Energy-sensitive regulation of Na\textsuperscript{+}/K\textsuperscript{+}-ATPase by Janus kinase 2

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The Janus kinase-2 (JAK2)-signal transducer and activator of transcription-5 (STAT5) pathway participates in the signaling of several hormones including leptin (61), erythropoietin (23, 45, 71, 85), and growth hormone (15, 16). Leptin (7), erythropoietin (60) and growth hormone (24, 57) are in turn known to protect cells against ischemic cell injury. Cell survival during energy depletion critically depends on electrolyte transport across the cell membrane (47), as ATP is required for the function of the Na\textsuperscript{+}/K\textsuperscript{+}-ATPase (75).

Recently, JAK2 has been shown to upregulate Cl\textsuperscript{−} channels (40), the Na\textsuperscript{+}-coupled glucose transporter SLC5A1 (41), several Na\textsuperscript{+}-coupled amino acid transporters (11, 41), the betaine/GABA transporter BGT1 (43), and the Na myoinositol transporter SMIT (39). JAK2 downregulates the Na\textsuperscript{+}-coupled creatine transporter SLC6A8 (73). Thus JAK2 participates in the regulation of several Na\textsuperscript{+}-coupled transporters. Nothing is known, however, about a role of JAK2 in the regulation of Na\textsuperscript{+}/K\textsuperscript{+}-ATPase (75), the most prominent member of the P-type ATPase family that includes further members, such as the sarcoplasmic reticulum Ca\textsuperscript{2+}-ATPase (59) and the gastric H\textsuperscript{+}/K\textsuperscript{+}-ATPase (72). Na\textsuperscript{+}/K\textsuperscript{+}-ATPase pumps three Na\textsuperscript{+} ions out of and two K\textsuperscript{+} ions into the cell per molecule of ATP hydrolyzed and thereby creates Na\textsuperscript{+} and K\textsuperscript{+} concentration gradients across the plasma membrane (1, 64). K\textsuperscript{+} can be substituted by other monovalent metal ions (including Na\textsuperscript{+} itself) and even organic cations (67), whereas only Li\textsuperscript{+} and H\textsuperscript{+} can substitute partially for Na\textsuperscript{+} (12). K\textsuperscript{+} ions accumulated by the Na\textsuperscript{+}/K\textsuperscript{+}-ATPase exit through K\textsuperscript{+} channels, thus maintaining the potential difference across the cell membrane (48). The Na\textsuperscript{+}/K\textsuperscript{+}-ATPase is a major cellular energy sink (6, 10, 17, 22, 25, 27–29). In cultured neurons, Na\textsuperscript{+}/K\textsuperscript{+}-ATPase inhibitor ouabain prevented ~50% of the ATP consumption (10). As much as 40% of metabolism in EEG-arrested barbiturate-inhibited brain could be related to Na\textsuperscript{+}/K\textsuperscript{+} fluxes and associated transport (5).

The present study explored the role of JAK2 in energy depletion and its role in the regulation of Na\textsuperscript{+}/K\textsuperscript{+}-ATPase activity. To this end, JAK2 tyrosine phosphorylation and activity as well as cytosolic ATP levels were determined following energy depletion in Jurkat T cells induced by exposure of the cells to sodium azide (NaN\textsubscript{3}) (44, 46, 53) or 2,4- dinitrophenol (DNP) (50, 69, 84). In addition, the effect of JAK2 on transcript and protein abundance determined by real-time PCR and Western blotting, respectively. Ouabain-sensitive K\textsuperscript{+}-induced currents (I\textsubscript{pump}) were measured by whole cell patch clamp. I\textsubscript{pump} was further determined by dual-electrode voltage clamp in Xenopus oocytes injected with RNA-encoding JAK2, active V617F-JAK2, or inactive K882E-JAK2. As a result, in Jurkat T cells, JAK2 activity significantly increased following energy depletion by sodium azide (NaN\textsubscript{3}) or 2,4- dinitro phenol (DNP). DNP- and NaN\textsubscript{3}-induced decrease of cellular ATP was significantly augmented by JAK2 inhibitor AG490 and blunted by Na\textsuperscript{+}/K\textsuperscript{+}-ATPase inhibitor ouabain. DNP-decreased and AG490-enhanced I\textsubscript{pump} as well as Na\textsuperscript{+}/K\textsuperscript{+}-ATPase α1-subunit transcript and protein abundance. The α1-subunit transcript levels were also enhanced by signal transducer and activator of transcription-5 inhibitor CAS 285986-31-4. In Xenopus oocytes, I\textsubscript{pump} was significantly decreased by expression of JAK2 and V617F-JAK2 but not of K882E-JAK2, effects again reversed by AG490. In V617F-JAK2-expressing Xenopus oocytes, neither DNP nor NaN\textsubscript{3} resulted in further decline of I\textsubscript{pump}. In Xenopus oocytes, the effect of V617F-JAK2 on I\textsubscript{pump} was not prevented by inhibition of transcription with actinomycin. In conclusion, JAK2 is a novel energy-sensing kinase that curtails energy consumption by downregulating Na\textsuperscript{+}/K\textsuperscript{+}-ATPase expression and activity.

