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Cytoprotection by lithium and valproate varies between cell types and cellular stresses

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Abstract

Despite much evidence that lithium and valproate, two commonly used mood stabilizers, exhibit neuroprotective properties against an array of insults, the pharmacological relevance of such effects is not clear because most of these studies examined the acute effect of these drugs in supratherapeutic doses against insults which were of limited disease relevance to bipolar disorder. In the present study, we investigated whether lithium and valproate, at clinically relevant doses, protects human neuroblastoma (SH-SY5Y) and glioma (SVG and U87) cells against oxidative stress and endoplasmic reticulum stress in a time-dependent manner. Pretreatment of SH-SY5Y cells for 7 days, but not 1 day, with 1 mM of lithium or 0.6 mM of valproate significantly reduced rotenone and H_2O_2-induced cytotoxicity, cytochrome *c* release and caspase-3 activation, and increased Bcl-2 levels. Conversely, neither acute nor chronic treatment of SH-SY5Y cells with lithium or valproate elicited cytoprotective responses against thapsigargin-evoked cell death and caspase-3 activation. Moreover, inhibitors of glycogen synthase kinase-3 (GSK-3), kenpaullone and SB216763, abrogated rotenone-induced, but not H_2O_2-induced, cytotoxicity. Thus the cytoprotective effects of lithium and valproate against H_2O_2-induced cell death is likely independent of GSK-3 inhibition. On the other hand, chronic lithium or valproate treatment did not ameliorate cytotoxicity induced by rotenone, H_2O_2, and thapsigargin in SVG astroglial and U87 MG glioma cell lines. Our results suggest that lithium and valproate may decrease vulnerability of human neural, but not glial, cells to cellular injury evoked by oxidative stress possibly arising from putative mitochondrial disturbances implicated in bipolar disorder.

Keywords: Lithium; Valproate; Cytotoxicity; Cytochrome *c*; Bcl-2; Caspase-3; Bipolar disorder

1. Introduction

Bipolar disorder is a life-threatening psychiatric illness characterized by mood disturbances with recurrent episodes of mania, hypomania, and depression. It is a major public health problem that affects approximately 1% of the population worldwise, and extracts a marked toll in terms of morbidity, mortality, and societal cost (Belmaker, 2004; Goodwin and Jamison, 1990). Although the etiology remains poorly understood, a large body of data has clearly implicated postreceptor signal transduction disturbances in the pathophysiology of bipolar disorder (Li et al., 2000b; Manji and Lenox, 2000; Warsh et al., 2004). Furthermore, cellular and molecular studies have also illuminated the key role of intracellular signaling proteins as targets for lithium, a mainstay in treatment for BD (Jope, 1999; Li, 2004; Phiel and Klein, 2001).

Converging evidence from structural neuroimaging and postmortem studies have identified anatomical and neuropathological abnormalities, including ventricular enlargement, decreased gray matter volume, lower levels of *N*-acetylaspartate (a marker of neuronal integrity), and reductions of number, size and/or density of neurons and glial cells in brain of patients with bipolar disorder (Rajkowska, 2002; Strakowski et

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al., 2005; Vawter et al., 2000). Interestingly, bipolar patients treated with lithium or valproate had modest but significantly greater gray matter volumes (Moore et al., 2000b; Sassi et al., 2002) and *N*-acetyl-aspartate levels (Moore et al., 2000a) compared with medication-free patients. These observations raise the possibility that the therapeutic effects of mood-stabilizing drugs, such as lithium and valproate, are mediated through mechanisms regulating cellular resilience (Chuang, 2004; Manji et al., 2000; Warsh et al., 2004). Indeed, lithium and valproate display cytoprotective properties in response to numerous cytotoxic insults in cultured cells and animal models (Chuang, 2004; Li et al., 2002; Yuan et al., 2004). However the relevance of these cytoprotective actions to their therapeutic effect in bipolar disorder is still uncertain. First, the clinical effects of lithium and valproate require chronic administration, with a lag period for onset of action of several days to weeks (Bowden, 1996). With a few exceptions (Hashimoto et al., 2002; Hiroi et al., 2005; Kanai et al., 2004) however, most of the in vitro studies of their action only examined the short-term (0.5–24 h) effect of these drugs (Kang et al., 2003; Kim et al., 2005; King et al., 2001; King and Jope, 2005; Li et al., 2000a, 2002; Linseman et al., 2003; Mora et al., 2001). Second, the concentrations of lithium and valproate used in these short-term in vitro studies (King et al., 2001; King and Jope, 2005; Li et al., 2000a, 2002; Linseman et al., 2003; Mora et al., 2001; Song et al., 2002) were substantially higher than the accepted therapeutic plasma ranges of 0.6–1.2 and 0.3–1.0 mM, respectively (Bowden, 1996). More importantly, many of these earlier studies (King et al., 2001; King and Jope, 2005; Li et al., 2000a, 2002; Linseman et al., 2003; Mora et al., 2001; Song et al., 2002) did not examine lithium/valproate in the context of their therapeutic actions in bipolar disorder but rather as inhibitor of glycogen synthase kinase (GSK)-3β, a multi-tasking protein kinase that has been identified as an important pro-apoptotic factor (Grimes and Jope, 2001). Third, the types of cytotoxic insults employed in these in vitro (e.g. trophic factor withdrawal, C_2-ceramide, β-amyloid, colchicine, β-bungarotoxin, ouabain, valinomycin and heatshock, etc.) or in vivo (e.g. excitotoxicity-induced striatal or basal forebrain lesions) studies (Chuang, 2004; Li et al., 2002; Yuan et al., 2004) lack specific disease relevance to bipolar disorder. Finally, it remains unclear as to whether lithium and valproate protect glial cells from cytotoxic signals, especially considering that reductions in number and density of glial cells have been demonstrated in brain of patients with bipolar disorder (Rajkowska, 2002).

