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Up-regulation of the betaine/GABA transporter BGT1 by JAK2

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ABSTRACT

Janus-activated kinase-2 JAK2 is activated by hyperosmotic shock and modifies the activity of several Na^+ coupled transporters. Carriers up-regulated by osmotic shock include the Na^+ coupled osmolyte transporter BGT1 (betaine/GABA transporter 1), which accomplishes the concentrative cellular uptake of γ-amino-butyric acid (GABA). The present study thus explored whether JAK2 participates in the regulation of BGT1 activity. To this end, cRNA encoding BGT1 was injected into *Xenopus* oocytes with or without cRNA encoding wild type JAK2, constitutively active ^{V617F}JAK2 or inactive ^{K882E}JAK2, and electrogenic GABA transport determined by dual electrode voltage clamp. In oocytes injected with cRNA encoding BGT1 but not in oocytes injected with water or with cRNA encoding JAK2 alone, the addition of 1 mM GABA to the extracellular fluid generated an inward current (I_{BGT}). In BGT1 expressing oocytes I_{BGT} was significantly increased by coexpression of JAK2 or ^{V617F}JAK2, but not by coexpression of ^{K882E}JAK2. According to kinetic analysis coexpression of JAK2 increased the maximal I_{BGT} without significantly modifying the concentration required for halfmaximal I_{BGT} (K_M). In oocytes expressing BGT1 and ^{V617F}JAK2 I_{BGT} was gradually decreased by JAK2 inhibitor AG490 (40 μM). The decline of I_{BGT} following disruption of carrier insertion with brefeldin A (5 μM) was similar in the absence and presence of the JAK2 inhibitor AG490 (40 μM). In conclusion, JAK2 is a novel regulator of the GABA transporter BGT1. The kinase up-regulates the carrier presumably by enhancing the insertion of carrier protein into the cell membrane.

1. Introduction

The Janus-activated kinase-2 JAK2 contributes to the signaling of several hormones and cytokines [1–3] including leptin [4], growth hormone [5,6], erythropoietin [2], thrombopoietin [2] and granulocyte colony-stimulating factor [2]. Excessive JAK2 activity may lead to the development of malignancy and JAK2 inhibitors may be effective in the treatment of myeloproliferative disorders [7–12]. The gain of function mutation ^{V617F}JAK2 has been detected in and presumably contributes to development of myeloproliferative disease [13–16].

JAK2 is activated by oxidative stress and ischemia [17] and by hypertonicity [18,19]. The sensitivity to osmotic cell shrinkage renders the kinase a candidate signaling molecule participating in the regulation of cell volume. Mechanisms up-regulated during osmotic cell shrinkage include the activity of Na^+ coupled osmolyte transporters, such as the betaine/GABA transporter BGT1 (SLC6A12) [20,21]. BGT1 belongs to superfamily of Na^+, Cl^- coupled transporters for neurotransmitters (e.g. dopamine, GABA, serotonin and norepinephrine), amino acids (e.g. glycine) [22], creatine [22], and the organic osmolytes betaine [23] and taurine [24]. BGT1 is expressed in liver [25–27], kidney [27], airways [28], and brain [27,29].

Osmotic cell shrinkage up-regulates BGT1 expression [30–32]. Beyond that, the carrier is regulated by ATP [33], Ca^{2+} [34], protein kinase C [35], prostaglandin E_2 [36,37] and the cytoskeleton [38,39].

Cellular accumulation of osmolytes protects against apoptosis and thus participates in the regulation of cell survival [40].

The present study explored whether the activity of BGT1 is influenced by regulated by JAK2. To this end, BGT1 was expressed in *Xenopus* oocytes with or without wild type JAK2, constitutively active ^{V617F}JAK2 or inactive ^{K882E}JAK2 and the electrogenic GABA transport determined utilizing dual electrode voltage clamp.

2. Materials and methods

2.1. Constructs

Constructs were used encoding wild type BGT1 [41], and wild-type human JAK2 (Imagenes, Berlin, Germany). Further, an inactive ^{K882E}JAK2 mutant [42] and the ^{V617F}JAK2 mutant [16] were generated by site-directed mutagenesis (QuikChange II XL Site-Directed Mutagenesis Kit; Stratagene, Heidelberg, Germany) according to

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the manufacturer's instructions [43]. The following primers were used:

- ^{V617F}JAK2: 5′-AGCATTTGGTTTTAAATTATGGAGTATGTTTCTGTGGAGACGAGA-3′;
- ^{V617F}JAK2: 5′-TCTCGTCTCCACAGAAACATACTCCATAATTTAAAACCAAATGCT-3′;
- ^{K882E}JAK2: 5′-GGGAGGTGGTCGCTGTAGAAAAGCTTCAGCATAGT-3′; and
- ^{K882E}JAK2: 5′-ACTATGCTGAAGCTTTTCTACAGCGACCACCTCCC-3′.

Underlined bases indicate mutation sites. The mutants were sequenced to verify the presence of the desired mutation. The mutants were used for generation of cRNA as described previously [44].

