Neuroscience 299 (2015) 1–17

ALTERED EXPRESSION OF NEUROPLASTICITY-RELATED GENES IN THE BRAIN OF DEPRESSED SUICIDES

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Abstract—*Background:* Expression of the neuronal membrane glycoprotein M6a (GPM6A), the proteolipid protein (PLP/DM20) family member, is downregulated in the hippocampus of chronically stressed animals. Its neuroplastic function involves a role in neurite formation, filopodium outgrowth and synaptogenesis through an unknown mechanism. Disruptions in neuroplasticity mechanisms have been shown to play a significant part in the etiology of depression. Thus, the current investigation examined whether GPM6A expression is also altered in human depressed brain. *Methods:* Expression levels and coexpression patterns of *GPM6A*, *GPM6B*, and *PLP1* (two other members of PLP/DM20 family) as well as of the neuroplasticity-related genes identified to associate with GPM6A were determined using quantitative polymerase chain reaction (qPCR) in postmortem samples from the hippocampus ($n = 18$) and the prefrontal cortex (PFC) ($n = 25$) of depressed suicide victims and compared with control subjects (hippocampus $n = 18$; PFC $n = 25$). Neuroplasticity-related proteins that form complexes with GPM6A were identified by coimmunoprecipitation technique followed by mass spectrometry. *Results:* Results indicated transcriptional downregulation of *GPM6A* and *GPM6B* in the hippocampus of depressed suicides. The expression level of calcium/calmodulin-dependent protein kinase II alpha (CAMK2A) and coronin1A (CORO1A) was also significantly decreased. Subsequent analysis of coexpression patterns demonstrated coordinated gene expression in the hippocampus and in the PFC indicating that the function of these genes might be coregulated in the human brain. However, in the brain of depressed suicides this coordinated response was disrupted. *Conclusions:* Disruption of coordinated gene expression as well as abnormalities in *GPM6A* and *GPM6B* expression and expression of the components of GPM6A complexes were detected in the brain of depressed suicides. **© 2015 IBRO. Published by Elsevier Ltd. All rights reserved.**

Key words: postmortem, mRNA expression, coexpression analysis, glycoprotein M6a, qPCR.

INTRODUCTION

Neuroplasticity is the mechanism by whichinformation is stored and maintained within individual synapses, neurons, and neuronal circuits to guide organism behavior. Several lines of evidence demonstrate impairment of neuroplasticity in depression (Pittenger and Duman, 2008; Christoffel et al., 2011). For example, in patients with different types of depression, the hippocampus and the prefrontal cortex (PFC) are both reduced in size and activity, and alterations in synaptic and morphological plasticity have been reported (Rajkowska et al., 1999; Stockmeier et al., 2004; Drevets et al., 2008; Pittenger and Duman, 2008; Kang et al., 2012). The histopathological correlates include reductions in synapses or synaptic proteins, reductions in neuronal size, and in neuropil (Drevets et al., 2008). Similar alterations have been observed in animal models of chronic stress: reductions in dendritic arborizations and a loss of highly specialized dendritic spines and synapses in regions that appear homologous to the areas where reductions are evident in depressed humans (i.e., PFC, hippocampus) (McKittrick et al., 2000; Radley et al., 2004; Drevets et al., 2008; Pittenger and Duman, 2008). The intracellular mechanisms underlying these alterations and their relevance to human depression are poorly understood.

The neuronal membrane glycoprotein M6a (GPM6A), a member of the myelin proteolipid protein (PLP/DM20) family, has been shown to play a role in stress response in different animal models (Alfonso et al., 2004a,b; Cooper et al., 2009; Monteleone et al., 2014). For example, chronic social and physical stress decreases Gpm6a mRNA levels in the hippocampus, and this downregulation is prevented by administration of antidepressants (Alfonso et al., 2004b, 2006). An association of the *GPM6A* gene with a depression subgroup of schizophrenia patients (Boks et al., 2008) as well as a critical role of GPM6A expression levels for cognitive function have been reported recently (Gregor et al., 2014). *Gpm6a*

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Abbreviations: ANCOVA, analysis of covariance; CAMK2A, calcium/calmodulin-dependent protein kinase II alpha; CORO1A, coronin1A; FC, fold change; GFP, green fluorescent protein; GPM6A, neuronal membrane glycoprotein M6a; PFC, prefrontal cortex; PLP, proteolipid protein; PMI, postmortem interval; qPCR, quantitative polymerase chain reaction; RT, reverse transcription.

http://dx.doi.org/10.1016/j.neuroscience.2015.04.057
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knockout mouse model is viable and shows no gross malformations or behavioral abnormalities (El-Kordi et al., 2013). However, after mild social stress by single housing, these mice displayed a claustrophobia-like phenotype. Interestingly, in humans a 3'UTR variant of *GPM6A* has been linked to claustrophobia in two pedigrees (El-Kordi et al., 2013).

