European Neuropsychopharmacology (2012) 22, 527–535

www.elsevier.com/locate/euroneuro

Chronic treatment with lithium or valproate modulates the expression of *Homer1b/c* and its related genes *Shank* and *Inositol 1,4,5-trisphosphate receptor*

Andrea de Bartolomeis [a,*], Carmine Tomasetti [a], Maria Cicale [a], Pei-Xiong Yuan [b], Husseini K. Manji [b,c]

[a] *Laboratory of Molecular Psychiatry and Psychopharmacotherapeutics, Department of Neuroscience, Section of Psychiatry, University School of Medicine "Federico II", Naples, Italy*
[b] *Laboratory of Molecular Pathophysiology, National Institute of Mental Health, National Institutes of Health, Bethesda, MD, USA*
[c] *Johnson and Johnson Pharmaceutical Research and Development, Titusville, NJ, USA*

Received 8 August 2011; received in revised form 28 October 2011; accepted 14 November 2011

KEYWORDS
Bipolar disorder;
Gene expression;
Glutamate;
Postsynaptic density;
Schizophrenia;
Striatum

Abstract

Homer proteins are associated with both dopaminergic and glutamatergic function. In addition, these proteins are implicated in many signal transduction pathways that are also putative targets of the mood stabilizers lithium and valproate (VPA). This study investigated the effect of *in vivo* chronic administration of therapeutically-relevant doses of lithium and VPA on the expression of the inducible (*Homer1a* and *ania-3*) and constitutive (*Homer1b/c*) isoforms of the *Homer1* gene in rat brain, and of two other *Homer*-related genes: *Inositol 1,4,5 trisphosphate receptor* (*IP3R*) and *Shank*. *Homer1b/c* was significantly decreased in cortex by VPA, and in striatal and accumbal subregions by both lithium and VPA. Both mood stabilizers reduced *Homer1b/c* expression in the dorsolateral caudate-putamen, while only VPA decreased gene expression in

Abbreviations AcCo, nucleus accumbens core; AcSh, nucleus accumbens shell; ANOVA, analysis of variance; Bcl-2, B-cell lymphoma 2 protein; BAD, Bcl-2-associated death promoter; BDNF, brain-derived neurotrophic factor; Cg, cingulate cortex; CPDM, dorsomedial caudate-putamen; CPDL, dorsolateral caudate-putamen; CPVL, ventrolateral caudate-putamen; CPVM, ventromedial caudate-putamen; CREB, c-AMP response element-binding protein; ERK, extracellular-signal regulated kinase; GSK-3β, glycogen synthase kinase 3β; I, insular cortex; IEGs, immediate-early genes; IP3, Inositol 1,4,5-trisphosphate; IP3Rs, IP3 receptors; M1, motor cortex; M2, medial agranular cortex; MAPK, mitogen-activated protein kinase; mGluRs, metabotropic glutamate receptors; NMDARs, N-methyl-D-aspartate glutamate receptors; PBS, phosphate buffered saline; PI, phosphoinositide; PI3K-Akt, phosphatidylinositol-3-kinase; PKC, protein kinase C; PSD, postsynaptic density; ROI, region of interest; SS, somatosensory cortex; SSC, saline sodium citrate solution; VPA, valproate

* Corresponding author at: Laboratory of Molecular Psychiatry and Psychopharmacotherapeutics, Department of Neuroscience, Section of Psychiatry, University School of Medicine "Federico II", Via Pansini 5, 80131 Naples, Italy. Tel.: +39 081 746 3673, +39 081 746 3884 (Lab); fax: +39 081 746 2378.
E-mail address: adebarto@unina.it (A. de Bartolomeis).

doi:10.1016/j.euroneuro.2011.11.006

all other striatal subregions. *Shank* and *IP3R* were downregulated by both mood stabilizers in the cortex. Neither chronic lithium nor VPA affected *Homer* immediate-early genes. These results suggest that lithium and VPA similarly modulate the expression of structural postsynaptic genes with topographic specificity in cortical and subcortical regions. Thus, *Homer* may represent an additional molecular substrate for mood stabilizers, and a potential link with dopaminergic function.

1. Introduction

The *Homer* family of genes encodes for scaffolding proteins that are localized at the glutamatergic postsynaptic density (PSD). These proteins have been implicated in the regulation of synaptic plasticity, as well as in animal behaviors considered relevant to the modeling of human psychiatric diseases (de Bartolomeis and Iasevoli, 2003). Moreover, several lines of evidence suggest that Homer proteins may play a potential role in some neuropsychiatric disorders, such as schizophrenia (Szumlinski et al., 2006).

