Glycogen synthase kinase-3β, β-catenin, and tau in postmortem bipolar brain

Short Communication

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Summary. Therapeutic concentrations of the anti-bipolar drug lithium inhibit the activity of glycogen synthase kinase-3β, which raises the possibility that this enzyme and its substrates may be altered in the brain of subjects with bipolar disorder. Therefore, in prefrontal cortical samples from subjects with bipolar disorder and age-matched control subjects, we examined the levels of glycogen synthase kinase 3β and of two proteins modified by it, β-catenin and the microtubule associated protein tau. There were no significant differences between subject groups among these measurements, but there was a tendency for the tau isoform profile to be modified in bipolar tissue. Thus, while there are no differences between bipolars and controls in prefrontal cortical levels of glycogen synthase kinase-3β, β-catenin, or tau, tau isoform levels or phosphorylation states may be modified in bipolar disorder.

Keywords: Bipolar disorder, glycogen synthase kinase-3β, β-catenin, tau.
described function of phosphorylating glycogen synthase, this kinase also phosphorylates several other proteins, including microtubule associated proteins (MAPs) such as tau (Lovestone et al., 1996) and MAP-1B (Lucas et al., 1998), which contribute to regulating neuronal cytoskeletal networks. Several recent studies found that inhibition of GSK-3β by lithium reduces tau phosphorylation (Klein and Melton, 1996; Stambolic et al., 1996; Hong et al., 1997; Munoz-Montano et al., 1997; Zheng-Fischhofer et al., 1998; Xie et al., 1998). GSK-3β also promotes the degradation of β-catenin, and lithium was shown to lead to elevated β-catenin levels apparently as a result of inhibition of GSK-3β (Stambolic et al., 1996).

These new findings raise the possibility that bipolar disorder may be associated with elevated GSK-3β and alterations of β-catenin and tau, since therapeutic concentrations of lithium inhibit GSK-3β and thereby may modulate β-catenin levels and the level and phosphorylation of tau. Therefore, the present study examined GSK-3β, β-catenin, and tau in postmortem prefrontal cortex from subjects with bipolar disorder and matched psychiatrically normal controls.

**Methods**

**Tissue**

Brain tissue was obtained at autopsy at the Cuyahoga County (Ohio) Coroner’s Office, in compliance with policies of the institutional review board and written consent from the next-of-kin. Tissue samples were obtained from 5 subjects with bipolar disorder (4 male/l female; 36 ± 4 years of age; 23 ± 3 hr postmortem interval; pH 6.8 ± 0.1; mean ± S.E.M.) and 5 matched psychiatrically normal control subjects (4 male/l female; 36 ± 3 years of age; 23 ± 1 hr postmortem interval; pH 6.7 ± 0.1). Brain lithium levels were measured as described previously (Jope et al., 1996) and were equal to, or less than, 0.8 μg/g tissue in all bipolar subjects, well below therapeutic concentrations. Diagnoses, medications, and toxicological analyses were obtained as described previously (Pacheco et al., 1996). The bipolar subjects (4 out of 5 died from suicide) generally had other existing complications (4 out of 5 were classified as psychoactive substance-dependent) and thus cannot be viewed as a “clean” bipolar population, as is often the case with this diagnostic group.

**Immunoblotting**

Samples (~60 mg wet weight) of prefrontal cortex (left Brodmann's areas 8 and 9) were homogenized (1:5, w/v) in 250 mM Tris-HCl, pH 6.8, 5 mM EDTA, 5 mM EGTA, 2% sodium dodecylsulfate (SDS) and 10% glycerol. Homogenates were sonicated briefly and clarified by centrifugation. Protein concentrations were determined using the bicinchoninic acid (BCA) assay kit (Pierce) using bovine serum albumin as the standard. Samples were diluted to 1 mg protein/ml in 2X Laemmli buffer (250 mM Tris-HCl, pH 6.8, 5 mM EDTA, 5 mM EGTA, 2% SDS, 10% glycerol, 25 mM dithiothreitol, and 0.01% bromophenol blue as the tracking dye), and incubated in a boiling water bath for 10 min. Proteins were resolved on 10% SDS-polyacrylamide gels and transferred to nitrocellulose. The membranes were blocked for 15 to 30 min in Tris-buffered saline/Tween 20 (137 mM NaCl, 20 mM Tris-base, pH 7.4, and 0.05% Tween 20) containing 5% nonfat dried milk. Blots were incubated overnight at 4°C in the blocking buffer with combined antibodies 5A6/Tau5 (both 1:1,000), which detect total tau levels (Johnson et al., 1997; Carmel et al., 1996), or with combined antibodies to GSK-3β (1:1,000) and β-catenin (1:2,000) (both from Transduction Labs). Blots were rinsed twice with Tris-buffered