Chronic Treatment with Mood Stabilizers Increases Membrane GRK3 in Rat Frontal Cortex

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Background: *G-protein receptor kinases (GRKs) are a family of serine/threonine kinases involved in the homologous desensitization of agonist activated G-protein coupled receptors (GPCRs). G-protein coupled receptor supersensitivity, possibly as a result of decreased GRK, has been suggested in affective disorders.*

Methods: *We used immunobloting to determine if chronic, therapeutically relevant doses of lithium (Li^+), carbamazepine (CBZ), and valproate (VPA), would increase GRK2/3 protein levels in rat frontal cortex.*

Results: *Chronic Li^+ (24%) and CBZ (44%) significantly increased GRK3 in the membrane but not cytosol fractions. Chronic VPA had no effect on GRK3. G-protein receptor kinase 2 protein levels were unchanged by all treatments. The GRK3 membrane to cytosol ratio was increased significantly in Li^+ and CBZ treated rats.*

Conclusions: *These results show that chronically administered Li^+ and CBZ, but not VPA, increase the translocation of GRK3 from cytosol to membrane, possibly correcting supersensitivity of GPCRs in bipolar disorder.*

Key Words: GRK3, GRK2, lithium, carbamazepine, valproate, brain

G-protein receptor kinases (GRKs) are a family of serine/threonine kinases involved in the homologous desensitization of agonist activated G-protein coupled receptors (GPCRs) (Krupnick and Benovic 1998; Palczewski et al 1991). Hundreds of different GPCRs are known to be regulated by seven types of GRKs (Gainetdinov et al 2004). G-protein receptor kinase 1 and GRK7 are strictly found in the retina. G-protein receptor kinase 4, GRK5, and GRK6 are not activated by the G-protein subunit βγ, whereas GRK2 and GRK3 are activated and translocated from the cytosol to the membrane by this subunit (Gainetdinov et al 2004; Koch et al 1993; Pitcher et al 1992; Premont et al 1995). G-protein receptor kinase 3 regulates several GPCRs, including the adrenergic (Carman and Benovic 1998), cholinergic muscarinic (Willets et al 2001), dopaminergic (Tiberi et al 1996), histaminergic (Shayo et al 2001), and corticotrophin regulating factor receptors (Dautzenberg et al 2001, 2002).

Studies of patients with bipolar disorder have demonstrated 1) abnormalities in serotonergic (Jope et al 1996), dopaminergic (Pantazopoulos et al 2004), and muscarinic receptor (Dilsaver 1986) mediated responses; 2) increased G-protein Gαs subunit in postmortem brain (Friedman and Wang 1996; Young et al 1993); 3) increased ^{35}[S]GTPγS binding to platelet membrane Gαs, Gαi, and Gαq/11 subunits (Hahn et al 2005); 4) increased serum phospholipase A_2 (PLA_2) activity (Noponen et al 1993); 5) a single nucleotide polymorphism in the promoter region of the GRK3 gene (Barrett et al 2003); and 6) a decreased GRK3 protein level in lymphocytes (Niculescu et al 2000). These studies imply overactivation of various GPCRs and decreased GRK3 protein level in bipolar disorder.

We therefore hypothesized that chronic mood stabilizers might increase GRK2/3 protein levels in the bipolar disorder brain and thereby reduce GPCR overactivation. To test this hypothesis, we determined whether chronic, therapeutically relevant doses of lithium (Li^+), carbamazepine (CBZ), or valproate (VPA) increase GRK2 and GRK3 protein levels in rat frontal cortex. We examined this region because studies indicate structural, metabolic, and signaling abnormalities in the frontal cortex of bipolar patients (Buchsbaum et al 1986; Lopez-Larson et al 2002; Lyoo et al 2004; Rajkowska 2002; Rubinsztein et al 2001; Suhara et al 1992).

