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Decreased Phosphorylated ERK 1/2 Related to GABA levels in Bipolar Disorder

A Preliminary Report

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ABSTRACT

Abstract

Introduction

MAP kinases and pathway proteins, such as ERK 1/2, may be associated with the etiology of mood disorders such as Bipolar Disorder, and these proteins have been identified as targets for mood stabilizers. There is evidence to suggest that ERK activation effects GABA secretion inhibition. This study was designed to measure phosphorylated ERK 1/2 and GABA in individuals with Bipolar Disorder and to begin to determine if there is a relationship between them.

Methods

We used ELISAs to measure the concentration of cellular, phosphorylated ERK ½ and plasma GABA levels in 34 individuals with Bipolar Disorder and 34 neurotypical, age and gender similar controls.

Results

We found that activated ERK 1/2 was significantly lower than in individuals with Bipolar Disorder compared to controls, and that these decreased levels correlated significantly with high GABA in these same patients.

Discussion

We suggest that in Bipolar Disorder, low ERK levels may result in high GABA, and that mood stabilizers, such as lithium and valproate, which act upon and raise ERK levels.

KEYWORDS *Major bleeding, Net clinical outcomes, MACE, Bivalirudin, Heparin*

Introduction

Severe mood alteration in mood disorders affects an individual's well being, as well as self esteem, judgment, attention, motivation, learning and memory, sleep, appetite, and overall psychomotor activity (1). Brain imaging studies demonstrate regional changes in CNS volume, and number and/or size of glia and neurons in discrete brain areas of patients with mood disorders. Data suggest neurotrophic function, cellular growth, death, and resilience are possible factors that contribute to mood disorders like Bipolar Disorder (2-4).

Growth factors, through receptor tyrosine kinases, recruit a large network of signaling proteins to execute their cellular programs. The first of these networks to be discovered was the Ras-Raf-ERK signal transduction cascade, defined by Extracellular Signal-regulated Kinase-1 (ERK1) and ERK2 (12). During growth factor stimulation, the ERK phosphorylation cascade is linked to cell surface receptor tyrosine kinases (RTKs) (11). ERK1 and ERK2 regulate transcription indirectly by phosphorylating the 90 kDa Ribosomal Protein S6 Kinases (RSKs), a family of broadly expressed serine/threonine kinases activated in response to mitogenic stimuli, including growth factors (13).

The ERK pathway is activated by a wide variety of receptors involved in growth and differentiation including receptor tyrosine kinases (RTKs), integrins, and ion channels (7-10).

Mood stabilizers. like lithium and valproate, are used in the treatment of manic-depressive illness. Lithium is a monovalent cation, and valproate is a branched chain fatty acid (5,6). These drugs affect the ERK pathway. Both drugs activate the ERK pathway in the rat frontal cortex and hippocampus, and they activate an ablated ERK pathway by facilitating ERK function at therapeutic doses. Also, genetic altercations of the ERK pathway in rodents cause mood behavioral changes (14).

Gamma-Aminobutyric acid (GABA) is the chief inhibitory neurotransmitter in the mammalian central nervous system. the MAPK pathway as a negative modulator of GABAA receptor function (26).

This study measured phosphrylated ERK 1/2 in individuals with bipolar disorder and compared these concentrations with levels of GABA.

Materials and Methods

Subjects

We used ELISAs to measure cellular phosphrylated ERK 1/2 in individuals with Bipolar Disorder (N=34; 19 male; mean age 31.2 years) and age/gender similar controls (N=34; 16 male; mean age 34.1 years).

White blood cells from consecutive individuals with diagnosed autism and controls were obtained from patients presenting at the Health Research Institute (HRI) * over a two-year period. All Bipolar individuals who presented to HRI were asked to participate. Plasma and cells from patients who participated in this study were randomly chosen from all patients who volunteered. Neurotypical control plasmas, were also obtained from HRI and randomly chosen from a selection of about 200 samples.

Patient consent was obtained from all patients involved in this study and the study was approved by the IRB of the HRI.

Enzyme Linked Immunosorbent Assays (ELISAs)

ELISAs were used to measure cellular ERK 1/2 (eBiosciences, San Diego, CA).