Multiple constitutive forms (Homer 1b/c, Homer2, and Homer 3) of Homer proteins, and two inducible forms (Homer1a and its splicing variant ania-3), which are induced in an immediate-early gene (IEG)-like fashion, have been described (Brakeman et al., 1997). Both constitutive and inducible forms have an EVH (Ena/Vasp homology) domain at the N-terminal, which allows these proteins to interact with metabotropic glutamate receptors (mGluRs) type I (mGlur1 and mGlur5) on the cell surface (Kammermeier and Worley, 2007). The constitutive forms also have a coiled-coil domain at the C-terminal that is responsible for self-multimerization, whereas the inducible forms (Homer 1a and ania-3)—which lack the coiled-coil domain—cannot multimerize. Homer1a disassembles the constitutive forms, acting as a "dominant negative", thereby modifying signal transmission initiated at mGluR1/5.

Several studies suggest that *Homer* expression can be modulated by psychotropic drugs that mainly, though not exclusively, impact dopaminergic and glutamatergic neurotransmission (Polese et al., 2002; Tomasetti et al., 2007; Zhang et al., 2007). In addition, previous studies from our laboratory demonstrated that antipsychotic drugs differentially affect *Homer1a* expression depending on the degree of dopamine D2 receptor blockade (Tomasetti et al., 2007).

Recently, we noted that the combined administration of antipsychotics and valproate (VPA) may induce differential modulation of PSD genes—particularly *Homer*-related genes—compared to the effects observed when these drugs were administered individually (Tomasetti et al., 2011). However, to the best of our knowledge, no study to date has investigated the manner in which the mood stabilizers lithium and VPA regulate *Homer* genes. This is unexpected, given that clinical (Moore et al., 2000) and preclinical (Du et al., 2004) evidence have implicated glutamate in the pathophysiology of bipolar disorder, and that dopamine neurotransmission is believed to play a role in the mechanism of action of mood stabilizers (Beaulieu et al., 2009). In addition, recent studies have directly implicated *Homer1* in the etiology of mood disorders (Rietschel et al., 2010). This dearth of information is even more surprising considering that Homer proteins modulate the phosphoinositide (PI) signaling pathway (Mao et al., 2008), one of the most extensively studied targets of lithium, and which has also recently been studied as a putative target of VPA (Tokuoka et al., 2008; Williams and Harwood, 2000). Furthermore, Homer has been implicated in the modulation of the phosphatidylinositol-3-kinase—Akt (PI3K-Akt) pathway (Ronesi and Huber, 2008), which, in turn, was shown to directly regulate the function of glycogen synthase kinase 3β (GSK-3β), a well-recognized molecular target of mood stabilizers (Gould and Manji, 2005).

An intriguing connection also appears to exist between the Homer proteins, the mGluRs, intracellular calcium concentrations, and the enzyme protein kinase C (PKC) (Sala et al., 2005), whose protein levels are also affected by chronic *in vivo* administration of lithium and VPA (Manji and Chen, 2002). In addition, recent observations suggest that Homers play a major role in modulating the complex signaling machinery that routes signals from postsynaptic receptors (mGluRs and N-methyl-D-aspartate receptors (NMDARs)) to the Mitogen activated protein kinase—extracellular signal-regulate kinase1/2 (MAPK-ERK1/2) pathway (Wang et al., 2007), which has been putatively implicated in mood modulation and in the mechanism of action of mood stabilizers (Einat et al., 2003; Yuan et al., 2001).

The present study investigated the manner in which chronic administration of lithium and VPA, at therapeutic doses, modulates the inducible and constitutive transcripts of *Homer1*. We evaluated the expression of *Homer* genes by means of *in situ* hybridization histochemistry, focusing on the cortex, caudate-putamen, and nucleus accumbens; all of these areas are believed to be involved in the pathophysiology of bipolar disorder (Green et al., 2007) and in the mechanism of action of mood stabilizers (Ramaprasad et al., 2005).

We chose to explore both inducible and constitutive Homer isoforms (i.e. *Homer1a* and *Homer 1b/c* respectively) based on previous studies (Tomasetti et al., 2007; Iasevoli et al., 2010) showing that chronic administration of antipsychotics may affect both transcripts. A recent study from our laboratory (Tomasetti et al., 2011) analyzed *Homer1a* following the administration of VPA—alone or in association with antipsychotic drugs—in both acute and chronic paradigm of injected administration; building on that work, the present study compared the effects of both lithium and VPA on *Homer1* transcripts in a chronic paradigm of oral administration. This model was chosen in order to more closely approximate the conditions necessary to obtain pharmacological effects with mood stabilizers in clinical practice, and to assess the impact of prolonged oral drug administration on *Homer* expression.