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Common effects of lithium and valproate on mitochondrial functions: protection against methamphetamine-induced mitochondrial damage

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Abstract

Accumulating evidence suggests that mitochondrial dysfunction plays a critical role in the progression of a variety of neurodegenerative and psychiatric disorders. Thus, enhancing mitochondrial function could potentially help ameliorate the impairments of neural plasticity and cellular resilience associated with a variety of neuropsychiatric disorders. A series of studies was undertaken to investigate the effects of mood stabilizers on mitochondrial function, and against mitochondrially mediated neurotoxicity. We found that long-term treatment with lithium and valproate (VPA) enhanced cell respiration rate. Furthermore, chronic treatment with lithium or VPA enhanced mitochondrial function as determined by mitochondrial membrane potential, and mitochondrial oxidation in SH-SY5Y cells. In-vivo studies showed that long-term treatment with lithium or VPA protected against methamphetamine (Meth)-induced toxicity at the mitochondrial level. Furthermore, these agents prevented the Meth-induced reduction of mitochondrial cytochrome c, the mitochondrial anti-apoptotic Bcl-2/Bax ratio, and mitochondrial cytochrome oxidase (COX) activity. Oligoarray analysis demonstrated that the gene expression of several proteins related to the apoptotic pathway and mitochondrial functions were altered by Meth, and these changes were attenuated by treatment with lithium or VPA. One of the genes, Bcl-2, is a common target for lithium and VPA. Knock-down of Bcl-2 with specific Bcl-2 siRNA reduced the lithium- and VPA-induced increases in mitochondrial oxidation. These findings illustrate that lithium and VPA enhance mitochondrial function and protect against mitochondrially mediated toxicity. These agents may have potential clinical utility in the treatment of other diseases associated with impaired mitochondrial function, such as neurodegenerative diseases and schizophrenia.

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Key words: Bcl-2, lithium, methamphetamine, mitochondria, valproate.

Introduction

In recent years, research has linked mood disorders with structural and functional impairments related to neuroplasticity in various regions of the central nervous system (CNS). Research on the biological underpinnings of mood disorders has therefore moved away from focusing on absolute changes in neurochemicals such as monoamines and neuropeptides, and instead has begun highlighting the role of neural circuits and synapses, and the plastic processes controlling their function. Indeed, accumulating evidence from microarray, biochemical, neuroimaging, and post-mortem brain studies all support the role of mitochondrial dysfunction in the pathophysiology of bipolar disorder (BPD) (Quiroz et al. 2008). Kato and colleagues recently found that mitochondrial DNA (mtDNA) polymorphisms and mutations in BPD affect mitochondrial calcium regulation (Kato, 2006, 2008). In addition, Konradi and colleagues found that among...
the 43 genes that were decreased in BPD compared with schizophrenia in post-mortem brain microarray studies, 42% coded for mitochondrial proteins, and were involved in regulating oxidative phosphorylation in the mitochondrial inner membrane (Konradi et al. 2004).

Key to the present discussion is that it is now clear that mitochondria regulate not only long-term cell survival and cell death, but also immediate synaptic function – both of which are clearly very relevant for BPD. It is important to emphasize at the outset that it is not our contention that BPD is necessarily a classical mitochondrial disorder. Indeed, the vast majority of BPD patients do not show the symptoms of classical mitochondrial disorders [e.g. optic and retinal atrophy, seizures, dementia, ataxia, myopathy, exercise intolerance, cardiac conduction defects, diabetes, lactic acidosis (Fadic & Johns, 1996)]. Instead, emerging data suggest that many of the upstream abnormalities (probably nuclear genome coded) converge to regulate mitochondrial function implicated both in acute abnormalities of neurotransmitter synaptic plasticity as well as in long-term cellular resilience (Quiroz et al. 2008).

The possible involvement of Bcl-2 in the pathophysiology and treatment of BPD initially arose from mRNA differential display studies suggesting that Bcl-2 might be a common target for the actions of both chronic lithium and valproate (VPA), mood stabilizers commonly used to treat BPD (Chen et al. 1999). Chronic treatment of rats with therapeutic doses of lithium and VPA doubled Bcl-2 levels in the frontal cortex, an effect due primarily to markedly increased numbers of Bcl-2 immunoreactive cells in layers II and III of the anterior cingulated cortex (Chen et al. 1999; Manji et al. 1999, 2000). Furthermore, at least in cultured cell systems, lithium reduces levels of the pro-apoptotic protein p53. As demonstrated recently, repeated electroconvulsive shock also significantly increases precursor cell proliferation in the dentate gyrus of the adult monkey, an effect that appears to be due to increased expression of Bcl-2 (Perera et al. 2007).

We therefore undertook this study to determine whether one of the major downstream actions of the mood stabilizers lithium and VPA is the enhancement of mitochondrial function. We used methamphetamine (Meth) treatment to investigate this issue because Meth induces mitochrondially mediated toxicity in the brain (Burrows et al. 2000; Cadet et al. 2005; Deng et al. 2002). The molecular mechanisms via which lithium and VPA protect against Meth-induced mitochondrial toxicity were investigated in vivo.

A mitochondria-related microarray study was conducted to explore the multiple functional groups of genes altered by Meth and protected by lithium and VPA. Finally, among these molecules, the role of Bcl-2 in lithium- and VPA-induced mitochondrial function was further investigated.

**Method**

**SH-SY5Y cell culture**

Cell cultures were prepared as previously described (Yuan et al. 1999, 2001). [See Supplementary Material (available online) for a detailed explanation of the procedure.]

**Preparation of mitochondrial fraction from SH-SY5Y cells**

Mitochondrial fractions were prepared as previously described with slight modifications (Almeida & Medina, 1998). (See Supplementary Material for a detailed explanation of the procedure.)

**Cellular respiration rate measurement**

Dulbecco’s Modified Eagle’s Medium (DMEM) was used for oxygen consumption measurements with the Clark electrode fitted to a small (200 µl) airtight chamber Oxygraph System (Hansatech, UK). The O₂ electrode was calibrated by immersion in electrode buffer and DMEM equilibrated with room air. The cell suspension in DMEM (10⁷ cells/ml) was slowly introduced to the bottom of the chamber to drive out micro-bubbles through a corresponding opening at the top. Cellular oxygen consumption was recorded with Oxygraph (Biaglow et al. 1998; Mamchaoui & Saumon, 2000) and replicated on three separate occasions. The respiration rate was normalized as percent of control for comparison.

