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Research Report

Decreased expression of insulin-like growth factor binding protein 2 in the prefrontal cortex of subjects with bipolar disorder and its regulation by lithium treatment

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ABSTRACT

Insulin-like growth factors (IGFs) regulate cellular proliferation and death, and their bioactivity is controlled by IGF binding proteins (IGFBPs). Since IGFBP-2 is the major brain resident IGFBP, and we have demonstrated lithium-mediated changes in its mRNA and protein levels in neuronal cultures, we examined IGFBP-2 expression in prefrontal cortex postmortem brain tissue from subjects with mood disorders. We found decreased IGFBP-2 expression in bipolar disorder patients compared with controls; this was especially pronounced in subjects not treated with lithium. These results suggest a role for IGFBPs in the etiology and pharmacotherapy of mood disorders.

Imaging and postmortem studies in subjects with mood disorders have revealed volumetric and glial abnormalities in various brain regions including the prefrontal cortex (PFC) (Rajkowska, 2002; Strakowski et al., 1999). These abnormalities are thought to involve changes in the expression of a number of neuroprotective and/or neurotrophic factors, such as brain derived neurotrophic factor (BDNF) (Gadient and Otten, 1997; Gruol and Nelson, 1997). The insulin-like growth factors (IGFs) I and II are components of yet another system which has been determined to be strongly neuroprotective (Cheng and Mattson, 1992). This system is regulated by IGF binding proteins (IGFBPs), a family of six structurally similar proteins which bind IGFs with high affinity and sequester them within tissues or organs and/or transport them between tissues or compartments (Leventhal et al., 1999). In the CNS, the principal IGFBP is IGFBP-2 (Aizenman and de Vellis, 1987), and we have previously observed a dose- and time-dependent decrease in the mRNA and protein levels of this factor with lithium treatment in primary cultured neurons (Bezchlibnyk et al., 2006). Therefore, we used real-time PCR to assess the expression of IGFBP-2 in postmortem brain samples from subjects with mood disorders in order to determine whether this factor plays a role in the etiology of mood disorders as well as pharmacotherapy.

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Abbreviations: PFC, prefrontal cortex; IGF, insulin-like growth factor; IGFBP, insulin-like growth factor binding protein; BD, bipolar disorder; CREB, cyclic AMP-response element-binding protein; BDNF, brain derived neurotrophic factor; MDD, major depressive disorder; PMI, postmortem interval; IGF-IR, insulin-like growth factor type I receptor; Trk, tyrosine kinase

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Frozen sections of human PFC (Brodmann's areas 9 and 42) were generously provided by the Stanley Foundation Neuropathology Consortium. The family of each deceased individual was contacted by a pathologist to make a preliminary diagnosis and to request permission for donation of the brain and release of the deceased's medical records. Diagnoses were retrospectively established by two senior psychiatrists using DSM-IV criteria, and detailed clinical information and diagnostic procedures are available (Dowlatshahi et al., 1999; Torrey et al., 2000). The sample consisted of 15 subjects with bipolar disorder (BD), 15 with major depressive disorder (MDD), and 14 non-psychiatric, non-neurologic comparison subjects; there were no differences between diagnostic groups in age ($F=1.463$, $df=2,43$, $p=0.243$), gender ($F=0.015$, $df=2,43$, $p=0.985$), postmortem interval (PMI) ($F=1.215$, $df=2,43$, $p=0.307$) (Table 1), or brain pH ($F=0.534$, $df=2,43$, $p=0.590$).

Total RNA was extracted from each subject using TriZol Reagent (Sigma), following the manufacturer's suggested protocol. Subsequently, RNA was purified using the RNeasy kit (Qiagen). Yield and purity of the RNA samples were determined by spectrophotometric analysis, and the integrity of rRNA bands was confirmed by electrophoretic separation. Subsequently, genomic DNA contamination was removed by treating 1 μg of each RNA sample with DNase I (Invitrogen) for 15 min at 25 °C. Double-stranded cDNA was then synthesized by RNA reverse transcription under MultiScribe reverse transcriptase (Applied Biosystems), primed with random hexamers. Real-time PCR analysis was performed on an ABI Prism 7000 Sequence Detector (Applied Biosystems). For each reaction, 50 ng of cDNA was combined with 25 μl of TaqMan Universal Master Mix (Applied Biosystems) and 2.5 μl of probe and primer mix specific for the human IGFBP-2 transcript (AssayID Hs00167151_m1; Applied Biosystems). In addition, 18S rRNA was assessed as an endogenous control for each sample. In either case, cycling parameters consisted of an initial incubation at 50 °C for 2 min, an additional incubation at 95 °C for 10 min, and 40 cycles of 95 °C for 15 s and 60 °C for 1 min. Each reaction was run in triplicate.