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JAK2-REGULATED Na⁺/K⁺-ATPase

penicillin/streptomycin at 37°C in a humidified atmosphere with 5% CO₂. Gradual energy depletion was induced in Jurkat T cells by incubating 2 × 10⁷ cells with 2 mM 2,4- dinitro phenol (DNP, Sigma-Aldrich) for different time points ranging from 0 to 8 h. In a separate series of experiments, energy depletion was induced by 5 mM sodium azide (Na₃N₃, Sigma-Aldrich) incubation (0–8 h). In some series, JAK2 inhibitor AG490 (40 μM, Calbiochem) was applied in the absence or in the presence of ouabain (100 μM, Calbiochem) and in the absence and presence of DNP (2 mM) or NaN₃ (5 mM). In one series, STAT5 inhibitor (CAS 285986-31-4, 50 μM, 8 h, Calbiochem) was used in the absence or in the presence of DNP (2 mM). At the end of the treatment, the cells were harvested and centrifuged to remove the medium and later used for analysis by radioactive kinase assay, ATP assay, RT-PCR, Western blotting, and patch clamp.

In vitro kinase assays. For determination of kinase activity, 2 × 10⁷ cells/time point were lysed in lysis buffer [0.2% Nonidet P40, 150 mM NaCl, 50 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), pH 7.5, 1 mM glycerol, 10 mM NaF, 10 mM β-glycerophosphate, 1 mM Na₃VO₄, and protease inhibitors]. JAK2 immunoprecipitation (Cell Signaling) was performed overnight at 4°C. Precipitates were washed and resuspended in kinase buffer [50 mM Tris-HCl (pH = 8.0), 5 mM MgCl₂, 2 mM MnCl₂, 0.5 mM DTT] plus 10 μCi γ³²P-ATP (GE HealthCare) and 5 μg bovine Myelin Basic Protein (MBP₁₃–₁₀₄) (Millipore). For the kinase reaction, incubation was 30 min at 30°C. Proteins were separated by SDSPAGE followed by autoradiography.

ATP assay. Intracellular ATP levels were measured by a luciferase-based assay (ATP Bioluminescence Assay kit CLS II, Roche Diagnostics) on a luminometer (Berthold Biotumat LB9500). To generate extracts for the ATP assay, Jurkat T cells (for each measurement 8 × 10⁶ cells per well in a 96-well plate) were preincubated 1 h without or with AG490, subsequently exposed to DNP (2 mM, 4 or 8 h) or NaN₃ (5 mM, 4 or 8 h), washed twice with ice-cold PBS, drained, solubilized into 100 μl of 100 mM Tris-HCl (pH 7.5) and 4 mM EDTA, and snap frozen in liquid nitrogen. Frozen cells were boiled for 5 min, placed on ice for 5 min, and then centrifuged at 2,000 revolution/min for 5 min at 4°C. The lysate (20 μl) was used for ATP determination in triplicate.

Real-time PCR. To determine α₁ subunit of the Na⁺/K⁺-ATPase (ATPα₁) mRNA abundance, total RNA was extracted from Jurkat T cells using the TriFast reagent (Peqlab) according to the manufacturer’s instructions. Subsequently ~2 μg of total RNA was reverse transcribed to cDNA using oligo-dT primers (Roche Diagnostics) and Transcriptor High Fidelity cDNA Synthesis Kit (Roche Diagnostics). Quantitative real-time PCR was applied on the CFX96 Real-Time System C1000 Thermal Cycler (Bio-Rad) using 500 nM forward and reverse primer and 2× GoTaq qPCR Master Mix SYBR Green (Promega) according to the manufacturer’s protocol. For the amplification, the following primers were used (5’→3’ orientation): ATPα₁ forward CAGGTCATTTGTGTAACAGGGCCAG, ATPα₁ reverse GCTGACTCAGAGGCATCTCCT.

The transcript levels of the housekeeping gene β-actin were determined for each sample using the following primers (5’→3’ orientation): β-actin forward CAGGGGGTGATGTTGGG, β-actin reverse CAAACATGATCGGCTCAGTC. Relative quantification of gene expression was achieved using the △△Ct method.