**JC-1, MitoTracker Red (MTR) and MitoTracker Green (MTG) staining**

For JC-1 staining, SH-SY5Y cells were cultured in four-well chamber glass slides and were treated (or transfected with siRNA-Bcl-2 and then treated) with various concentrations of lithium or VPA for 3–7 d. A total of 10 nm JC-1 (Molecular Probes, USA) was applied to the SH-SY5Y cells and incubated for 20 min at 37°C. The cells were washed twice with Phenol Red-free DMEM plus 10 mm Hepes, then viewed under a Zeiss 510 confocal microscope (Zeiss, USA) in Phenol Red-free DMEM with the excitation and emission wavelength for the red fluorescence (excitation
543 nm, emission 560 nm) and for the green fluorescence (excitation 488 nm, emission 530 nm).

The use of MTR and MTG to determine mitochondrial oxidation has been well-established (Buckman et al. 2001; Shanker et al. 2005). MTR CM-H2XRos (M7513) is a derivative of dihydro-X-rosamine compound. The reduced probe does not fluoresce until it enters an actively respiring cell, where it is oxidized to the corresponding fluorescent mitochondrial-selective probe and then sequestered in the mitochondria. We confirmed that it is a mitochondrial selective probe and then sequestered in the mitochondria. We confirmed that it is a mitochondrial dye by co-localization of MTR dye with MTG. The co-localization efficiency of these two dyes was >80%.

For MTR (Molecular Probes) and MTG (Molecular Probes) staining, 300 nM of MTR and 60 nM of MTG (dissolved in 1 × PBS) were added to the cells and incubated at 37 °C for 20 min. The cells were washed twice with Phenol Red-free DMEM plus 10 mM Hepes. The live cell images were randomly taken under exactly the same conditions for each group using LSM 510 software under a Zeiss 510 confocal microscope. For each well, only four images were taken to ensure the fluorescent signal was not quenched.

Qualitative analysis was performed using LSM 510 software (Zeiss). To avoid bias, all cells touching the line of a four-line ‘#’-shaped grid on the image were quantified for fluorescent intensity. For quantification of both JC-1 and MTR/MTG signals, red and green fluorescent intensities on the whole cell region were determined by LSM 510 software (Zeiss). For each experimental condition, 34–59 cells were quantified. The experiment was performed at least 2–3 times.

**Animals and drug treatments**

Adult male Wistar Kyoto rats (Charles River Laboratories, USA) weighing 200–250 g were housed 2–3 per cage with free access to food and water (12 h light/dark cycle; lights on 06:00 hours). All animal procedures were conducted according to the Guide for the Care and Use of Laboratory Animals and were approved by the NIMH Animal Care Committee. The Supplementary Material provides a detailed explanation of the treatment procedure, which was similar to that used in a previous study (Du et al. 2004).

**Preparation of mitochondrial fractions from brain tissue**

See the Supplementary Material for a detailed explanation of the procedure for preparing the mitochondrial fractions from brain tissue, which was similar to a previously published method (Zini et al. 1998).

**Western blot analysis**

Tissue or cell homogenates and mitochondrial fraction samples were prepared and analysed as previously described (Du et al. 2004). (See Supplementary Material for a detailed explanation of the procedure.)

**Cytochrome c oxidase activity measurements**

A Cytochrome c Oxidase Activity Assay kit (Cytocox I; Sigma, USA) was used to measure the activity of electron transport chain (ETC) complex IV according to the manufacturer’s instructions (see Supplementary Material for a detailed explanation of the procedure).

**RNA extraction and oligo-microarray analysis**

Total RNA of the rat brain cortex was isolated using Trizol according to the manufacturer’s protocol (Invitrogen, USA), and was further purified using the RNeasy Mini kit according to the manufacturer’s recommendations (Qiagen, USA), with the addition of DNase digestion with a RNase-Free DNase set (Qiagen). The concentration of RNA was determined spectrophotometrically at a ratio of 260/280 nm.

The oligo-microarrays used for this study contained 603 custom-designed rat mitochondria-related gene 70-mer oligos spotted onto poly-L-lysine-coated slides at the National Human Genome Research Institute (NHGRI) using an OmniGrid arrayer (GeneMachines, USA). Methods for probe-labelling reaction and microarray hybridization have been described elsewhere (Wang et al. 2003). Microarrays were scanned at 10 μm resolution on a Scanarray-5000 scanner (PerkinElmer, USA) and images were organized in IPLab software (BD Biosciences, USA). The Cy5- and Cy3-labelled cDNA samples were scanned at 635 nm and 532 nm, respectively. The resulting TIFF images were analysed by IPLab software (BD Biosciences).

Each sample was tested in duplicate by alternating the dyes. Thus, a total of eight microarrays for each group was completed, which provided enough data-points for statistical significance. The ratios of the sample intensity to the reference intensity (green [Cy3]/red [Cy5]) for all targets were determined. Because a normal distribution could not be applied to all components of the dataset, a Mann–Whitney test was used to ascertain statistical significance among microarray replicates (Wang et al. 2003). Well fluorescence was corrected for background fluorescence, and ratios of intensity were established relative to appropriate controls. We selected a 1.3-fold threshold difference because the multiple repeats in our experimental scheme increased the likelihood of statistical reliability.
**Bcl-2 siRNA transfection**

siRNA expression vectors against Bcl-2 were constructed. Bcl-2 gene 423–440 and +6 to +12 were selected as the siRNA targeting sequences. A scrambled sequence for Bcl-2 was used as the control sequence. Oligonucleotides were designed using the software provided by Ambion (Austin, USA) and were synthesized by Sigma Genosys (USA). siRNA oligos were then inserted into linearized P-Silencer 3.0 (Ambion, USA) according to the manufacturer’s instructions. SH-SY5Y cells were cultured in six-well plates or four-well glass slides for 24–48 h. When SH-SY5Y cells reached >90% confluence, they were transfected with 1 μg Bcl-2 siRNA pSilencer or scrambled siRNA in pSilencer using Lipofectamine™ 2000 (Invitrogen). A cyan fluorescent protein (CFP) plasmid (Invitrogen) was co-transfected as a marker for confocal image analysis. The transfection procedures followed the manufacturer’s instructions (Invitrogen). After transfection, the cells were treated with 1.2 mM lithium or 1.0 mM VPA the next day for 3 or 7 d. The down-regulation of Bcl-2 protein expression was determined by Western blot, and mitochondrial functions were analysed by mitochondrial dye staining.