Methods and Materials

Animals

The study was conducted following the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals (Publication no. 80-23) and was approved by the National Institutes of Child Health and Human Development Animal Care and Use Committee. Adult male CDF-344 rats (200–250 g) from Charles River Laboratories, (Wilmington, Massachusetts) received chronic lithium chloride (LiCl) (n = 7) (1.70 g/kg, 4 weeks, and 2.55 g/kg, 2 weeks in their chow), CBZ (n = 8) (25 mg/kg body weight dissolved in .9% saline/DMSO 50/50 [vol/vol], 30 days, daily intraperitoneal [IP] injection), or sodium VPA (n = 8) (200 mg/kg per day dissolved in .9% saline, 30 days, daily IP injection) to produce therapeutically relevant plasma concentrations of Li^+ (730 ± 30 μm) (Bosetti et al 2002), CBZ (53.6 ± 5.2 μm) (Ghelardoni et al 2004), and VPA (186.5 ± 37.9 μm) (Chang et al 2001). Control rats (n = 7, 8, 8) received appropriate vehicle. Animals were anesthetized by carbon dioxide (CO_2) inhalation and killed by decapitation. The brain was rapidly excised and the frontal cortex was dissected and frozen in 2-methylbutane at −50°C, then stored at −80°C until use.

Preparation of Cytosolic and Membrane Fractions

Cytosolic and membrane extracts were prepared from the frontal cortex of drug-treated and control rats as described (Dwivedi et al 2000). Protein concentrations were determined using Bio-Rad protein reagent (Bio-Rad, Hercules, California).

Western Blot Analysis

Cytosolic or membrane extracts (75 μg) were separated on a 10% to 20% SDS-Polyacrylamide gel (Bio-Rad) and transferred to a nitrocellulose membrane. Blots were incubated overnight with primary antibody (1:200) for GRK2 or GRK3 (Abgent, San Diego, California) in TBS buffer containing 5% nonfat dried milk and .1% Tween-20, followed by horseradish peroxidase (HRP)-conjugated secondary antibody (1:1000) (Bio-Rad). Blots were visual-

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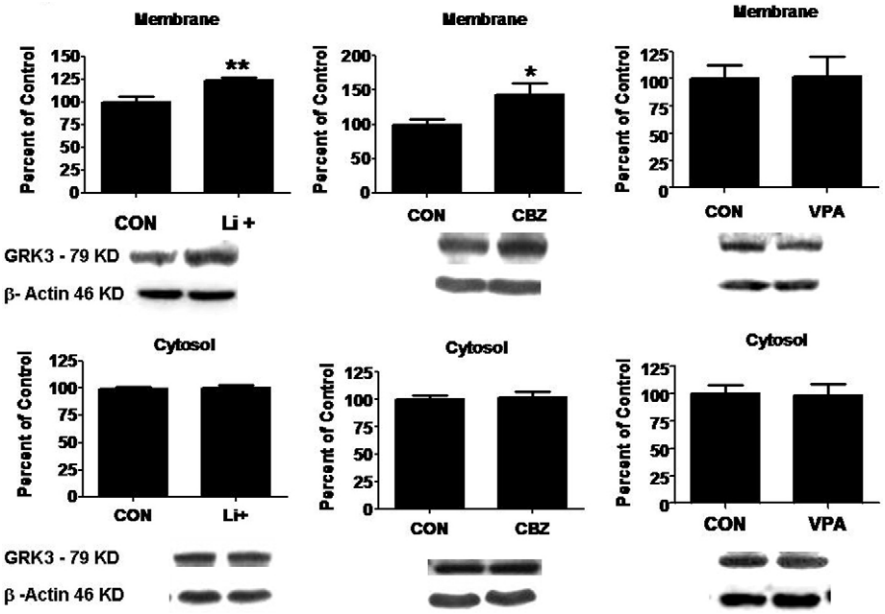

Figure 1. Representative immunoblots of membrane and cytosolic G-protein receptor kinase 3 (GRK3) protein levels in frontal cortex from control and mood stabilizer treated rats. Data are ratios of optical density of GRK3 to β-actin, expressed as percent of control rats and compared using a two-tailed, unpaired *t* test (mean ± SEM, *$p < .05$, **$p < .005$). GRK, G-protein receptor kinase.

ized and quantified after correcting for β-actin as described (Rao et al 2005).

Statistical Analysis

Data are expressed as mean ± SEM. Statistical significance was calculated using a two-tailed, unpaired *t* test set at $p < .05$.