Western blotting. To examine phosphorylation of the activation loop of JAK2 and protein abundance of Na⁺/K⁺-ATPase α₁-subunit, Jurkat T cells were lysed in lysis buffer (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1% Triton X-100, 0.5% SDS, 1 mM NaF, 1 mM Na₃VO₄, 0.4% β-mercaptoethanol) containing protease inhibitor cocktail (Sigma). Fifty micrograms of protein were solubilized in Laemmli sample buffer at 95°C for 5 min and resolved by 10% SDS-PAGE. For immunoblotting, proteins were electrotransferred onto a nitrocellulose membrane and blocked with 5% nonfat milk in Tris-buffered saline (TBS)-0.1% Tween 20 (TBST) at room temperature for 1 h. The membranes were incubated with affinity-purified phospho-JAK2 (Tyr1007/1008) rabbit monoclonal (1:1,000; 125 kDa, Cell Signaling) or ATPase-α1 rabbit (1:1,000; 100 kDa, Cell Signaling), or GAPDH monoclonal rabbit (1:1,000; 37 kDa, Cell Signaling) antibody at 4°C overnight. To determine total JAK2 protein, the same membrane that was used for the detection of phospho-JAK2 was stripped with reblot stripping buffer (Millipore) for 15 min followed by washing and blocking with 5% milk in TBST. The membrane was then incubated with affinity-purified JAK2 rabbit monoclonal antibody (1:1,000; 125 kDa, Cell Signaling) at 4°C overnight. After being washed three times with TBST (10 min each), the blots were incubated with horseradish peroxidase-conjugated secondary anti-rabbit antibody (1:2,000; Cell Signaling) for 1 h at room temperature. After the washing, antibody binding was detected with the ECL detection reagent (Amersham). Antibody binding was quantified with Quantity One Software (Bio-Rad).

Patch-clamp experiments in Jurkat cells. Ouabain-sensitive K⁺-induced currents (ifamp) reflecting Na⁺/K⁺-ATPase activity were measured for determination of intracellular Na⁺/K⁺ ratios.

Fig. 1. Activation and phosphorylation of Janus kinase-2 (JAK2) in Jurkat T cells following ATP depletion induced by the electron transport chain uncoupler 2,4-dinitro phenol (DNP). Jurkat T cells were treated for the indicated time points with DNP (2 mM). Cells were lysed and subjected to immunoprecipitation by JAK2-specific antibodies. The kinase activity of precipitated JAK2 was determined in a radioactive in vitro kinase assay by phosphorylation of myelin basic protein (MBP) followed by radioautography. The phosphorylation at the activation loop was determined by Western blotting. A: representative blot displaying the radioactive kinase assay for JAK2 activation following ATP depletion by DNP (2 mM). Top: kinase assay with substrate MBP₁₃–₁₀₄. Bottom: total JAK2 protein in the samples detected by immunoprecipitation (IP) followed by Western blotting (WB). This blot is representative of 5 independent experiments. B: original Western blot of tyrosine-phosphorylated JAK2 protein in Jurkat cells following ATP depletion by DNP (2 mM). The blot was probed with phospho-JAK2 (Tyr1007/1008) antibody and then stripped and reprobed with JAK2 antibody. The data represent 3 independent repetitions. C: arithmetic means (± SE, n = 3) of phospho-JAK2/JAK2 ratio analyzed from Western blots as shown in B. *P < 0.05, ANOVA.
determined by whole cell patch-clamp recording in human Jurkat T cells before and following energy depletion with 2 mM DNP for 4 h in the presence and absence of JAK2 inhibitor AG490 (40 μM). Alternatively, Jurkat cells were pretreated with AG490 (40 μM) alone for 4 h. Patch-clamp experiments were performed at room temperature in voltage-clamp, fast whole cell mode (35, 70, 79). Cells were continuously superfused through a flow system inserted into the dish. The bath was grounded via a bridge filled with the external solution. Borosilicate glass pipettes (2- to 4-MΩ resistance; Harvard Apparatus) manufactured by a microprocessor-driven DMZ puller (Zeitz) were used in combination with a MS314 electrical micromanipulator (MW, Märzhäuser). The currents were recorded by an EPC-9 amplifier (Heka) and analyzed with Pulse software (Heka) and an ITC-16 Interface (Instrutech). Currents were recorded at an acquisition frequency of 10 kHz and 3 kHz low pass filtered.

To measure Na+/K+-ATPase activity, ouabain (100 μM)-sensitive K+-induced outward currents were recorded. The pipette solution used contained (in mM): 30 NaCl, 20 KCl, 70 CsCl, 5 MgCl₂, 5 HEPES, 5 Na₂ATP, and 5 ethylene glycol tetraacetic acid (EGTA)

The external solution contained (in mM): 60 NaCl, 80 TEA-Cl, 1 MgCl₂, 2.5 CaCl₂, 5 NiCl₂, 5 glucose, 10 HEPES (pH 7.4, CsOH).

