouabain, a short term application of ouabain (4.5 min) did not significantly modify Kv1.5 channel activity. Instead the voltage-gated conductance in Kv1.5 expressing

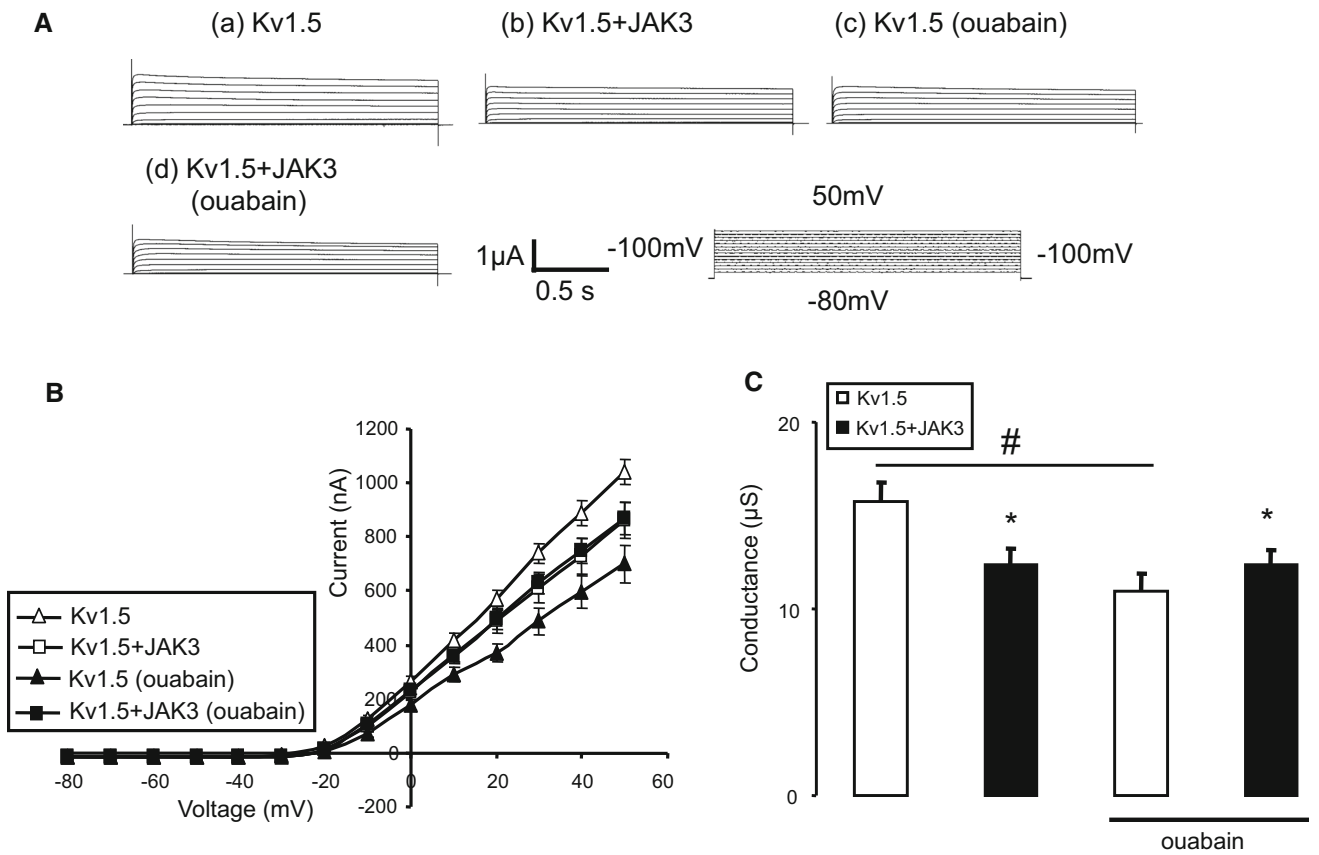

Fig. 8 Effect of ouabain on voltage-gated current in Kv1.5 and wild-type JAK3 expressing oocytes. **a** Representative original tracings showing currents in *Xenopus* oocytes expressing Kv1.5 alone (*a*, ***c***) or together with constitutively active ^{A568V}JAK3 (*b*, *d*), without (*a*, *b*) or with (*c*, *d*) a prior 24 h treatment with ouabain (100 μM). **b** Arithmetic means ± SEM ($n = 14$–24) of the current (*I*) as a function of the potential difference across the cell membrane (*V*) in *Xenopus laevis* oocytes injected with Kv1.5 alone (*triangles*) or together with constitutively active JAK3 (*squares*) without (*white symbols*) and with (*black symbols*) a prior 24 h treatment with ouabain (100 μM). **c** Arithmetic means ± SEM ($n = 14$–24) of the conductance calculated by linear fit of respective *I/V*-curves between 20 and 50 mV in *Xenopus* oocytes injected with Kv1.5 alone (*white bars*) or expressing Kv1.5 together with constitutively active JAK3 (*black bars*) incubated for 24 h in the absence (*left bars*) and presence (*right bars*) of ouabain (100 μM). *($p < 0.05$) indicates statistically significant difference from oocytes expressing Kv1.5 alone, and #($p < 0.05$) indicates statistically significant difference from the respective oocytes in the absence of ouabain

oocytes was similar in the absence (24.1 ± 2.0 μS, $n = 10$) and in the presence (23.0 ± 2.3 μS, $n = 10$) of ouabain.