**Results**

**Long-term lithium or VPA increases cell respiration rate in SH-SY5Y cells**

Oxygen consumption of cells is closely related to mitochondrial function for ATP production (McCully et al. 2007), thus we first sought to determine whether lithium and VPA had a common effect on oxygen consumption in human neuroblastoma SH-SY5Y cell lines, using Oxygraph. We chose this cell line because of its human origin, neuronal phenotype, and its demonstrated usefulness as an experimental model of mitochondria-mediated toxicity (Bar-Am et al. 2005; Kluck et al. 1997). We used therapeutically relevant concentrations of lithium and VPA in human plasma for both in-vitro and in-vivo studies. The clinical therapeutic ranges are 0.7 ± 0.3 mM for lithium (Lauritsen et al. 1981; Vestergaard & Schou, 1984) and 39 ± 3 μg/ml for VPA (Bowden et al. 1996). We found that treatment with either lithium or VPA increased oxygen consumption time- and dose-dependently in SH-SY5Y cells (Fig. 1).

When SH-SY5Y cells were exposed to therapeutically relevant concentrations of lithium (1.2 mM) and VPA (1.0 mM), cell oxygen consumption increased significantly after 1 d treatment, and these effects were sustained for 6 d (Fig. 1a). We also found that oxygen consumption increased after 0.5 mM of lithium treatment, and peaked at 1 mM of lithium treatment (Fig. 1b). The increase in oxygen consumption fell slightly after 2.0 mM of lithium treatment, progressing...
in an inverted U-shaped pattern (Fig. 1b). Similarly, VPA also regulated respiration rate in an inverted U-shaped manner, showing a narrow concentration window for this biological effect (Fig. 1c).

**Long-term lithium or VPA enhances mitochondrial membrane potential in SH-SY5Y cells**

Next, we investigated the effects of lithium and VPA on additional mitochondrial functions. Because mitochondrial membrane potential is a key indicator of mitochondrial function, we assessed the effects of long-term lithium or VPA on this important parameter. We found that long-term treatment with lithium significantly increased the green and red fluorescence ratio in JC-1-stained SH-SY5Y cells, indicating dose-dependently increased mitochondrial membrane potential (*p* < 0.01) (Fig. 2). VPA treatment also enhanced the red/green ratio; this effect reached a plateau after 0.8 mM treatment, which is a therapeutically relevant concentration.

**Common effect of lithium and VPA on mitochondrial oxidation in SH-SY5Y cells**

Mitochondrial oxidation is one of the key mitochondrial functions involved in ATP synthesis. We therefore investigated mitochondrial oxidation after treatment with either lithium or VPA using MTR and MTG staining. MTR dye will become a red fluorescent colour only when oxidized. We measured both the fluorescent intensity of MTR and the MTR/MTG ratio; this ratio filtered the bias caused by quenching of the fluorescence signals and the alteration in MTR and MTG dye uptake. We found that after 7 d treatment with VPA (1.0 mM), MTR staining was significantly enhanced, indicating increased mitochondrial oxidation (Fig. 3c,d). After VPA treatment, the MTR/MTG ratio also showed significant enhancement (Fig. 3e). Seven days of lithium treatment similarly enhanced MTR intensity (Fig. 3a,b) and MTR/MTG ratio (Fig. 3e).

**Lithium and VPA attenuate Meth-induced decreases in mitochondrial cytochrome c and mitochondrial Bcl-2/Bax ratio in vivo**

We further investigated the protective effects of lithium and VPA on mitochondrial function against Meth-induced neurotoxicity. The doses of lithium and VPA in rodent chow were established by previous studies from our laboratory that found that, after chronic treatment with lithium- and VPA-containing chow, plasma concentrations of lithium and VPA in rats were within the range of human therapeutically relevant concentrations (Du *et al*., 2004). Rat serum drug concentrations were in the range of 0.74 ± 0.25 mM for lithium and 0.55 ± 0.17 mM for VPA. Because the prefrontal cortex is highly implicated in the pathophysiology of mood disorders (Adler *et al*., 2006; Nitschke & Mackiewicz, 2005), we investigated the protective effects of lithium and VPA in this brain region.

Cell survival is thought to be critically dependent on a molecular balancing act regulating the ratio of anti-apoptotic protein Bcl-2 to pro-apoptotic protein Bax; imbalance of Bcl-2 family members results in the consequent release of cytochrome c and activation of effector caspases that cause apoptosis (Kluck *et al*., 1997). In this study, Meth treatment indeed caused a marked decrease of the anti-apoptotic protein Bcl-2 and induction of the pro-apoptotic protein Bax, resulting in a significant decrease in Bcl-2/Bax ratio in the mitochondrial fractions of rat frontal cortex (Fig. 4). After Meth administration, cytochrome c levels also decreased in the mitochondrial fraction of rat frontal cortex, indicating that some of the cytochrome c was released into cytoplasm as an apoptotic signal. Four weeks of pretreatment with lithium or VPA prevented Meth’s effects on mitochondrial Bcl-2/Bax and cytochrome c (Fig. 4), suggesting that these agents play a protective role in mitochondrial homeostasis. Porin was used as a loading control for Western blot analysis of mitochondrial fractions.
Lithium and VPA preserve rat frontal cortex mitochondrial function by inhibiting the Meth-induced reduction of cytochrome oxidase (COX) activity

COX is one of the key mitochondrial enzymes and a reliable marker of mitochondrial efficiency. Cyclosporin A (CsA) was selected as a positive control because it exerts its effect by inhibiting permission transition pore (PTP), thereby preventing cytochrome c release from the mitochondria. In the present study, we found that Meth profoundly decreased COX activity in the mitochondrial fraction of rat frontal cortex. CsA treatment prevented Meth’s effect on COX activity (Fig. 5), and chronic pretreatment with lithium or VPA prevented the reduction of COX activity induced by Meth (Fig. 5). COX activity was measured as an arbitrary optic density reading/μg protein per minute. Treatment with lithium or VPA alone did not significantly affect COX activity (Fig. 5).

Lithium and VPA prevent Meth-induced reduction of tyrosine hydroxylase (TH), a functional enzyme in dopaminergic neurons

Previous studies found that Meth decreases TH staining as a marker for dopaminergic neurons (Deng et al. 2007). We investigated TH levels in the prefrontal cortex after Meth treatment with or without chronic pretreatment with lithium or VPA. We found that TH levels were significantly lower in prefrontal cortex after 1 d of Meth treatment, an effect that was prevented by pretreatment with lithium or VPA; this suggests that these agents protect against Meth-induced reductions in TH levels (Fig. 6). β-actin was used as a loading control. Under similar conditions, we also investigated the general neuronal marker neuron-specific enolase (NSE). Levels of NSE and actin remained unchanged, suggesting that the effects of lithium and VPA on TH are relatively specific (data not shown).