Fig. 2. Activation and phosphorylation of JAK2 in Jurkat T cells following energy depletion induced by sodium azide (NaN₃). Jurkat T cells were treated for the indicated time periods with NaN₃ (5 mM). Cells were lysed and subjected to immunoprecipitation by JAK2-specific antibodies. The kinase activity of precipitated JAK2 was determined in a radioactive in vitro kinase assay by phosphorylation of MBP followed by radioautography. The phosphorylation at the activation loop was determined by Western blotting. A: representative blot showing the results of the radioactive kinase assay on JAK2 activation following ATP depletion by NaN₃ (5 mM). Top: kinase assay with substrate MBP₁₃–₁₀₄. Bottom: total JAK2 protein in the samples detected by immunoprecipitation followed by Western blotting. This blot is representative of 5 independent experiments. B: original Western blot of tyrosine-phosphorylated JAK2 protein in Jurkat cells following ATP depletion by NaN₃ (5 mM). The blot was probed with phospho-JAK2 (Tyr1007/1008) antibody and then stripped and reprobed with JAK2 antibody. The data represent 3 independent repetitions. C: arithmetic means (± SE, n = 3) of phospho-JAK2/JAK2 ratio analyzed from Western blots as shown in B. **P < 0.01, ANOVA.

Fig. 3. Cellular ATP level in the presence and in the absence of JAK2 inhibitor AG490 upon energy depletion induced by DNP or NaN₃ in Jurkat T cells. A: arithmetic means ± SE (n = 4) of intracellular ATP levels as measured by firefly luciferin-luciferase luminescence intensity in Jurkat T cells treated with (solid bars) or without (open bar) JAK2 inhibitor AG490 (40 μM) upon energy depletion induced by DNP (2 mM) for 0–8 h. ATP levels were normalized to respective values in untreated cells (first open bar). *P < 0.05, **P < 0.01, ***P < 0.001, ANOVA. B: arithmetic means ± SE (n = 4) of intracellular ATP levels in Jurkat T cells treated with (solid bars) or without (open bar) JAK2 inhibitor AG490 (40 μM) upon energy depletion induced by NaN₃ (5 mM) for 0–8 h. ATP levels were normalized to respective values in untreated cells (first open bar). *P < 0.05, **P < 0.01, ***P < 0.001, ANOVA. C: arithmetic means ± SE (n = 8–12) of intracellular ATP levels in Jurkat T cells treated with (solid bars) or without (open bar) JAK2 inhibitor AG490 (40 μM) upon energy depletion induced by DNP (2 mM) for 4 h in the absence or in the presence of ouabain (100 μM, 4 h). ATP levels were normalized to respective values in untreated cells (first open bar). *P < 0.05, **P < 0.01, ***P < 0.001, ANOVA.
and 0.5 EGTA, Na⁺/K⁺-ATPase currents were elicited by switching to a bath solution that contained 60 NaCl, 80 TEA-Cl, 5 KCl, 1 MgCl₂, 2.5 CaCl₂, 5 NiCl₂, 5 glucose, and 10 HEPES (pH 7.4, to a bath solution that contained 60 NaCl, 80 TEA-Cl, 5 KCl, 1 MgCl₂, 2.5 CaCl₂, 5 NiCl₂, 5 glucose, and 10 HEPES (pH 7.4, CsOH). The currents were measured at −40 mV.

**Voltage-clamp experiments in Xenopus oocytes.** Xenopus oocytes were explanted from adult Xenopus laevis (NASCO). Xenopus laevis frogs were anaesthetized by a 0.1% Tricain solution. After confirmation of anesthesia and disinfection of the skin, a small abdominal incision was made and oocytes were removed, followed by closure of the skin with sutures. All animal experiments were conducted according to the German law for the welfare of animals, and the surgical procedures on the adult Xenopus laevis were reviewed and approved by the respective government authority of the state Baden-Württemberg (Regierungspräsidium) before the start of the study (Anzeige für Organentnahme nach §6).

For generation of cRNA, constructs were used encoding wild-type human JAK2 (Imagenes). The inactive K882EJAK2 mutant (30) and the gain of function V617FJAK2 mutant (42, 52) were generated by site-directed mutagenesis (QuikChange II XL Site-Directed Mutagenesis Kit; Stratagene) as described previously (58).

