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Molecular effects of lithium are partially mimicked by inositol-monophosphatase (IMPA)1 knockout mice in a brain region-dependent manner

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Abstract
We have previously shown that homozygote knockout (KO) of inositol-monophosphatase1 (*IMPA1*) results in lithium (Li)-like behavior. We now aimed to find out whether Li-treated mice and *IMPA1* KO mice exhibit neurochemical similarity at the gene- and protein-expression level. Hippocampal and frontal cortex B-cell lymphoma (*Bcl-2*), Bcl-2-associated X protein (*BAX*), *P53*, Perodoxin2 (*PRDX2*), myristoylated alanine-rich C kinase substrate (*MARCKS*) and neuropeptide Y (*NPY*) mRNA levels, and hippocampal, frontal cortex and hypothalamic cytokine levels, all previously reported to be affected by lithium treatment, were measured in three groups of mice: wildtype (WT) on regular-food (RF), WT on Li-supplemented food (Li-treated) and *IMPA1*-KOs.
Hippocampal and frontal cortex *Bcl-2* and *MARCKS* were the only genes commonly affected (downregulated) by Li and *IMPA1* KO; *Bcl-2* - by 28% and 19%, respectively; *MARCKS* – by about 20% in both regions.
The effect of Li and of *IMPA1* KO on cytokine levels differed among the three brain areas studied. Only in the hippocampus both interventions exerted similar effects. Frontal cortex cytokine levels were unaffected neither by Li nor by *IMPA1* KO.
Similar changes in *Bcl-2* and *MARCKS* but not in *PRDX2* and *NPY* following both Li-treatment and *IMPA1* KO suggest a mechanism different than inositol-monophosphatase1 inhibition for Li's effect on the latter genes. The cytokine levels results suggest that the mechanism mediating

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Li's effect on the inflammatory system differs among brain regions. Only in the hippocampus the results favor the involvement of the phosphatidylinositol (PI) cycle.

1. Introduction

Despite long-lasting use of lithium salts (Li) to treat bipolar disorder (BD) its mechanism of action is still not fully understood. The two main hypotheses for the molecular mechanism of its therapeutic beneficial effect are inhibition of glycogen synthase kinase (GSK)-3 (Klein and Melton, 1996) and inositol depletion as a consequence of inhibition of the brain-abundant inositol monophosphatase (IMPase) (Berridge and Irvine, 1989) encoded by *IMPA1*.

GSK-3 is constitutively active under basal conditions. Lithium inhibits GSK-3 in a direct and indirect manner (Klein and Melton, 1996). Inhibition of GSK-3 leads to activation of cell-survival transcription factors such as CREB, AP-1, β-catenin and heat-shock factor-1 (HSF-1) (Chiu and Chuang, 2010) and to inhibition of pro-apoptotic factors such as P53 (Watcharasit et al., 2002). Many of these effector systems, in turn, regulate members of the B-cell lymphoma (Bcl-2) protein family including Bcl-2 itself and Bcl-2-associated X protein (BAX) resulting in an overall anti-apoptotic effect (Chiu and Chuang, 2010).

Another anti-apoptotic target of lithium is Perodoxin 2 (PRDX2), a member of the peroxiredoxin family of antioxydative enzymes. PRDXs play a role in protein and lipid protection against oxidative injury, in cell proliferation and differentiation and in intracellular signaling pathways regulating apoptosis (Kim et al., 2000). Increased cellular levels of antioxidant enzymes confer protection against apoptosis. *PRDX2* was reported to be upregulated in a microarray study of lithium-modulated gene expression in human neuronal cells (Seelan et al., 2008).

Myristoylated alanine-rich C kinase substrate (MARCKS) is downregulated by lithium in an inositol-dependent manner (Lenox and Wang, 2003). It plays a role in CNS development, in secretion, phagocytosis, neuroplasticity and cytoskeleton remodeling (Lenox and Wang, 2003).

Neuropeptides such as neuropeptide Y (NPY), vascular endothelial growth factor (VEGF), neurokinin A and others have been reported to be altered in specific brain regions under mood stabilizers treatment and in animal models of depression (Chiu and Chuang, 2010; Mathe et al., 1990). Lithium was found to alter mRNA levels of *NPY* in specific brain regions (Zachrisson et al., 1995) possibly due to region-specific differences in the abundance of neuropeptide receptors and their subtypes throughout the brain.

Chronic inflammation has been suggested to be involved in the pathophysiology of many neuropsychiatric disorders, in general, and in bipolar disorder, in particular (Goldstein et al., 2009; Rapoport et al., 2009). Cytokines access the brain and interact with multiple pathophysiological domains relevant to BD (Leboyer et al., 2012). The effects of cytokines in the CNS include an array of metabolic, endocrinic [hypothalamic-pituatary-adrenal (HPA) axis] and behavioral alterations (Goldstein et al., 2009; Raison et al., 2006). In bipolar patients during episodes of mania and depression elevated plasma pro-inflammatory cytokine levels are consistently reported while reports related to anti-inflammatory cytokines are inconsistent (Goldstein et al., 2009). In major depression the most robust findings relate to IL-1β and IL-6 (Raison et al., 2006).

Lithium has been reported to affect immune modulation via second messenger systems and transcription factors (Rapaport and Manji, 2001). The drug exerts an immunosuppressive effect in immune system-related diseases such as HIV, autoimmune diseases and during transplantation (Rapaport and Manji, 2001). The drug's effect on plasma cytokine levels has been intensively studied (Goldstein et al., 2009) but its effect on brain levels was scarcely investigated (Rao et al., 2010).

The availability in our lab of *IMPA1* homozygote knockout (KO) mice exhibiting lithium-like behavioral phenotype in the pilocarpine-induced seizures paradigm and in the forced-swim test (FST) (Agam et al., 2009) provided the opportunity to assess the possibility that IMPase1 inhibition mediates lithium's molecular effects. To this end we compared brain mRNA levels of genes (*Bcl-2*, *BAX*, *P53*, *MARCKS*, *PRDX2* and *NPY*) previously reported to be altered by chronic lithium treatment (Chen and Chuang, 1999; Seelan et al., 2008) and levels of a wide array of cytokines in the brain of chronically Li-treated mice and *IMPA1* KOs.

2. Experimental procedure

2.1. Animals

Male, 10–12 weeks old mice were used for the experiments. Animals were maintained on a 12 h/12 h light/dark cycle (lights on between 8:00 a.m. and 8:00 p.m.) with *ad libitum* access to food and water. All tests were performed during the light phase of the cycle between 9:00 am and 7:00 pm. *IMPA1* KO mice were originally generated as previously described (Cryns et al., 2008). Homozygote *IMPA1* KO mice and wildtype (WT) mice were maintained in our animal-care facility by breeding heterozygote males and females. *Myo*-inositol supplementation in drinking water (3.5% w/v) to pregnant and lactating dams was used to rescue the KO mice. Genotyping was carried out as previously described (Cryns et al., 2008).

2.2. Chronic lithium treatment

WT mice were divided to two groups (control and Li-treatment) and subjected to lithium-supplemented food or regular food (RF) for two weeks, as previously described (Bersudsky et al., 2007). *IMPA1* KOs received regular food, identical to that of their WT-untreated littermates. Lithium plasma levels were measured in an ion-selective electrode apparatus ISE (AVL 9180 Electrolyte Analyzer, Hoffmann-La Roche, Basel, Switzerland). Lithium blood levels were in the range of 0.52–0.91 mM.