2.2. Voltage clamp in Xenopus oocytes

Xenopus oocytes were prepared as previously described [45]. Where not indicated otherwise, 15 ng BGT1 cRNA on the first day and 10 ng of wild type JAK2 cRNA were injected on the second day or same day after preparation of the oocytes [46]. The oocytes were maintained at 17 °C in ND96 solution containing (in mM): 96 NaCl, 4 KCl, 1.8 $MgC1_2$, 0.1 $CaC1_2$, 5 HEPES, pH 7.4, gentamycin (100 mg/L), tetracycline (100 mg/L), ciprofloxacin (1.6 mg/L), theophylline (90 mg/L) where indicated, the JAK2 inhibitor AG490 (40 μM), actinomycin D (10 μM) or brefeldin A (5 μM) were added to the respective solutions. The voltage clamp experiments were performed at room temperature 4 days after injection [47]. Two-electrode voltage-clamp recordings were performed at a holding potential of −60 mV. The data were filtered at 10 Hz and

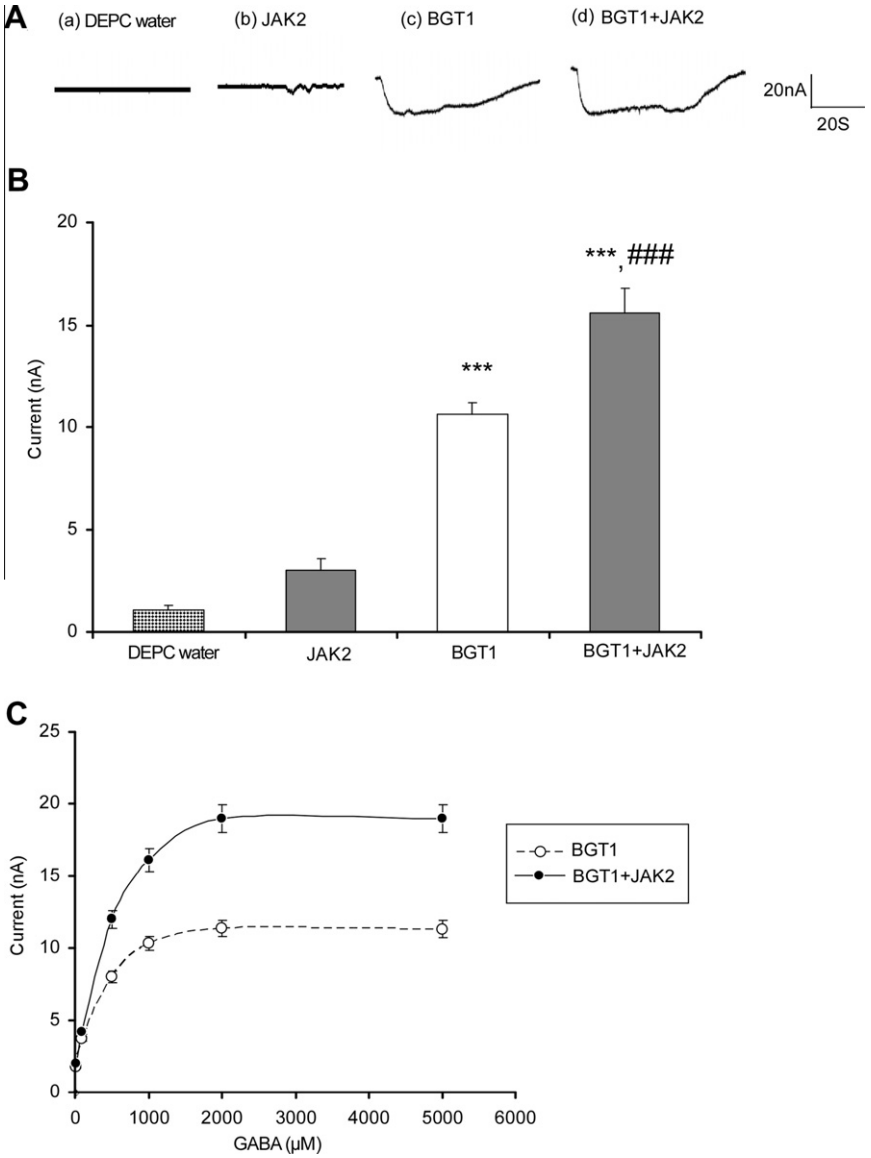

Fig. 1. Coexpression of JAK2 increases electrogenic GABA transport in BGT1-expressing *Xenopus* oocytes. (A) Representative original tracings showing GABA (1 mM)-induced current (I_{BGT}) in *Xenopus* oocytes injected with water (a), expressing wild type JAK2 alone (b), or expressing BGT1 without (c) or with (d) additional coexpression of wild type JAK2. (B) Arithmetic means ± SEM (n = 7) of GABA (1 mM)-induced current (I_{BGT}) in *Xenopus* oocytes injected with water (H_2O, dotted bar), expressing JAK2 alone (JAK2, dark gray bar), or expressing BGT1 without (BGT1, white bar) or with (BGT1 + JAK2, dark gray bar) additional coexpression of wild type JAK2. *** Indicates statistically significant ($p < 0.001$) difference from the absence of BGT1. ### Indicates statistically significant ($p < 0.001$) difference from the absence of JAK2. (C) Arithmetic means ± SEM (n = 3) of GABA-induced current (I_{BGT}) as a function of GABA concentration in *Xenopus* oocytes expressing BGT1 without (open circles, dashed line), or with (closed circles, solid line) additional coexpression of wild type JAK2.