The cRNA was generated, and the Xenopus oocytes were prepared for voltage-clamp analysis, as previously described (13). Oocytes were injected with wild-type JAK2 cRNA, V617FJAK2, or K882EJAK2 cRNA (10 ng) on the first day after preparation of the Xenopus oocytes. All experiments were performed at room temperature (about 22°C) 3 days after the injection. Two-electrode voltage-clamp recordings were performed (55) at a holding potential of −30 mV for determination of the endogenous Na⁺/K⁺-ATPase. The data were filtered at 10 Hz and recorded with a GeneClampex 500 amplifier, a DigiData 1300 A/D-D/A converter, and the pClampfit 9.2 software packages for data acquisition and analysis (Axon Instruments) (38). The control bath solution (superfusate/ND96) contained (in mM): 96 NaCl, 1.8 CaCl₂, 1 MgCl₂, and 5 HEPES, pH 7.4 (NaOH), supplemented with gentamycin (100 mg/l), tetracycline (50 mg/l), and ciprofloxacin (1.6 mg/l). The flow rate of the superfusion was 20 ml/min, and a complete exchange of the bath solution was reached within about 10 s (2). To determine electrotransport by Na⁺/K⁺-ATPase, the oocytes were incubated for 4 h in a K⁺-free solution containing (in mM): 96 NaCl, 1.8 CaCl₂, 1 MgCl₂, 5 HEPES, and 25 sucrose, pH 7.4 (NaOH) (3). Subsequently, the oocytes were exposed to the same solution containing, in addition, 5 mM BaCl₂ (replacing 10 mM sucrose) for inhibition of K⁺ channels. Then 5 mM KCl (replacing 10 mM sucrose) was added in the continuous presence of BaCl₂. Where indicated, ouabain (1 mM) was added to inhibit the K⁺-induced outward current. The ND96 solution was supplemented where indicated, with AG490 (40 μM) actinomycin D (50 nM), DNP (2 mM), and NaN₃ (5 mM).

**Statistical analysis.** Data are provided as means ± SE; n represents the number of oocytes, cells, or individual experiments. All voltage-clamp experiments were repeated with at least two to three batches of oocytes; in all repetitions, qualitatively similar data were obtained. Data were tested for significance using ANOVA, Bonferroni multiple-comparisons test, or t-test, as appropriate. Results with P < 0.05 were considered statistically significant.

**RESULTS**

**JAK2 phosphorylation and activation following ATP depletion by DNP or NaN₃ treatment of Jurkat T cells.** Exposure of Jurkat T cells to DNP (2 mM) resulted in a gradual activation of JAK2 in a time-dependent manner, as measured by the radioactive kinase assay (Fig. 1A). The maximal activation of JAK2 was observed 8 h after DNP treatment of Jurkat T cells (Fig. 1A). JAK2 is known to be activated by phosphorylation at the tyrosine in position 1007/1008. Thus Western blot analysis was performed to test whether JAK2 phosphorylation is modified by energy depletion. To this end, Jurkat T cells were pretreated for 0–8 h with DNP. As illustrated in Fig. 1, B and C. DNP treatment indeed significantly increased (Tyr 1007/1008) JAK2 phosphorylation.

Additional experiments were performed to elucidate whether JAK2 activation and phosphorylation are similarly influenced by sodium azide (NaN₃, 5 mM). As illustrated in Fig. 2. JAK2 kinase activity as well as JAK2 phosphorylation at tyrosine

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**Fig. 4. Sensitivity of Na⁺/K⁺-ATPase currents in Jurkat T cells to DNP-induced energy depletion and JAK2 inhibitor AG490.** A: original whole cell tracings recorded at −40 mV in untreated (control) Jurkat cells in the absence (Na⁺) and in the presence of 5 mM K⁺ (K⁺) in the bath solution. K⁺ was added either in the absence (left) or in the presence (right) of ouabain (100 μM). B: original whole cell tracings recorded at −40 mV in Jurkat T cells following 4-h incubation with 2 mM DNP (left), 40 μM JAK2 inhibitor AG490 (middle), or AG490 and DNP together (right). The currents were recorded in the absence (Na⁺) and in the presence of 5 mM K⁺ (K⁺) in the bath solution. C: mean whole cell current at −40 mV normalized to cell capacitance (current density ± SE, n = 7–42) in Jurkat cells treated as in B. ***P < 0.01, ***P < 0.001, ANOVA; #P < 0.01, at comparison with unpaired t-test.
1007/1008 were significantly increased by 2 h of incubation with NaN3 (5 mM).

Enhanced ATP loss in Jurkat T cells pretreated with the JAK2 inhibitor AG490. Pretreatment of Jurkat T cells with DNP (2 mM, Fig. 3A) or NaN3 (5 mM, Fig. 3B) resulted in a gradual decrease of the ATP level. In the presence of the JAK2 inhibitor AG490 (40 μM, added 1 h before addition of DNP or NaN3), the decline of the ATP level was significantly enhanced (Fig. 3, A and B). Accordingly, JAK2 activity apparently contributed to conservation of cellular ATP levels during energy depletion. In the presence of the Na+/K+-ATPase inhibitor ouabain (100 μM, 4 h), the augmentation of DNP-induced ATP loss by AG490 was virtually abolished (Fig. 3C). Accordingly, JAK2 may accomplish ATP conservation by inhibition of the Na+/K+-ATPase.