Fig. 3. Lithium (Li) and valproate (VPA) enhance mitochondrial oxidation in SH-SY5Y cells. SH-SY5Y cells were treated with lithium (1.2 mM) or VPA (1.0 mM) for 3–7 d. The mitochondrial oxidation of VPA- or lithium-treated SH-SY5Y cells was determined by MitoTracker Red (MTR) staining. After 6–8 d of treatment, lithium (a, b) and VPA (c, d) significantly enhanced mitochondrial oxidation (N = 2–4, n = 39–53, Student’s t test: * p < 0.05). The MTR/MTG ratio showed similar changes (e). Data are expressed as mean ± S.E.M.
Mitochondria-relevant microarray analysis for lithium and the protective effects of VPA against Meth-induced neurotoxicity

We used customized ‘mitochondria-relevant’ oligo cDNA expression for protein profiling to investigate the protective effects conferred by lithium and VPA against Meth-induced neurotoxicity. The microarrays used for this study included 603 custom-designed rat mitochondria-related genes, including ETC proteins, enzymes for tricarboxylic acid (TCA) cycle and substrate metabolism, the Bcl-2 family proteins, and chaperone proteins. Cluster analysis using GeneSpring revealed different patterns of gene expression in rats 1 d after Meth administration with or without pretreatment with lithium or VPA. The positive targets were identified based on eight independent tests (n = 8 animals per group). In addition, fold-change levels were set at >30%, and stringent statistical tests were performed in accordance with previously established methods (Jin et al. 2001).

Specifically, Meth up-regulated (>1.3-fold) 39 genes (Table 1) and down-regulated 20 genes (Table 2). Among the 39 genes up-regulated by Meth, Meth’s effects on 11 genes were also significantly prevented by lithium and VPA. These 11 genes belonged to three major gene groups: (1) apoptotic genes, including caspase-3, caspase 11, and cytochrome c; (2) ETC genes, including NADH dehydrogenase, NADH dehydrogenase 1 alpha subcomplex 5, cytochrome b5, cytochrome P450, and COX10 homolog cytochrome c assembly protein fernesyltransferase; and (3) chaperone interacting protein genes, including FK506 binding protein 4, suppression of tumorigenicity 13 (Hsp70...
interacting protein), and stress-induced phosphoprotein 1 (Hsp70/Hsp90 organizing protein) (Table 1). For the 20 genes down-regulated by Meth, Meth’s effects on seven genes were prevented by lithium or VPA. These seven genes belonged to two major groups: (1) anti-apoptotic genes, including cyclin D1 and cyclin A1; and (2) enzymes involved in substrate metabolism, such as glutamic-pyruvate transaminase, glutamate oxaloacetate transaminase 2, glutaminyl-tRNA synthase, arylalkylamine N-acetyl-transferase, and vitamin D(3)-25 hydroxylase (Table 2).

Western blot analysis was performed to assess whether the changes in mRNA levels were reflected in protein expression. For confirmation, five targets were selected from the pro-apoptotic gene group (caspase-3, caspase-11, and cytochrome c), the ETC group (ETC complex I NADH–ubiquinone oxidoreductase 1 beta subcomplex 10), and the anti-apoptotic gene group (cyclin D1). Meth significantly induced the gene expression of apoptosis-related proteins caspase-3 (full-length form, 35 kDa) and 48-kDa subunit caspase-11, and showed a clear trend to increase cytochrome c. Pretreatment with lithium or VPA attenuated Meth’s increase in the expression of the 48-kDa subunit of caspase-11 and caspase-3, and showed a trend to attenuate Meth’s increase of cytochrome c expression. Up-regulation of caspase-3, caspase-11, and cytochrome c proteins was consistent with up-regulation of the corresponding genes in the microarray finding. In addition, Meth significantly reduced ETC complex I NADH–ubiquinone oxidoreductase I beta subcomplex 10, a protein crucial for mitochondrial respiration. Levels of β-actin showed no change. These changes in protein levels were reflected in mRNA levels in the microarray findings (Fig. 7).

Bcl-2, a common target of lithium and VPA, is critical to the effects of lithium and VPA on mitochondrial function

Among the mitochondrial proteins regulated by mood stabilizers, Bcl-2 is known to be a key regulator of mitochondrial function as well as a common target for lithium and VPA in vivo (Chen et al. 1999). Here, we further investigated whether Bcl-2 is the critical molecule in the up-regulation of mitochondrial function by lithium and VPA. Western blot analysis showed that long-term treatment with lithium or VPA at therapeutically relevant concentrations increased Bcl-2 levels in SH-SY5Y cell homogenates by 35% and 40% respectively; these levels reached their peak after 6 d treatment (Fig. 8a). β-actin was used as a loading control. In addition, in mitochondrial fractions of human neuroblastoma SH-SY5Y cells, Bcl-2 levels increased to 339% of control after lithium treatment, and to 426% of control after VPA treatment (Fig. 8a). Porin was used as a loading control for mitochondrial fractions.

Next, we investigated the role of Bcl-2 in enhancing mitochondrial function after long-term treatment with lithium or VPA. Bcl-2 siRNA in a vector, P-silencer, was transfected into SH-SY5Y cells following treatment with lithium (1.2 mM) or VPA (1.0 mM) for 3 d or 7 d. CFP was used as an indicator for the transfected SH-SY5Y cells. After transfection of Bcl-2 siRNA or scrambled siRNA (SCR) vectors, Bcl-2 siRNA significantly reduced Bcl-2 protein levels (Fig. 8b), and also attenuated MTR staining (Fig. 8c); these results suggest reduced oxidation in mitochondria. MTR staining was also attenuated in the Bcl-2 siRNA-transfected SH-SY5Y cells treated with lithium or VPA by about 30%, suggesting that Bcl-2 is one of the key players in allowing lithium and VPA to increase mitochondrial function (Fig. 8c).
Discussion

Mitochondrial dysfunction in the CNS appears to be a pathogenic pathway for a variety of disorders associated with progressive atrophic/degenerative changes, including Parkinson’s disease, Alzheimer’s disease, Huntington’s disease, amyotrophic lateral sclerosis (ALS), BPD, and schizophrenia (Browne & Beal, 2004; Karry et al. 2004; Kato & Kato, 2000; Mecocci et al. 1994; Orth & Schapira, 2001; Stavrovskaya & Kristal, 2005). In the present study, we investigated whether the mood stabilizers lithium and VPA increase key mitochondrial functions and protect against Meth-induced functional damage to mitochondria. We found that (i) long-term lithium or VPA enhanced mitochondrial function as assessed by mitochondrial membrane potential and mitochondrial oxidation; (ii) chronic lithium or VPA protected against Meth-induced decreases in mitochondrial indexes in the prefrontal cortex in vivo; e.g., these agents prevented Meth-induced changes in mitochondrial Bcl-2/Bax ratio and COX activity; (iii) a mitochondria-relevant oligoarray showed that Meth up-regulated the expression of 39 genes (>1.3-fold) and down-regulated 20 genes. Lithium and VPA protected against the alteration of some of these genes; and (iv) this regulation of mitochondrial functions by lithium or VPA was partially mediated through Bcl-2; Bcl-2 knock-down attenuated the effects of lithium or VPA on the regulation of mitochondrial functions.