1. 50 µL/well of 1X Cell Lysis Mix (negative control) and 50 µL/well Positive
Control Cell Lysate (positive control) to separate assay wells for controls.
2. 40 µl of lysis buffer (contains a combination of detergents, phosphatase inhibitors, salts and buffers) was added to each of the control and experimental wells.
3. 10 µl of buffy coat cells (experimental and controls) were added to appropriate wells and mixed gently.
2. 50 µL/well of Antibody Cocktail mix (detection antibody and HRP conjugated antibody) was added to all the assay test wells. The plate was incubated for 1 hr at room temperature on a microplate shaker (~300 rpm).
3. Wells were washed with 300 µL/well 1X Wash Buffer 4 times.
4. 100 µL of Detection Reagent (TMB) was added to each well and the wells were incubated for 10-30 minutes.
5. After color development, 100 µL of Stop Solution was added to each well.
6. Absorbance was measured using a colorimetric (spectrophotometric) plate reader (BioRad) set at 450 nm.

To ensure reproducibility of results, samples were run in duplicate and reported concentrations were the result of the average of at least two separate assays.

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Plasma and buffy coat (white blood cells) Preparation

Plasma and buffy coat cells, obtained from the patients at the Health Research Institute, were treated in an

identical fashion - frozen at -70C immediately after collection and cell/serum separation, then stored at -70C until thawed for use in ELISAs.

Results

Using an ELISA, we measured phosphorylated ERK 1/2 in patients diagnosed with Bipolar Disorder (N=34) and age and gender similar neurotypical controls (N=34). Individuals with Bipolar Disorder had significantly lower phosphorylated ERK ½ (mean 149.14 +/- 21.38 pg/ml) when compared to neurotypical controls (mean 254.91 +/- 34.40 pg/ml) (Figure 1). We also found that phosphorylated ERK 1/2 concentration was not associated with drugs, as ERK levels of those who were taking pharmaceuticals did not differ from those who were nor taking drugs (p=0.9) (Figure 1). When we compared those taking individual drugs (antidepressant, antipsychotic, antianxiety, antimanic and anticonvulsant) we found no significant difference between ERK levels of individuals taking these drugs and levels of those taking all other drugs.

We also found that the decreased activated ERK levels in these Bipolar individuals correlated significantly with increased GABA ($r = 0.37$; $p = 0.05$).

Statistics

Inferential statistics were derived from unpaired t-test and odds ratios with 95% confidence intervals. Pearson moment correlation test was used to establish degree of correlation between groups using 95% confidence intervals.

*The Health Research Institute, Warrenville, Il., is a comprehensive treatment and research center, specializing in the care of with neurological disorders, including Bipolar Disorder.

Discussion

Activated ERK phosphorylates numerous proteins involved in a diverse array of cellular processes including epigenetic, transcriptional and translational regulation, dendritic organization, cellular excitability, long-term potentiation and long-term depression, neuronal survival and synaptogenesis, and neurotransmitter release (15-18).

The ERK pathway is a major intracellular signaling cascade mediating the biological effects of neurotrophic factors such as brain-derived neurotrophic factor (19), and neurotrophic factors have increasingly been implicated as playing important roles in the pathophysiology and treatment of mood disorders (20-22).

A number of studies have been undertaken to investigate the role of the ERK pathway in mediating cognitive function (15-17).

Previous studies have implicated ERK signaling in learning and LTP, a synaptic plasticity mechanism thought to underlie learning & memory (23). Enhanced learning and memory may be associated with GABAergic synaptic transmission (24). There is evidence to suggest that ERK activation effects GABA secretion inhibition (25), and that the ERK pathway regulates GABAA receptors (26).

In this study, we showed that ERK 1/2 concentration is decreased in individuals with Bipolar Disorder compared to controls, and that these levels correlate with high GABA. We didn't find a significant difference in ERK levels associated with the taking of any type of mood stabilizer.