The aim of the present study was to discern whether lithium and valproate, administered within therapeutically relevant concentrations exhibit time-dependent cytoprotective actions against cellular stresses arising as the consequences of putative cellular perturbations implicated in the pathophysiology of bipolar disorder. In light of the reported disturbances in endoplasmic reticulum (Hough et al., 1999; Kakiuchi et al., 2003; Kato et al., 2003; Warsh et al., 2004) and mitochondrial (Kato and Kato, 2000; Stork and Renshaw, 2005) function in bipolar disorder, oxidative stress and endoplasmic reticulum stress were used to model potential intracellular stress signals implicated in bipolar disorder in selected neuronal (SH-SY5Y neuroblastoma) or glial cell models (SVG p12 [Simian Virus 40 transformed astroglial] and U87 MG glioma) of human origin. Oxidative stress was induced either by the mitochondrial complex I inhibitor rotenone (Wolvetang et al., 1994), or by the application of hydrogen peroxide (H_2O_2) (Ruffels et al., 2004), while endoplasmic reticulum stress was induced by thapsigargin (Wei et al., 1998). We report here that lithium and valproate exert differential protective effects against endoplasmic reticulum stress- and oxidative stress-induced cell death in human SH-SY5Y cells. The selective protective effect against oxidative stress-evoked cell death appears to be dependent on Bcl-2 upregulation but independent of GSK-3β suppression. In comparison lithium and valproate failed to protect SVG p12 and U87 MG glioma cells against cell death induction by either endoplasmic reticulum stress or oxidative stress, suggesting that the cytoprotective actions of lithium and valproate may not be evident in cells of astroglial phenotype.

2. Materials and methods

2.1. Materials

SH-SY5Y, SVG p12, and U87 MG cells were obtained from the American Tissue Culture Collection (Manassas, VA). Lithium chloride, sodium valproate, rotenone, H_2O_2, and kenpaullone were purchased from Sigma-Aldrich (Oakville, ON., Canada). Thapsigargin was purchased from Alomone Labs (Jerusalem, Israel). The GSK-3 inhibitor SB216763 [3-(2,4-dichlorophenyl)-4-(1-methyl-1*H*-indol-3-yl)-1*H*-pyrrole-2,5-dione] was obtained from Tocris Cookson Inc. (Ellisville, MO). Cell culture media, penicillin and streptomycin were purchased from GIBCO/Invitrogen (Burlington, ON., Canada) whereas fetal bovine serum (FBS) was from Hyclone (Logan, UT). Primary polyclonal antibodies against Bcl-2 and β-tubulin were acquired from Santa Cruz Biotechnology (Santa Cruz, CA), whereas primary monoclonal antibody raised against cytochrome *c* was from BD Biosciences (Mississauga, ON., Canada). Secondary antibodies used included horseradish peroxidase-conjugated anti-mouse (Southern Biotechnology, Birmingham, AL) and anti-rabbit immunoglobulin (Vector Laboratories, Burlington, ON., Canada), and protein A (BioRad, Hercules, CA). ECL Plus Western blot detection kits were purchased from Amersham Biosciences (Bsie d'Urfe, QC., Canada).

2.2. Cell culture and drug treatment

SH-SY5Y neuroblastoma cells were cultured at 37 °C in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% FBS, 100 U/ml penicillin and 100 μg/ml streptomycin in a 95% air, 5% CO_2 humidified incubator. Cells were seeded at 2×10^6 cells per 75-cm^2 flask. The culture medium was changed every 3 days. Upon reaching 95% confluence, cells were washed with Dulbecco's Phosphate Buffered Saline (PBS: 1.7 mM NaH_2PO_4, 150 mM NaCl, 9.1 mM Na_2HPO_4) followed