Common effects of lithium and VPA on mitochondrial function

Using multiple measures, we found a robust enhancement of mitochondrial function by long-term treatment with lithium or VPA at therapeutically relevant concentrations. Recent studies have shown that lithium desensitizes brain mitochondria to calcium, antagonizes permeability transition, and diminishes cytochrome c release (Shalbuyeva et al. 2007); it also inhibits mitochondria-generated reactive oxygen species (ROS) and nitric oxide (NO) in an animal model of ischaemia (Plotnikov et al. 2007). However, VPA has been found to inhibit substrate-specific oxygen consumption and mitochondrial ATP synthesis in rat hepatocytes, and VPA metabolites have been found to inhibit dihydrolipoamide dehydrogenase activity leading to impaired oxidative phosphorylation (Luis et al. 2007). This is supported by evidence that VPA therapy may cause inborn errors of metabolism (IEMs) and acute liver toxicity, potentially because of its interference with mitochondrial β-oxidation (Silva et al. 2008).

Because mood stabilizers exhibit neuroprotective properties against an array of insults in cells of neuronal origin, this toxic effect seems cell-type specific. Recent studies suggest that lithium and VPA may decrease the vulnerability of human neural, but not glial, cells to cellular injury evoked by oxidative stress possibly arising from putative mitochondrial disturbances implicated in BPD (Lai et al. 2006). The studies found that pretreatment of SH-SY5Y cells for 7 d, but not 1 d, with 1 m M lithium or 0.6 m M VPA significantly reduced rotenone- and hydrogen peroxide-induced cytotoxicity, cytochrome c release, and caspase-3 activation, and increased Bcl-2 levels; these results are in agreement with the findings of the present study (Lai et al. 2006). Also key to this discussion is VPA’s narrow therapeutic window. Although both lithium and VPA have neuroprotective mechanisms that are indirectly exerted through mitochondrial enhancement, clinical studies have shown that VPA in the toxic range impairs mitochondrial β-oxidation in patients (Eyer et al. 2005).

Lithium and VPA protect against Meth-induced damage to mitochondrial function

To examine the utility of lithium- and VPA-induced enhancement of mitochondrial function, we conducted Meth experiments in vivo, and found that both lithium and VPA prevented Meth-induced reduction of mitochondrial function. Meth is a neurotoxin known to exert mitochondrially mediated toxicity, and has been shown to directly inhibit COX, complex IV of the ETC (Burrows et al. 2000), and succinate dehydrogenase, complex II of the ETC (Brown et al. 2005). In addition Meth induces apoptosis by activating the mitochondrial cell death pathway (Cadet et al. 2005; Deng et al. 2001, 2002) and has been shown to decrease mitochondrial membrane potential (Riddle et al. 2006; Wu et al. 2007) and increase ROS (Wu et al. 2007) followed by apoptosis. In previous studies, Meth significantly decreased the anti-apoptotic genes Bcl-2 and Bcl-XL, which may contribute to its cytotoxic effect in mouse neocortex (He et al. 2004; Jayanthi et al. 2001). Notably, lithium and VPA exhibit neuroprotective properties against an array of insults. Our in-vivo studies showed that chronic treatment with lithium or VPA attenuated the Meth-induced decrease of Bcl-2/Bax ratio in the mitochondrial fraction of prefrontal cortex.

Meth-induced toxicity probably occurs by increasing oxidative stress and free radical production through inhibition of COX. This inhibition eventually leads to a decrease in mitochondrial respiration,
Table 1. Oligo-microarray analysis: Meth up-regulated genes