Apart from *Gpm6a*, other members of the family, the closely related *Gpm6b* and *Dm20* (*Plp1* transcript variant), but not *Plp1* itself, have been shown to be downregulated by chronic stress (Fernandez et al., 2010). Remarkably, the myelin proteolipid protein (PLP/DM20) family members, such as GPM6A, GPM6B, and PLP1 transcript variant DM20, but not PLP1 (Fernandez et al., 2010), have been shown to be involved in the processes of neurite outgrowth and filopodium formation (Lagenaur et al., 1992; Mukobata et al., 2002; Alfonso et al., 2005; Michibata et al., 2008; Zhao et al., 2008; Fuchsova et al., 2009; Brocco et al., 2010; Scorticati et al., 2011). GPM6A, in particular, is also required for filopodium motility and synaptogenesis (Fuchsova et al., 2009; Brocco et al., 2010), and it has been implicated in neuronal differentiation of human stem cells (Michibata et al., 2009) and PC12 cells (Mukobata et al., 2002). When siRNA methodology is used, GPM6A low-expressing neurons display decreased filopodia numbers and a lower density of synaptophysin clusters (Alfonso et al., 2005).

Neurite growth and remodeling, as well as filopodium and spine formation, represent fundamental processes during neuroplasticity. Thus, we hypothesized that alterations in the expression of the stress responsive neuroplasticity-related genes such as the members of the PLP family could suggest that the cellular pathways that involve these genes are sensitive to disease condition. This would result in dysregulation of neuroplasticity mechanisms involved in the etiology of this disease. Therefore, we examined in the present study the expression of PLP family members *GPM6A*, *GPM6B*, and *PLP1* in the hippocampus ($n = 18$) and the PFC ($n = 25$) of depressed suicides. The mechanisms and signaling pathways that mediate GPM6A neuroplastic effects are still unknown. Thus, we also identified the neuroplasticity-related proteins that form complexes with GPM6A and examined their expression as well.

EXPERIMENTAL PROCEDURES

Subjects

The study was performed in PFC (Brodmann area 9) samples from suicide ($n = 25$) and matched nonpsychiatric control subjects ($n = 25$). Brain tissues from the same cohort have been used previously in various studies published by our group (Dwivedi et al., 2008; Dwivedi et al., 2009, 2010; Pandey et al., 2014). Dissected regions of interest included predominantly cortical gray matter. The white matter was removed as much as possible, but there was still some white matter left. Hippocampi were available for 18 suicide subjects and 18 nonpsychiatric controls. Postmortem brain samples were obtained from the Maryland Brain Collection at the Maryland Psychiatric Research Center, Baltimore. Tissues were collected only after a family member gave informed consent. Brain samples were free of neuropathologic abnormalities or human immunodeficiency virus antibodies. Toxicological data were obtained by an analysis of urine and blood samples. Psychiatric diagnoses in suicide and control subjects were evaluated with the Diagnostic Evaluation After Death (Salzman et al., 1983) and the Structured Clinical Interview for *DSM-IV* (Spitzer et al., 1995). Family members gave permission for clinical records to be obtained from mental health treatment providers in all cases of suicide. Control subjects were verified to be free from mental illnesses using a consensus diagnostic procedure. All procedures were approved by the Institutional Review Board of the University of Illinois at Chicago. Detailed demography of subjects is provided in Table 1.

RNA isolation and reverse transcription

Total RNA was extracted from 100 mg of tissue using the Trizol® (Invitrogen, Carlsbad, California) according to the manufacturer's directions. RNA concentration and purity were determined by measuring the OD A260/A280 and A260/A230 using NanoDrop®ND-1000 (NanoDrop Technologies, Montchanin, Delaware). All samples were free of contaminants with absorbance ratios close to two. RNA quality was assessed using Agilent Bioanalyzer 2100. All samples had 28S/18S ratios > 1.2 and RNA integrity number (RIN) above 6.6. First-strand cDNA was synthesized from 1 μg of total RNA using MMLV-reverse transcriptase (Invitrogen) in the presence of random hexamers (2.5 μM) (Invitrogen) according to manufacturer's instructions.

Oligonucleotide primers

Primer Express 3.0 software (Applied Biosystems, Foster City, CA, USA) was used to design primers for the amplification of human *GPM6A*, *GPM6B*, *PLP1*, *CORO1A*, *GIT1*, *CAMK2A*, *BDNF* and five candidate internal reference genes (*GAPDH*, *YWHAZ*, *CYC1*, *PPIA*, and *EIF4A2*). Primer sequence, full gene name, accession number, function, and chromosomal localization are listed in Table 2 (reference genes) and Table 3 (target genes).

Quantitative polymerase chain reaction (qPCR)

To determine mRNA levels, qPCR reactions were performed using SYBR®Select Master Mix (Applied Biosystems) according to the manufacturer's instructions. Measurements were done on Stratagene Mx3005P equipped with MxPro software (Stratagene, La Jolla, CA, USA). All reactions were performed in duplicate. No-RT control, no-template control and three different inter-run calibrators were always included for each individual plate and gene assay. After the final cycle of PCR, reactions were subjected to a heat dissociation protocol to verify primer specificity. A single peak corresponding to the melting temperature (Tm) of

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