We suggest that in Bipolar Disorder, low ERK levels may result in high GABA, and that mood stabilizers, such as lithium and valproate, which act upon and raise ERK levels, may change behavior by altering GABA levels. Given that a high percentage of individuals with Bipolar Disorder don't respond well to current available drugs, and the fact that we didn't find a significant relationship between mood stabilizers and ERK levels, we suggest seeking new therapy that raises ERK levels, and changes GABA levels in an attempt to improve symptoms of individuals with Bipolar Disorder.

References

1. Goodwin FK, Jamison KR (1990) Manic-depressive illness. New York: Oxford UP.
2. Manji HK, Drevets WC, Charney DS (2001) The cellular neurobiology of depression. Nat Med 7:541–547.
3. Nestler EJ, Barrot M, DiLeone RJ, Eisch AJ, Gold SJ, Monteggia LM Neurobiology of depression. Neuron 2002;34:13–25.
4. Coyle JT, Duman RS Finding the intracellular signaling pathways affected by mood disorder treatments. Neuron 2003;38:157–160.
5. Gould TD, Chen G, Manji HK Mood stabilizer psychopharmacology. Clin Neurosci Res 2002;2:193–212.
6. Manji HK, Chen G (2002) PKC, MAP kinases and the bcl-2 family of proteins as long-term targets for mood stabilizers. Mol Psychiatry 2002;7 [Suppl 1]:S46 –S56.
7. Anjum R, Blenis J The RSK family of kinases: emerging roles in cellular signalling. Nat. Rev. Mol. Cell Biol. 2008;9(10), 747–58.

8. Kim EK, Choi EJ Pathological roles of MAPK signaling pathways in human diseases. Biochim. Biophys. Acta 2010;1802(4), 396–405.
9. Keyse SM Dual-specificity MAP kinase phosphatases (MKPs) and cancer. Cancer Metastasis Rev. 2008;27(2), 253–61.
10. De Luca A, Maiello MR, D'Alessio A, Pergameno M, Normanno N The RAS/RAF/MEK/ERK and the PI3K/AKT signaling pathways: role in cancer pathogenesis and implications for therapeutic approaches. Expert Opin. Ther. Targets 2012;16 Suppl 2, S17–27.
11. Blume-Jensen, P. & T. Hunter Oncogenic kinase signaling Nature 2001;411:355.
12. Seger, R. & E. Krebs The MAPK signaling cascade. FASEB J. 1995;9:726.
13. Chen, R.-H. et al. Regulation of pp90OSk phosphorylation and S6 phosphotransferase activity in Swiss 3T3 celsby growth factor-,phorbol ester-, and cyclic AMP-mediated signal transduction. Mol. Cell. Biol. 1991;11:1861.
14. Haim Einat et al, The Role of the Extracellular Signal-Regulated Kinase Signaling Pathway in Mood Modulation The Journal of Neuroscience, August 13, 2003;23(19):7311–7316.
15. Kelleher III RJ, Govindarajan A, Tonegawa S. Translational regulatory mechanisms in persistent forms of synaptic plasticity. Neuron 2004; 44: 59–73.
16. Thomas GM, Huganir RL. MAPK cascade signalling and synaptic plasticity. Nat Rev Neurosci 2004; 5: 173–183.
17. Sweatt JD. Mitogen-activated protein kinases in synaptic plasticity and memory. Curr Opin Neurobiol 2004; 14: 311–317.
18. Chen G, Manji HK. The extracellular signal-regulated kinase pathway: an emerging promising target for mood stabilizers. Curr Opin Psychiatry 2006;19: 313–323.
19. Huang EJ, Reichardt LF. Trk receptors: roles in neuronal signal transduction. Annu Rev Biochem 2003;72: 609–642.
20. . Coyle JT, Duman RS. Finding the intracellular signaling pathways affected by mood disorder treatments. Neuron 2003;38:157–160.
21. Berton O, Nestler EJ. New approaches to antidepressant drug discovery: beyond monoamines. Nat Rev Neurosci 2006; 7: 137–15.
22. . Manji HK, Drevets WC, Charney DS. The cellular neurobiology of depression. Nat Med 2001;7: 541–547.
23. Selcher JC, Atkins CM, Trzaskos JM, Paylor R, Sweatt JD. Learning & memory. Vol. 6. Cold Spring Harbor, NY: 1999. A necessity for MAP kinase activation in mammalian spatial learning; pp. 478–490.
24. Collinson N et al Enhanced learning and memory and altered GABAergic synaptic transmission in mice lacking the alpha 5 subunit of the GABAA receptor. J Neurosci. 2002;22(13):5572-80.