<table>
<thead>
<tr>
<th>Acc. no.</th>
<th>Title</th>
<th>Meth vs. Con</th>
<th>Li + Meth vs. Con</th>
<th>VPA + Meth vs. Con</th>
<th>Li + Meth vs. Meth</th>
<th>VPA + Meth vs. Meth</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Avg</td>
<td>p(a)</td>
<td>Avg</td>
<td>p(b)</td>
<td>p(b)</td>
</tr>
<tr>
<td>Proapoptotic genes</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>NM_012922.1</td>
<td><em>Rattus norvegicus</em> caspase-3 (Casp3)</td>
<td>1.5210</td>
<td>0.0025</td>
<td>0.9964</td>
<td>0.9453</td>
<td>0.9865</td>
</tr>
<tr>
<td>M20622.1</td>
<td>Cytochrome c, somatic</td>
<td>1.4589</td>
<td>0.0089</td>
<td>1.0470</td>
<td>0.0905</td>
<td>1.0546</td>
</tr>
<tr>
<td>NM_053736.1</td>
<td><em>Rattus norvegicus</em> caspase-11 (Casp11)</td>
<td>1.4495</td>
<td>0.0001</td>
<td>0.6975</td>
<td>0.0001</td>
<td>0.8810</td>
</tr>
<tr>
<td>Electron transfer chain</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>XM_343910.1</td>
<td><em>predicted: Rattus norvegicus</em> COX10 homolog, cytochrome c oxidase assembly protein, heme A: farnesyltransferase (yeast) (predicted)</td>
<td>2.0246</td>
<td>8.3002</td>
<td>1.3490</td>
<td>0.0086</td>
<td>1.0818</td>
</tr>
<tr>
<td>XM_216378.2</td>
<td><em>Rattus norvegicus</em> similar to NADH dehydrogenase</td>
<td>1.4437</td>
<td>0.0001</td>
<td>1.1437</td>
<td>0.1732</td>
<td>0.9241</td>
</tr>
<tr>
<td>NM_012985.1</td>
<td><em>Rattus norvegicus</em> NADH dehydrogenase (ubiquinone) 1 alpha subcomplex 5 (Ndufa5)</td>
<td>1.3732</td>
<td>0.0005</td>
<td>1.0380</td>
<td>0.5197</td>
<td>0.9871</td>
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<tr>
<td>NM_030586.1</td>
<td><em>Rattus norvegicus</em> cytochrome b5, outer mitochondrial membrane isoform (omb5)</td>
<td>1.3627</td>
<td>0.0067</td>
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<td>0.8822</td>
<td>1.0065</td>
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<td>NM_017286.1</td>
<td><em>Rattus norvegicus</em> cytochrome P450, subfamily 11A (Cyp11a)</td>
<td>1.3164</td>
<td>0.0001</td>
<td>1.0876</td>
<td>0.0918</td>
<td>1.0756</td>
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<tr>
<td>XM_227084.2</td>
<td><em>Rattus norvegicus</em> Nicotinamide nucleotide transhydrogenase (NAD(P)(^+) transhydrogenase) (Nnt)</td>
<td>1.3039</td>
<td>0.0076</td>
<td>1.0891</td>
<td>0.4014</td>
<td>0.9819</td>
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<td>Chaperone interacting proteins</td>
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<tr>
<td>S7856.1</td>
<td>Grp75 = 75 kDa glucose regulated protein</td>
<td>1.4050</td>
<td>0.0031</td>
<td>1.3189</td>
<td>0.0027</td>
<td>1.5038</td>
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<tr>
<td>XM_342763.1</td>
<td>FK506 binding protein 4, 59 kDa</td>
<td>1.3278</td>
<td>0.0037</td>
<td>0.9453</td>
<td>0.6196</td>
<td>0.9245</td>
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<tr>
<td>NM_031122.1</td>
<td>Suppression of tumorigenicity 13 (colon carcinoma) (Hsp70 interacting protein)</td>
<td>1.3261</td>
<td>0.0001</td>
<td>0.9212</td>
<td>0.2403</td>
<td>0.9601</td>
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<tr>
<td>NM_138911.2</td>
<td>Stress-induced-phosphoprotein 1 (Hsp70/ Hsp90-organizing protein)</td>
<td>1.3227</td>
<td>0.0010</td>
<td>0.9106</td>
<td>0.1744</td>
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<td>Enzymes for metabolism</td>
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<td>NM_053607.1</td>
<td><em>Rattus norvegicus</em> fatty acid coenzyme A ligase, long chain 5 (Facl5)</td>
<td>1.4552</td>
<td>0.0002</td>
<td>1.2884</td>
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<td>XM_217611.2</td>
<td><em>Rattus norvegicus</em> similar to carbonic anhydrase 5b, mitochondrial</td>
<td>1.3782</td>
<td>0.0079</td>
<td>1.1034</td>
<td>0.5435</td>
<td>1.0858</td>
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<tr>
<td>XM_217181.2</td>
<td><em>Rattus norvegicus</em> similar to serine beta lactamase-like protein LACT-1</td>
<td>1.3739</td>
<td>0.0001</td>
<td>1.4016</td>
<td>0.0003</td>
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<td>Accession</td>
<td>Description</td>
<td>Mean (SD)</td>
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<tr>
<td>XM_226211.2</td>
<td>Rattus norvegicus similar to thymidine kinase 2, mitochondrial; thymidine kinase 2</td>
<td>1.3371 0.0014 0.9054 0.3324 1.2136 0.1327 0.0133 0.6483</td>
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<td>NM_130795.1</td>
<td>Rattus norvegicus citrate synthase (Cs)</td>
<td>1.339 0.0016 1.0361 0.5139 1.0687 0.4587 0.0186 0.0377</td>
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<tr>
<td>XM_343764.1</td>
<td>Rattus norvegicus monoamine oxidase A (MAOA)</td>
<td>1.314 0.0024 1.0768 0.1971 1.0671 0.5794 0.1072 0.0920</td>
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<tr>
<td>NM_053592.1</td>
<td>Rattus norvegicus Deoxyuridinetriphosphatase (dUTPase) (Dut)</td>
<td>1.307 0.0081 1.2282 0.1606 1.0645 0.3420 0.7732 0.1989</td>
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<td>NM_019168.1</td>
<td>Rattus norvegicus arginase 2 (Arg2),</td>
<td>1.326 0.0021 1.2234 0.0156 1.2514 0.0372 0.0186 0.0377</td>
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<td>M_031720.2</td>
<td>Deiodinase, iodothyronine, type II</td>
<td>1.320 0.0017 1.0729 0.1635 1.0878 0.4394 0.0838 0.1086</td>
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<td>XM_223499.1</td>
<td>Rattus norvegicus similar to apurinic/apyrimidinic endonuclease 2</td>
<td>1.312 0.0002 1.0869 0.6406 1.0423 0.3923 0.3319 0.2132</td>
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<td>XM_341628.1</td>
<td>Rattus norvegicus similar to malic enzyme 2, NAD(+) - dependent, mitochondrial</td>
<td>1.304 0.0047 0.9787 0.5792 1.0132 0.8618 0.0046 0.0110</td>
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<tr>
<td>Others</td>
<td>Proenkephalin</td>
<td>1.4176 0.0023 1.2298 0.0728 1.0861 0.5093 0.4531 0.1021</td>
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<tr>
<td>XM_214751.1</td>
<td>Rattus norvegicus similar to mitochondrial ribosomal protein L18</td>
<td>1.6222 0.0016 1.2615 0.1107 1.0373 0.7301 0.1279 0.0090</td>
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<td>XM_216010.2</td>
<td>Rattus norvegicus similar to mitochondrial ribosomal protein L41</td>
<td>1.4442 0.0040 1.1631 0.3343 1.2224 0.0066 0.2158 0.3744</td>
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<tr>
<td>XM_344002.1</td>
<td>Rattus norvegicus similar to GA binding protein alpha chain (GABP-alpha subunit) (transcription factor E4TF1-60) (Nuclear respiratory factor-2 subunit alpha)</td>
<td>1.4272 0.0003 0.8527 0.0159 0.7324 0.0089 0.0000 3.6004</td>
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<tr>
<td>XM_343135.1</td>
<td>Unactive progesterone receptor, 23 kDa</td>
<td>1.3733 0.0039 1.1737 0.3904 1.0098 0.8864 0.5164 0.1301</td>
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<tr>
<td>XM_217179.2</td>
<td>Rattus norvegicus similar to ClpX protein</td>
<td>1.3669 0.0076 1.0847 0.3975 1.2241 0.1413 0.1949 0.6407</td>
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<tr>
<td>NM_053610.1</td>
<td>Rattus norvegicus peroxiredoxin 5 (Prdx5)</td>
<td>1.3587 0.0034 0.8464 0.1154 1.1156 0.3029 0.0019 0.1681</td>
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<tr>
<td>NM_053842.1</td>
<td>Mitogen-activated protein kinase 1</td>
<td>1.3463 0.0043 1.0694 0.3518 1.0466 0.5974 0.0561 0.0368</td>
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<td>NM_053357.2</td>
<td>Catenin (cadherin-associated protein), beta 1, 88 kDa</td>
<td>1.3408 0.0063 1.0885 0.0469 1.1042 0.2799 0.0693 0.0923</td>
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<tr>
<td>XM_214491.2</td>
<td>Rattus norvegicus similar to mitochondrial ribosomal protein L32</td>
<td>1.3403 0.0022 1.0475 0.4889 1.0457 0.5563 0.0210 0.0202</td>
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<tr>
<td>XM_236263.2</td>
<td>Rattus norvegicus similar to Ddx10 protein</td>
<td>1.3348 0.0032 1.1298 0.2182 1.1018 0.1154 0.1757 0.1116</td>
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<td>NM_031818.1</td>
<td>Rattus norvegicus chloride intracellular channel 4 (Clic4)</td>
<td>1.3240 0.0056 1.0856 0.2837 0.9362 0.2581 0.0649 0.0023</td>
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<tr>
<td>XM_342712.1</td>
<td>Rattus norvegicus similar to mitochondrial ribosomal protein L53</td>
<td>1.3203 0.0062 1.0861 0.0295 1.1565 0.1659 0.1077 0.3158</td>
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<tr>
<td>XM_221202.2</td>
<td>Rattus norvegicus similar to mitochondrial ribosomal protein L12</td>
<td>1.3178 0.0040 1.0410 0.7843 1.1087 0.4890 0.2955 0.4894</td>
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<tr>
<td>M18331.1</td>
<td>Protein kinase C, epsilon</td>
<td>1.3142 0.0010 1.3292 0.0046 1.1009 0.3262 0.9902 0.1656</td>
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</table>