A further series of experiments was performed to test whether ouabain influences the cell membrane surface abundance of Kv1.5-HA protein. To this end, oocytes expressing Kv1.5-HA alone and oocytes expressing both, Kv1.5-HA and JAK3, were exposed to ouabain (100 μM, for 24 h prior to quantification of Kv1.5-HA protein surface abundance). As illustrated in Fig. 9, a 24 h ouabain treatment significantly decreased the cell membrane surface abundance of Kv1.5-HA protein in oocytes expressing Kv1.5-HA with or without JAK3, and dissipated the differences between oocytes expressing Kv1.5-HA alone and oocytes expressing both, Kv1.5-HA and JAK3.

Discussion

The present study identifies a novel effect of JAK3, i.e., the down-regulation of the voltage-gated K^+ channel Kv1.5. The effect of wild-type JAK3 is mimicked by the gain of function mutant ^{A568V}JAK3, but not by the inactive kinase mutant ^{K851A}JAK3, indicating that the effect requires kinase activity. JAK3/^{A568V}JAK3 is at least partially effective by decreasing the channel protein abundance in the cell membrane. As co-expression of ^{A568V}JAK3 did not accelerate the retrieval of channel protein from the cell membrane, it may be partially effective by inhibiting insertion of new channel protein into the cell membrane. JAK3 co-expression similarly down-regulated heteromeric channels composed of Kv1.5 and Kv1.2.

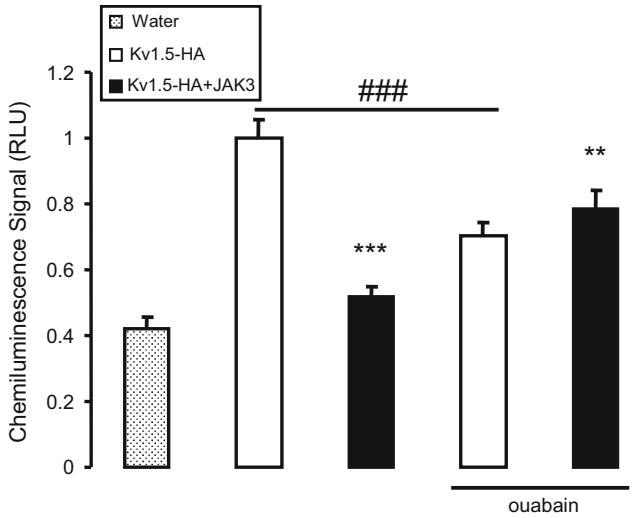

Fig. 9 Effect of ouabain on cell membrane surface protein abundance in Kv1.5-HA and wild-type JAK3 expressing oocytes. Arithmetic means ± SEM ($n = 71$–78) of normalized Kv1.5-HA chemiluminescence in *Xenopus* oocytes injected with water (*dotted bar*), expressing Kv1.5-HA alone (*white bar*) or expressing Kv1.5-HA together with wild-type JAK3 (*black bar*) incubated for 24 h in the absence (*left bars*) and presence (*right bars*) of ouabain (100 μM). For normalization, the chemiluminescence of each oocyte was divided by the mean chemiluminescence of oocytes expressing Kv1.5-HA alone. ***($p < 0.001$) indicates statistically significant difference from oocytes expressing Kv1.5-HA alone, and ###($p < 0.001$) indicates statistically significant difference from the respective oocytes in the absence of ouabain

The experiments with Na^+/K^+ ATPase inhibitor ouabain suggest that JAK3 may be effective through inhibition of Na^+/K^+ ATPase. Most recent experiments revealed that JAK3 down-regulates Na^+/K^+ ATPase activity (Hosseinzadeh et al. 2015). Pretreatment with the Na^+/K^+ ATPase inhibitor ouabain mimicked the effect of JAK3 co-expression and similarly decreased the voltage-gated K^+ current in Kv1.5 expressing oocytes. The effects of JAK3 and ouabain on voltage-gated K^+ current in Kv1.5 expressing oocytes were apparently not additive. Acute exposure of Kv1.5 expressing oocytes to ouabain did not significantly interfere with voltage-gated K^+ current, indicating that ouabain did not directly block the channel. Instead, long-term inhibition of Na^+/K^+ ATPase leads to cellular K^+ loss, which in turn decreases the activity or conductance of K^+ channels (Lang and Rehwald 1992; Messner et al. 1985).

The present study did not address the functional significance of JAK3-sensitive Kv1.5 channel activity. As Kv1.5 channels contribute to cell volume regulation (Barfield et al. 2005a, b; Felipe et al. 2012, 1993; Yeung and Cooper 2008), the inhibition of Kv1.5 K^+ channels could serve to counteract cell shrinkage by curtailing cellular loss of K^+ ions. Kv1.5 channels have further been shown to participate in the regulation of cell proliferation and survival of tumor cells (Comes et al. 2013; Leanza et al. 2014, 2012). While inhibition of K^+ channels may indeed counteract apoptosis (Lang and Hoffmann 2012), cell proliferation is usually paralleled by activation of K^+ channels (Lang and Stournaras 2014; Pardo and Stuhmer 2014; Turner and Sontheimer 2014). Thus, the presently observed down-regulation of Kv1.5 channels hardly contributes to the stimulating effect of JAK3 on cell proliferation (de Totero et al. 2008; Fainstein et al. 2008; Kim et al. 2010; Nakayama et al. 2009; Uckun et al. 2007). Clearly, additional experimental information is required prior to understanding the functional significance of JAK3-sensitive regulation of Kv1.5.

In conclusion, wild-type JAK3 and constitutively active ^{A568V}JAK3 down-regulate Kv1.5, an effect mimicked and presumably caused by inhibition of Na^+/K^+ ATPase.

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

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