Sensitivity of Na+/K+-ATPase activity in Jurkat T cells to energy depletion and JAK2. According to whole cell patch clamp, addition of K+ to the extracellular solution triggered an ouabain-sensitive outward current in Jurkat T cells reflecting the electrogenic transport by the Na+/K+-ATPase (Fig. 4A). The current following addition of K+ reflected electrogenic extrusion of 3 Na+ in exchange for 2 K+ (Fig. 4A). The outward current generated by addition of K+ to the extracellular solution (I_pump) was strongly increased by the JAK2 inhibitor AG490 (40 μM, 4 h, Fig. 4, B and C). When Jurkat T cells were pretreated with DNP (2 mM, 4 h) to induce energy depletion, I_pump was slightly reduced compared with untreated cells (Fig. 4, B and C). Pretreatment of Jurkat T cells with AG490 (40 μM) 1 h before addition of DNP not only completely rescued I_pump from DNP-induced inhibition (Fig. 4, B and C), but also resulted in enhanced I_pump compared with untreated cells (Fig. 4C).

JAK2-dependent regulation of transcript and protein abundance of the α1-subunit of the Na+/K+-ATPase in Jurkat T cells. JAK2 may influence Na+/K+-ATPase in part by stimulation of Na+/K+-ATPase expression. As illustrated in Figs. 5 and 6, transcript (Fig. 5A) and protein (Fig. 6) levels of the Na+/K+-ATPase α1-subunit were increased by JAK2 inhibitor AG490 (40 μM, 8 h). Moreover, energy depletion induced by DNP (2 mM, 8 h) resulted in a decrease in transcript (Fig. 5A) and protein (Fig. 6) abundance of the Na+/K+-ATPase α1-subunit, an effect abolished in the presence of JAK2 inhibitor (Figs. 5A and 6). The transcript abundance of the Na+/K+ pump α1-subunit was also increased by STAT5 inhibitor (CAS 285986-31-4, 50 μM, 8 h, Fig. 5B), suggesting JAK2-STAT5-mediated inhibition of Na+/K+-ATPase transcription.

JAK2 sensitivity of Na+/K+-ATPase activity in Xenopus oocytes. To confirm that Na+/K+-ATPase activity is regulated by JAK2, K+-induced and ouabain-sensitive pump currents were measured in Xenopus oocytes, heterologously expressing JAK2. To measure Na+/K+-ATPase activity, Xenopus oocytes were first preincubated for 4 h in K+-free solution and thereafter superfused for a few minutes with K+-free bath solution. Subsequently, the K+-channel blocker Ba2+ (5 mM) was added to abrogate K+ fluxes through K+ channels. The readdition of K+ was followed by an outwardly directed pump current (I_pump), which was inhibited by ouabain (1 mM) (Fig. 7A). Both I_pump and the ouabain-sensitive current (I_ouabain) were significantly smaller in Xenopus oocytes injected with cRNA encoding JAK2 than in oocytes injected with diethyl pyrocarbonate (DEPC) water (Fig. 7). Thus JAK2 inhibited the endogenous Na+/K+-ATPase in Xenopus oocytes. Similar to expression of wild-type JAK2 in Xenopus oocytes, expression of the active mutant V617F JAK2 resulted in a significant reduction of I_pump and I_ouabain (Fig. 8). In contrast, expression of the inactive mutant K882E JAK2 did not significantly alter I_pump and I_ouabain (Fig. 8).

Sensitivity of Na+/K+-ATPase activity in V617F JAK2-expressing Xenopus oocytes to pharmacological JAK2 inhibition. Incubation of oocytes expressing V617F JAK2 with JAK2 inhibitor AG490 (40 μM) was followed by a gradual increase in I_pump, reaching statistical significance at 6-h and 24-h incubation (Fig. 9B). The effect of the inhibitor on I_ouabain in Xenopus oocytes reached statistical significance within 24 h of drug exposure (Fig. 9C).

Effect of NaN3 and DNP on Na+/K+-ATPase activity in Xenopus oocytes. The effects of NaN3 (5 mM) and DNP (2 mM) on Na+/K+-ATPase activity were further analyzed at...
different exposure times (3–8 h) in water-injected and in V617FJAK2-expressing Xenopus oocytes. Incubation with NaN3 significantly reduced I_pump in water-injected oocytes (Fig. 10A). The inhibitory effect of NaN3 was completely absent in V617FJAK2-expressing Xenopus oocytes (Fig. 10A). Similar effects on I_pump in water-injected oocytes and V617FJAK2-expressing Xenopus oocytes were observed upon incubation with DNP (Fig. 10B).

Effect of actinomycin D on Na+/K+-ATPase activity in V617FJAK2-expressing Xenopus oocytes. To test whether the effect of V617FJAK2 on Na+/K+-ATPase activity of Xenopus oocytes required transcription, experiments were performed in the presence of actinomycin D (50 nM, added simultaneously with the mRNA injection). As a result, in the continued presence of actinomycin, I_pump (Fig. 11A) and I_ouabain (Fig. 11B) were both significantly lower in oocytes expressing active V617FJAK2 than in oocytes expressing inactive K882EJAK2. Thus JAK2 is effective even in the absence of transcription.