Meth, Methamphetamine treatment; Li, lithium pretreatment; VPA, valproate pretreatment; Con, control.

*a p value of Student’s t test to compare each treatment and the corresponding control.

*b p value of one-way ANOVA Turkey post hoc to compare lithium or VPA pretreatment vs. pure amphetamine treatment.
Table 2. Oligo-microarray analysis: Meth down-regulated genes

<table>
<thead>
<tr>
<th>Acc. no.</th>
<th>Title</th>
<th>Meth vs. Con</th>
<th>Li + Meth vs. Con</th>
<th>VPA + Meth vs. Con</th>
<th>Li + Meth vs. Meth</th>
<th>VPA + Meth vs. Meth</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Avg</td>
<td>$p_a$</td>
<td>Avg</td>
<td>$p_a$</td>
<td>$p_b$</td>
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<td><strong>Antiapoptotic genes</strong></td>
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<tr>
<td>NM_171992.2</td>
<td>Cyclin D1 (PRAD1: parathyroid adenomatosis 1)</td>
<td>0.6284</td>
<td>0.0021</td>
<td>0.9187</td>
<td>0.3748</td>
<td>0.7825</td>
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<tr>
<td>XM_215565.2</td>
<td>Cyclin A1</td>
<td>0.6883</td>
<td>0.0003</td>
<td>0.8556</td>
<td>0.0103</td>
<td>0.8301</td>
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<td><strong>Enzymes for metabolism</strong></td>
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<td>AF111160.1</td>
<td>Glutathione S-transferase A3</td>
<td>0.5862</td>
<td>1.5701</td>
<td>0.8755</td>
<td>0.0712</td>
<td>0.7933</td>
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<tr>
<td>NM_031039.1</td>
<td><em>Rattus norvegicus</em> glutamic-pyruvate transaminase (Gpt)</td>
<td>0.5997</td>
<td>0.0010</td>
<td>0.9231</td>
<td>0.1189</td>
<td>0.9707</td>
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<tr>
<td>NM_012569.1</td>
<td><em>Rattus norvegicus</em> glutaminase (Gls)</td>
<td>0.6384</td>
<td>0.0003</td>
<td>0.8017</td>
<td>0.0382</td>
<td>0.9262</td>
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<tr>
<td>NM_133598.1</td>
<td><em>Rattus norvegicus</em> glycine cleavage system protein H (Gcs)</td>
<td>0.6579</td>
<td>0.0071</td>
<td>0.8119</td>
<td>0.0412</td>
<td>0.8852</td>
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<tr>
<td>NM_013177.1</td>
<td><em>Rattus norvegicus</em> glutamate oxaloacetate transaminase 2 (Got2)</td>
<td>0.6714</td>
<td>0.0015</td>
<td>0.9570</td>
<td>0.3572</td>
<td>0.8828</td>
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<tr>
<td>Y07534.1</td>
<td><em>Rattus norvegicus</em> mRNA for mitochondrial vitamin D(3) 25-hydroxylase</td>
<td>0.6728</td>
<td>0.0018</td>
<td>0.8476</td>
<td>0.0076</td>
<td>0.9235</td>
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<tr>
<td>NM_012504.1</td>
<td>ATPase, Na$^+$ /K$^+$ transporting, alpha 1 polypeptide</td>
<td>0.6762</td>
<td>0.0034</td>
<td>0.8418</td>
<td>1.9419</td>
<td>0.8228</td>
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<tr>
<td>NM_017290.1</td>
<td>ATPase, Ca$^{2+}$ transporting, cardiac muscle, slow twitch 2</td>
<td>0.6783</td>
<td>0.0034</td>
<td>0.8843</td>
<td>0.0179</td>
<td>0.8708</td>
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<td>XM_228810.2</td>
<td><em>Rattus norvegicus</em> similar to Nucleolar RNA helicase II</td>
<td>0.6793</td>
<td>0.0044</td>
<td>0.9502</td>
<td>0.3904</td>
<td>0.8003</td>
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<td>(Nucleolar RNA helicase Gu) (RH II/Gu) (DEAD-box protein 21)</td>
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<td>XM_214381.2</td>
<td><em>Rattus norvegicus</em> similar to glutaminyl-tRNA synthetase</td>
<td>0.6811</td>
<td>0.0004</td>
<td>1.0574</td>
<td>0.3460</td>
<td>0.9798</td>
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<tr>
<td>(glutamine-tRNA ligase) (GlnRS)</td>
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<tr>
<td>D90401.1</td>
<td><em>Rattus norvegicus</em> mRNA for dihydrolipoamide succinyltransferase</td>
<td>0.6912</td>
<td>0.0037</td>
<td>0.7964</td>
<td>1.7801</td>
<td>0.9125</td>
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<tr>
<td>NM_012818.1</td>
<td>Arylalkylamine N-acetyltransferase</td>
<td>0.6954</td>
<td>0.0001</td>
<td>0.8863</td>
<td>0.0111</td>
<td>0.9379</td>
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</tbody>
</table>
proton leakage across the mitochondrial membrane, and uncoupling. Uncoupling subsequently causes energetic inefficiency and increases metabolic heat, thereby increasing temperature. It has also been reported that VPA (only at the high concentration of 10 mM) causes uncoupling of mitochondrial respiration in liver cells (Jimenez-Rodriguezvila et al. 1985); however, this may not apply to the low VPA concentration (0.3–2.0 mM) used in the present study.