25. Zhang ZJ et al ERK activation effects on GABA secretion inhibition induced by SDF-1 in hippocampal neurons of rats. Zhongguo Ying Yong Sheng Li Xue Za Zhi. 2015;31(5):443-7.
26. Bell-Horner CL et al ERK/MAPK pathway regulates GABAA receptors. J Neurobiol. 2006;66(13):1467-74.

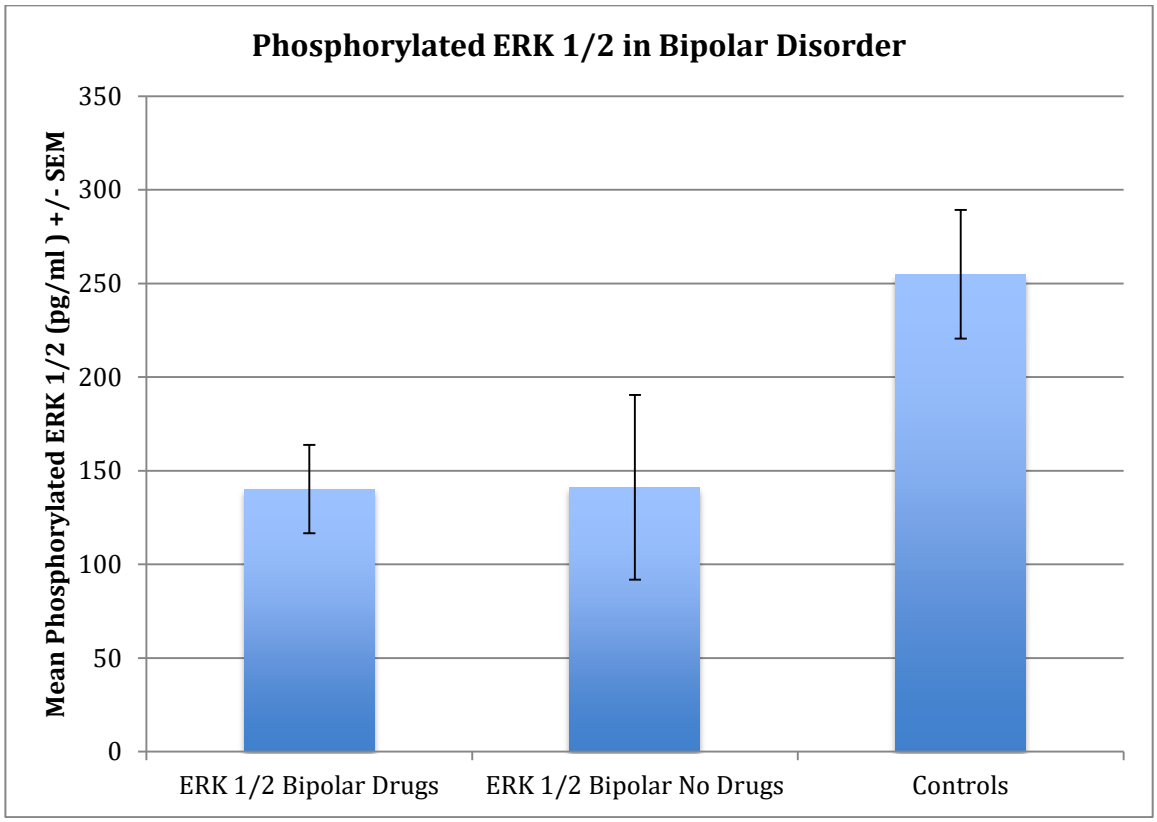

Figure 1 Phosphorylated ERK 1/2 in neurotypical controls, Individuals with Bipolar Disorder not taking drugs, Individuals with Bipolar Disorder taking drugs. ERK 1/2 levels in individuals with Bipolar Disorder (taking drugs or not) were significantly lower than controls. ANOVA p=0.03.