DISCUSSION

The present observations disclose a completely novel property and function of JAK2, i.e., energy sensitivity and regulation of Na+/K+-ATPase abundance and activity. Our data reveal a gradual increase in cellular JAK2 activity by mitochondrial uncoupler DNP and respiratory chain inhibitor NaN3, as determined by both radioactive kinase assay and Western blotting of JAK2 phosphorylated at the activation loop Tyr1007/1008. Tyrosine phosphorylation of the JAK2 activation loop is essential for a high-activity catalytic state but is dispensable for basal catalytic activity (18).

JAK2 is widely expressed (81) and could serve as an energy sensor in a multitude of cells. Hypoxia leads to enhanced JAK2

Activation of the JAK2/STAT5 axis has been proposed to mediate cell tolerance to hypoxia (26). In cardiomyocytes undergoing hypoxia reperfusion, JAK2/STAT5 propagate a prosurvival signal (86). Consistently, apoptosis of renal cells induced by ATP depletion was promoted by inhibition of JAK2 with AG490 (83). Moreover, tissue hypoxia is characteristic of solid tumors, and hence the activation of JAK2 upon energy depletion could serve as a mechanism conferring tumor cell survival. One established mechanism of JAK2/STAT5-mediated cell survival is the suppression of proapoptotic Fas (CD95)/FasL (CD95L) (26).

The inhibition of the Na+/K+-ATPase by JAK2 during cellular energy depletion may represent another prosurvival mechanism. The Na+/K+-ATPase is responsible for 20% to 80% of cellular resting metabolic rate (56) and for ~30% of cellular ATP consumption (31, 65, 66, 78). Impaired Na+/K+-ATPase activity decreases energy consumption and may thus help the cell to overcome a transient period of energy shortage.

Fig. 1. Representative photomicrograph of the Xenopus oocyte injection chamber. A: before injection. B: after injection. C: after lysis of the injected oocyte.
Fig. 8. Decrease of Na\(^+\)/K\(^+\)-ATPase activity in *Xenopus* oocytes by active \(V_{617}^{\text{F}}\)JAK2 but not by inactive \(K_{882}^{\text{E}}\)JAK2. A: representative original tracings in *Xenopus* oocytes injected with DEPC water alone (a), with cRNA-encoding JAK2 (b), with cRNA-encoding inactive \(K_{882}^{\text{E}}\)JAK2 (c), or with cRNA-encoding active \(V_{617}^{\text{F}}\)JAK2 (d). B: arithmetic means (± SE, \(n = 13–14\)) of K\(^+\)-induced currents measured in *Xenopus* oocytes injected with water (open bars), with cRNA-encoding wild-type JAK2 (solid bars), constitutive active \(V_{617}^{\text{F}}\)JAK2 (dark gray bars), or inactive mutant \(K_{882}^{\text{E}}\)JAK2 (light gray bars). ***\(P < 0.001\), ANOVA. C: arithmetic means (± SE, \(n = 13–14\)) of ouabain-sensitive currents measured in *Xenopus* oocytes injected with water (open bars), with cRNA-encoding wild-type JAK2 (solid bars), active \(V_{617}^{\text{F}}\)JAK2 (dark gray bars), or inactive \(K_{882}^{\text{E}}\)JAK2 (light gray bars). ***\(P < 0.001\), ANOVA.

Fig. 9. Effect of JAK2 inhibitor AG490 on Na\(^+\)/K\(^+\)-ATPase activity in \(V_{617}^{\text{F}}\)JAK2-expressing *Xenopus* oocytes. A: representative original tracings recorded in *Xenopus* oocytes injected with DEPC water (a) or with cRNA-encoding \(V_{617}^{\text{F}}\)JAK2 without (b) or with a 6-h (c) or 24-h (d) pretreatment with JAK2 inhibitor AG490 (40 μM). B: arithmetic means (± SE, \(n = 9–14\)) of K\(^+\)-induced currents measured in *Xenopus* oocytes injected with water (open bars) or with cRNA-encoding active \(V_{617}^{\text{F}}\)JAK2 in the absence of inhibitor (solid bars) or following pretreatment with the JAK2 inhibitor AG490 (40 μM) (shaded bars) for the indicated time periods. *\(P < 0.05\), ***\(P < 0.001\), ANOVA. C: arithmetic means (± SE, \(n = 9–14\)) of ouabain-sensitive currents measured in *Xenopus* oocytes injected with water (open bars) or with cRNA-encoding active \(V_{617}^{\text{F}}\)JAK2 in the absence of inhibitor (solid bars) or following pretreatment with the JAK2 inhibitor AG490 (40 μM) (shaded bars) for the indicated time periods. ***\(P < 0.001\), ANOVA.
We show here that JAK2 suppresses the Na+/K+ -ATPase protein abundance and activity. This suppressive effect occurs already under resting conditions, as evident from the stimulating effect of the JAK2 inhibitor AG490 on the pump current and the transcript and protein levels of the Na+/K+ -ATPase \( \alpha 1 \)-subunit. The decrease of the pump current and \( \alpha 1 \)-subunit transcript/protein level following energy depletion by DNP could be completely reversed by AG490, suggesting that JAK2 regulates Na+/K+ -ATPase \( \alpha 1 \)-subunit expression presumably in part via STAT5 because pharmacological inhibition of STAT5 enhances transcription of the Na+/K+ -ATPase \( \alpha 1 \)-subunit. Similarly, STAT5 inhibition could reverse DNP-induced downregulation of the \( \alpha 1 \)-subunit transcription. The decay of Na+/K+ -ATPase activity following treatment of Xenopus oocytes with actinomycin was, however, not significantly different in oocytes expressing active \( ^{V617F} \)JAK2 and inactive \( ^{K882E} \)JAK2, suggesting that non-genomic effects did significantly contribute to JAK2-dependent regulation of Na+/K+ -ATPase activity.