Meth was originally shown to inhibit COX activity following administration in rats (Burrows et al. 2000), suggesting that it has a direct inhibitory effect on mitochondrial function exerted through incapacitation of one of the enzymes responsible for oxidative phosphorylation and energy production. In the present study, we found that Meth significantly decreased COX activity in the homogenate of prefrontal cortex and hippocampus. Chronic lithium preserved the activity of mitochondrial COX (Fig. 5).

TH positive dopaminergic and norepinephrinergic axons are distributed in prefrontal cortex (Divac et al. 1994; Lewis et al. 1998; Miner et al. 2003). Previous TH immunostaining studies revealed that Meth is toxic to dopaminergic neurons (Davidson et al. 2007; Deng et al. 2007). Therefore, we also assessed levels of the dopaminergic neuronal marker TH in prefrontal cortex, because TH levels may reflect loss of dopaminergic terminals. We found that TH levels in prefrontal cortex were significantly reduced after Meth; notably, chronic lithium or VPA pretreatment protected against the Meth-induced TH reductions (Fig. 6).

Mitochondria-related groups of genes involved in the protective effects of lithium and VPA against Meth

The mitochondria-related gene array studies provide an overall picture of mitochondria-related gene changes involved in the protective effects of lithium and VPA against Meth. Three major groups of mitochondrial genes – including apoptotic, ETC, and chaperone interacting protein genes – were up-regulated by Meth treatment; Meth’s effect was significantly prevented by lithium or VPA (Table 1). Mitochondrial functions, such as oxidation or ATP production are performed by groups of genes. As discussed above, Meth disturbed energy production, therefore, it is not surprising that five ETC genes were altered by Meth, including NADH dehydrogenase, NADH dehydrogenase 1 alpha subcomplex 5, cytochrome b5, cytochrome P450, and COX10 homolog cytochrome c. This Meth-induced increase was attenuated by treatment with lithium or VPA (Table 1). These effects on ETC genes provide the molecular basis for the alteration in
mitochondrial functions, and support the mitochondrial oxidation and membrane potential alterations we observed in vitro. In the apoptotic group, it is notable that mRNA and protein levels of caspase-3, a pro-apoptotic marker used to detect early apoptosis (Belloc et al. 2000), were significantly enhanced by Meth; lithium or VPA prevented these increases, suggesting that they offer protection against mitochondrially mediated cell apoptosis.

Several mitochondria-related genes were downregulated by Meth, including anti-apoptotic genes and enzymes involved in metabolism; Meth’s effects were prevented by lithium and VPA. Both lithium and VPA pretreatment attenuated the Meth-induced downregulation of the anti-apoptotic genes cyclin D1 and cyclin A1 (Table 2), suggesting a mechanism of neuroprotection. Overall, these microarray results help to explain the mechanism whereby lithium and VPA are involved in the protection of mitochondrial functions.

**Bcl-2 is involved in the regulation of mitochondrial function induced by lithium and VPA**

Finally, to elucidate the mechanisms underlying the enhancement of mitochondrial functions, we examined the role of Bcl-2. It is now clear that Bcl-2 is a protein that robustly enhances mitochondrial function, thereby inhibiting both apoptotic and necrotic cell death induced by diverse stimuli (Adams & Cory, 1998; Bruckheimer et al. 1998; Merry & Korsmeyer, 1997). Recent studies suggest that lithium’s effects on Bcl-2 may be mediated through the expression of p53 (Chen & Chuang, 1999). Previous studies from our laboratory and others have shown that lithium and VPA increase cellular levels of Bcl-2 (Bush & Hyson, 2006; Chen et al. 1999; Ghribi et al. 2002; Zhang et al. 2003). However, mitochondrial Bcl-2 and its impact on mitochondrial function remain unclear. In the present study, we found that treatment with lithium or VPA produced a marked (>300%) increase in mitochondrial Bcl-2 levels.

We therefore used a variety of independent measures to determine whether the lithium- or VPA-induced increases in Bcl-2 levels did, in fact, translate into enhanced mitochondrial function. Both lithium and VPA treatment increased cellular respiratory rate, enhanced mitochondrial membrane potential, and increased mitochondrial oxidation. In addition, Bcl-2 siRNA significantly knocked down Bcl-2 gene expression and clearly reduced mitochondrial oxidation parameters, suggesting that Bcl-2 is a key modulator of mitochondrial function regulation by lithium and VPA. It is likely that several cellular mechanisms are involved in mediating Bcl-2’s protective effects, including sequestering the proforms of caspases, inhibiting the effects of caspase activation, producing antioxidant effects, enhancing mitochondrial calcium uptake, and attenuating the release of calcium and...
cytochrome c from mitochondria (reviewed in Adams & Cory, 1998; Bruckheimer et al. 1998; Li et al. 1999; Sadoul, 1998).

Taken together, the evidence suggests that the mood stabilizers lithium and VPA have a common effect on the regulation of mitochondrial functions.
in vitro and in the CNS. This modulation of mitochondrial function may ultimately be useful in protecting mitochondria from pathological mitochondrial dysfunctions in the brain. The anti-apoptotic protein Bcl-2 appears to be one of the key players in this regulation of mitochondrial function. Indeed, this protective effect is quite profound, as highlighted by the fact that in this study, multiple functional groups of mitochondria-related genes were protected by lithium and VPA in the CNS in vivo.

Note
Supplementary material accompanies this paper on the Journal’s website (http://journals.cambridge.org).

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Statement of Interest
None.

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Stavrovskaya IG, Kristal BS (2005). The powerhouse takes control of the cell: is the mitochondrial permeability transition a viable therapeutic target against neuronal dysfunction and death? Free Radical Biology and Medicine 38, 687–697.