For data analysis, the IGFBP-2 expression was determined according to the relative quantification (ΔΔCT) method. Briefly, the average number of cycles required to attain a threshold level of log-based fluorescence (CT value) was normalized to the average CT value of the endogenous control [ΔCT(sample) = CT(target) – CT(endogenous control)] in each sample. The ΔCT value for IGFBP-2 in each sample was normalized to its value in a standardized sample, consisting of cDNA obtained from RNA pooled across all subjects, run in triplicate simultaneously with the experimental samples [ΔΔCT = ΔCT(sample) – ΔCT (standard)]. The ΔΔCT represents the logarithm (base 2) of the average change in expression between the control and experimental samples.

Results were expressed as the mean ± SEM and are representative of duplicate determinations. Two-tailed independent-samples Student's *t*-tests were used to assess the effect of gender in the general subject pool, while contributions of age, PMI, and pH were examined by Pearson's correlation analysis. Statistical significance between groups was determined by Bonferroni-corrected one-way ANCOVAs, with age, gender, PMI, and brain pH as covariates. Results were assumed to be significant if $p<0.05$ in all cases. All statistics were done using SPSS version 12.01 for Windows (SPSS Inc. Chicago, IL, USA).

These experiments indicate a significant effect of diagnosis on IGFBP-2 mRNA expression (Fig. 1a; $F=3.252$, $df=2,43$, $p=0.050$). Specifically, levels of IGFBP-2 were decreased (33.9%) in subjects with BD compared to controls ($p=0.024$); no differences between control and MDD, or BD and MDD subjects were observed. Moreover, there was no significant difference in IGFBP-2 expression across gender and no significant correlations were observed between this factor and age, PMI, or pH (Fig. 2).

When subjects with BD were stratified by lithium treatment at time of death, we observed a significant effect of treatment across groups (see Fig. 1b; $F=3.889$, $df=2,28$, $p=0.036$), manifested as a decrease in IGFBP-2 mRNA expression (42.4%) in subjects not treated or remotely treated with lithium at time of death compared to the control group ($p=0.024$). Although IGFBP-2 mRNA expression in the lithium-treated group was increased relative to non lithium-treated BD subjects (43.6%), this difference did not attain statistical significance. We found no effect of treatment with either antidepressants or anticonvulsants (i.e. valproate or carbamazepine) at the time of death. In addition, we were unable to observe an association between IGFBP-2 mRNA expression and suicide as a cause of death or family history of either BD or MDD (data not shown).

In summary, we report that subjects with BD exhibit decreased IGFBP-2 mRNA expression in the PFC, and these effects are observed predominantly in subjects not treated or remotely treated with lithium. Of relevance, administration of IGF has been shown to reduce neuronal damage deriving from ischemic, hypoxic, or physical brain injury (Cheng and Mattson, 1992; Schabitz et al., 2001; Wang et al., 2000), and IGF-I, the IGF-I receptor (IGF-IR), and IGFBP-2 are all highly expressed in regions of the brain undergoing plasticity (Bondy and Lee, 1993; D'Ercole et al., 1996; Lee et al., 1993). Moreover,

Table 1 – Subject demographics (IGFBP-2 mRNA expression)

	Control $n=14$	BD $n=15$	MDD $n=15$
Age (mean ± SEM, years)	48.4 ± 2.9	42.3 ± 3.0	46.5 ± 2.4
Range	[29–68]	[25–61]	[30–65]
Gender (male/female)	8/6	9/6	9/6
Treated with lithium (+/–)	–	5/10	2/13
PMI (mean ± SEM, hours)	24.7 ± 2.5	32.5 ± 4.2	27.5 ± 2.8
Range	[8–42]	[13–62]	[7–47]
Brain pH (mean ± SEM)	6.26 ± 0.66	6.18 ± 0.60	6.18 ± 0.55
Range	[5.8–6.6]	[5.8–6.5]	[5.8–6.5]

SEM, standard error of the mean; PMI, postmortem interval; BD, bipolar disorder; MDD, major depressive disorder; SCZ, schizophrenia; +, treated with lithium at time of death; –, not treated with lithium at time of death

This table shows the demographic characteristics of all subjects included in analyses of insulin growth factor binding protein 2 (IGFBP-2) mRNA expression in postmortem brain ($n=57$), comparing age, sex distribution, treatment with lithium, and postmortem interval (PMI) between non-psychiatric controls and subjects with bipolar disorder (BD) or major depressive disorder (MDD).