Results

Chronic administration of Li^+, CBZ, or VPA had no effect on the GRK2 protein level either in membrane (Control, 100 ± 9.6 vs. Li^+, 99 ± 6.4; Control, 100 ± 8.1 vs. CBZ, 130 ± 19.1; Control, 100 ± 16.4 vs. VPA, 88 ± 17.2) or cytosol (Control, 100 ± 7.5 vs. Li^+, 99 ± 9.2; Control, 100 ± 8.9 vs. CBZ, 102 ± 11.9; Control, 100 ± 15.5 vs. VPA, 102 ± 14.4). However, chronic administration of Li^+ (24%) and CBZ (44%) significantly increased the protein level of membrane GRK3 but not of cytosolic GRK3 (Figure 1). Chronic administration of VPA had no effect on membrane or cytosolic GRK3 protein level (Figure 1). Chronic administration of Li^+ (Control, 1.00 ± .063 vs. Li^+, 1.2 ± .061) and CBZ (Control, 1.00 ± .061 vs. CBZ, 1.4 ± .13) significantly increased the membrane to cytosol GRK3 ratio ($p < .01$), suggesting an increased translocation of GRK3 from the cytosol to membrane. The results were similar if the samples were not corrected for β-actin.

Discussion

Alterations in functions and regulation of serotonergic (Jope et al 1996), muscarinic (Dilsaver 1986; Tollefson and Senogles 1983), and dopaminergic (Pearlson et al 1995; Wong et al 1997) GPCRs have been reported in postmortem bipolar brain. These GPCRs are coupled to multiple effectors including PLA_2 (Basselin et al 2003, 2005a; Bhattacharjee et al 2005; Felder 1995; Felder et al 1990; Qu et al 2003). Reported GPCR overactivation was associated with increased Gαs and heterotrimeric G-protein levels in platelets of bipolar disorder patients (Friedman and Wang 1996; Manji and Lenox 2000; Vawter et al 2000).

The present study demonstrated that chronic administration of therapeutically relevant doses of Li^+ or CBZ to rats resulted in a statistically significant selective increase in membrane but not cytosolic GRK3 protein in rat frontal cortex. Because chronic Li^+ and CBZ increased the membrane to cytosol GRK3 ratio, this suggests an increased translocation of GRK3. The lack of a corresponding decrease in cytosolic GRK3 by Li^+ or CBZ may be due to the much larger cytosolic fraction or de novo synthesis. Because GRK3 is activated by the G protein subunits βγ (Koch et al 1993), the observed increase in membrane GRK3 might be secondary to increased expression of the G-β subunit in the frontal cortex after chronic Li^+ or CBZ adminstration (Jakobsen and Wiborg 1998). Despite a lack of effect of VPA on GRK3, VPA regulation of GPCRs by GRK3-independent mechanisms cannot be ruled out. The lack of an effect on GRK2 by all treatments may be due to differences in βγ isoform selectivity for GRK2 and GRK3. For instance, in Cos-7 cells, G-β3 binds to GRK3 but not to GRK2 (Daaka et al 1997). Brain levels of GRK2 are increased relative to control subjects in the prefrontal cortex of depressed patients and are lower in those who received antidepressant therapy (Grange-Midroit et al 2003). Further characterization, including studies examining the time course, a dose response of mood stabilizers, as well as other brain regions, are warranted to better understand direct involvement of the G protein subunit and GRK 2/3 protein levels interactions after mood stabilizer treatment.

Common effects of these mood stabilizers have been reported. Chronic Li^+ and CBZ administration selectively reduced arachidonic acid turnover as well as activity, protein, and messenger RNA (mRNA) levels of cytoplasmic PLA_2 ($cPLA_2$) in rat brain (Bazinet et al 2006a; Chang et al 1996, 1999; Ghelardoni et al 2004; Rao et al 2005; Rintala et al 1999; Weerasinghe et al 2004). Chronic VPA also selectively decreased arachidonic acid turnover in rat brain phospholipids but did not alter $cPLA_2$ expression or activity (Bazinet et al 2005; Chang et al 2001) and likely targeted a long-chain acyl-CoA synthetase (Bazinet et al 2006b). Furthermore, chronic Li^+ and CBZ but not VPA downregulated the brain activator protein (AP)-2 transcription factor, which was thought to account for the reduction in $cPLA_2$ gene expression (Rao et al 2005, Rao et al, in press[a], in press[b]).

The present study supports the hypothesis that increased GRK3 protein translocation from cytosol to membrane may be part of the therapeutic effect of lithium and carbamazepine.