It is worth noting that the observed DNP-induced decline of the Na+/K+ -ATPase activity in the present experiments is not just due to the ATP loss upon DNP treatment because the intracellular ATP concentration in whole cell patch-clamp experiments was maintained constant by the pipette solution. In unpatched cells, the intracellular ATP level declined moderately upon DNP or NaN3 treatment, an effect significantly enhanced by the JAK2 inhibitor AG490. Importantly, the decline of cytosolic ATP following DNP treatment in the presence of AG490 could be virtually abolished by ouabain, suggesting that ATP depletion in the presence of AG490 was in large part attributable to Na+/K+ -ATPase activity.

Recently, JAK2 has been shown to upregulate the Na+-coupled glucose transporter SLC5A1 (41) and several Na+-coupled amino acid transporters (11, 41). On the other hand, JAK2 downregulates the Na+-coupled creatine transporter SLC6A8 (73). During energy depletion, Na+-coupled transport could further be inhibited by depolarization of the cell membrane (48). Impairment of the Na+/K+ -ATPase either by pharmacological inhibition (54), lowering of temperature (82), or cellular energy depletion (68) decreases the K+ conductance, thus leading to depolarization with the respective decrease of driving force for Na+-coupled transport processes. Thus, in addition to more direct effects of JAK2 on individual carriers, the inhibitory effect of JAK2 on Na+/K+ -ATPase activity is expected to curtail Na+ entry via electrogenic Na+-coupled transporters. Clearly, further studies are required to elucidate the potential effect of JAK2 on K+-ATPase activity.

JAK2 may not only be important for regulation of electrolyte transport during energy depletion. Acute administration of leptin has been shown to trigger natriuresis (8, 14, 34), which may at least in theory reflect decreased renal tubular Na+ reabsorption attributable to JAK2-mediated decrease of Na+/K+ -ATPase activity. JAK2 is activated by further hormones...
such as erythropoietin (23, 45, 71, 85). Thus JAK2 may participate in the regulation of Na/K+-ATPase activity, not only in energy-depleted cells, but also in hormone-stimulated cells.

The present study did not address the putative interaction of the AMP-activated kinase (AMPK) and JAK2 in energy sensing and regulation of Na/K+-ATPase activity. AMPK has been shown to downregulate Na/K+-ATPase by activation of PKC-ζ (19, 21, 32, 80). Along those lines, in alveolar epithelial cells, hypoxia leads to Na/K+-ATPase ubiquitination, endocytosis, and degradation (33, 37). Whereas acute hypoxia alters only the membrane abundance of the Na/K+-ATPase (21), long-term hypoxia leads to decreased total Na/K+-ATPase levels (20). On the other hand, AMPK has been reported to upregulate Na+/K+-ATPase activity (4, 9), the opposite of what is seen following expression of JAK2. The AMPK-induced downregulation of Na/K+-ATPase surface abundance was attributed to AS160, a Rab-GTPase-activating protein fostering internalization of the pump (4). To the best of our knowledge, activation of STAT5 by AMPK has never been shown. In the contrary, AMPK has been shown to inhibit JAK/STAT signaling (36).

In conclusion, JAK2 is activated by energy depletion and in turn downregulates the Na+/K+-ATPase, which is expected to decrease energy expenditure. Thus JAK2 may actively participate in the regulation of cell survival during energy depletion. JAK2-dependent inhibition of Na+/K+-ATPase may similarly participate in the cell-protective effects of the hormones leptin and erythropoietin during energy depletion.

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DISCLOSURES

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AUTHOR CONTRIBUTIONS


REFERENCES


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