Progress in Neuro-Psychopharmacology & Biological Psychiatry 35 (2011) 1774–1779

Contents lists available at ScienceDirect

Progress in Neuro-Psychopharmacology & Biological Psychiatry

journal homepage: www.elsevier.com/locate/pnp

The mTOR signaling pathway in the prefrontal cortex is compromised in major depressive disorder

Courtney S. Jernigan [a,1], Dharmendra B. Goswami [a,1], Mark C. Austin [a], Abiye H. Iyo [a], Agata Chandran [a], Craig A. Stockmeier [a,b], Beata Karolewicz [a,*]

[a] Department of Psychiatry and Human Behavior, University of Mississippi Medical Center, Jackson, MS, USA
[b] Department of Psychiatry, Case Western Reserve University, Cleveland, OH, USA

ARTICLE INFO

Article history:
Received 22 April 2011
Received in revised form 17 May 2011
Accepted 17 May 2011
Available online 23 May 2011

Keywords:
Major depressive disorder
Postmortem
Prefrontal cortex
Translation initiation pathway

ABSTRACT

Recent studies demonstrate that rapid antidepressant response to ketamine is mediated by activation of the mammalian target of rapamycin (mTOR) signaling pathway, leading to increased synaptic proteins in the prefrontal cortex (PFC) of rats. Our postmortem studies indicate robust deficits in prominent postsynaptic proteins including N-methyl-D-aspartate (NMDA) receptor subunits (NR2A, NR2B), metabotropic glutamate receptor subtype 5 (mGluR5) and postsynaptic density protein 95 kDa (PSD-95) in the PFC in major depressive disorder (MDD). We hypothesize that deficits in the mTOR-dependent translation initiation pathway contribute to the molecular pathology seen in the PFC of MDD subjects, and that a rapid reversal of these abnormalities may underlie antidepressant activity. The majority of known translational regulation occurs at the level of initiation. mTOR regulates translation initiation *via* its downstream components: p70-kDa ribosomal protein S6 kinase (p70S6K), and eukaryotic initiation factors 4E and 4B (eIF4E and eIF4B). In this study, we examined the expression of mTOR and its core downstream signaling targets: p70S6K, eIF4E, and eIF4B in the PFC of 12 depressed subjects and 12 psychiatrically healthy controls using Western blot. Levels of eIF4E phosphorylated at serine 209 (p-eIF4E-Ser209) and eIF4B phosphorylated at serine 504 (p-eIF4B-Ser504) were also examined. Adjacent cortical tissue samples from both cohorts of subjects were used in our previous postmortem analyses. There was a significant reduction in mTOR, p70S6K, eIF4B and p-eIF4B protein expression in MDD subjects relative to controls. No group differences were observed in eIF4E, p-eIF4E or actin levels. Our findings show deficits in mTOR-dependent translation initiation in MDD particularly *via* the p70S6K/eIF4B pathway, and indicate a potential association between marked deficits in synaptic proteins and dysregulation of mTOR signaling in MDD.

1. Introduction

A major limitation of established antidepressants is the delayed onset of therapeutic response, resulting in non-compliance and dramatically increased risk for suicidal behavior. Of particular relevance is the demonstration that a single dose of ketamine, a glutamate N-methyl-D-aspartate (NMDA) receptor antagonist, induced a rapid (within hours), long lasting (up to 1 week), and robust antidepressant effect in treatment-resistant cases of MDD (Berman et al., 2000; Zarate et al., 2006). Recent animal studies indicate that the fast antidepressant response to NMDA receptor antagonists (ketamine and Ro 25–6981) is mediated by rapid activation of the mammalian target of rapamycin (mTOR) pathway leading to an increase in synaptic signaling proteins and increased number and function of new spine synapses in the prefrontal cortex (PFC) of rats (Li et al., 2010). Moreover, it has been demonstrated that a single dose of these antagonists rapidly reversed the chronic stress-induced behavioral and synaptic deficits in an mTOR-dependent manner (Li et al., 2011). Our recent postmortem studies show significant reductions in the expression of prominent postsynaptic proteins involved in glutamate neurotransmission, including NMDA receptor subunits (NR2A and NR2B), metabotropic glutamate receptor subtype 5 (mGluR5) and postsynaptic density 95 kDa (PSD-95) in the PFC from depressed subjects (Deschwanden et al., 2011; Feyissa et al., 2009). These studies may indicate an association between marked deficits in synaptic proteins and dysregulation of mTOR signaling in MDD (Karolewicz et al., 2011).

Abbreviations: MDD, major depressive disorder; PFC, prefrontal cortex; NMDA, N-methyl-D-aspartate; NR2A, NMDA receptor subunit 2A; NR2B, NMDA receptor subunit 2B; mGluR5, metabotropic glutamate receptor subtype 5; PSD-95, postsynaptic density-95 kDa; mTOR, mammalian target of rapamycin; p70S6K, 70-kDa ribosomal protein S6 kinase; eIF4B, eukaryotic initiation factor 4B; eIF4E, eukaryotic initiation factor 4E; p-eIF4E (Ser209), eukaryotic initiation factor 4E phosphorylated at serine 209; p-eIF4B (Ser504), eukaryotic initiation factor 4B phosphorylated at serine 504.

* Corresponding author at: Department of Psychiatry, University of Mississippi Medical Center, 2500 North State Street, Jackson, MS 39216-4505, USA. Tel.: +1 601 984 5896; fax: +1 601 984 5899.
E-mail address: bkarolewicz@umc.edu (B. Karolewicz).

[1] Mrs. Jernigan and Dr. Goswami contributed equally to this article.

0278-5846/$ – see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.pnpbp.2011.05.010

Traditionally, it was thought that the change in the proteome is caused by transcriptional activity. Now it is known that regulation of translation is another way of altering protein production (Nilsson et al., 2004). Protein synthesis is a highly regulated process that can be separated into three general phases: initiation, elongation and termination (Hoeffer and Klann, 2010; Klann et al., 2004). The rate-limiting step in the process of protein synthesis is translation initiation (Hoeffer and Klann, 2010; Holz et al., 2005). The activity of mTOR, an ubiquitously expressed serine/threonine kinase, is central to the regulation of translation initiation and, consequently, protein synthesis required for long-term potentiation and new synaptic connections (Hashimoto, 2011; Hoeffer and Klann, 2010; Klann et al., 2004; Tang et al., 2002; Tang and Schuman, 2002).

It has been reported that neuronal mTOR function is influenced by the activity of growth factors, insulin, cytokines, as well as glutamate activity *via* NMDA receptors and metabotropic glutamate receptors (mGluR) (Antion et al., 2008; Gong et al., 2006; Hay and Sonenberg, 2004; Hoeffer and Klann, 2010) (Fig. 1). Activated mTOR phosphorylates p70-kDa ribosomal protein S6 kinase (p70S6K) followed by p70S6K-induced phosphorylation of eukaryotic initiation factor 4B (eIF4B) which promotes the initiation of protein translation (Raught et al., 2004). mTOR also phosphorylates and inactivates eukaryotic initiation factor 4E-binding protein 1 (4E-BP1), reducing its affinity for eIF4E and releasing eIF4E to facilitate translation initiation. Activated translation initiation factors, particularly eIF4E and eIF4B, are responsible for ribosome recruitment to the 5′ end of mRNA. The 5′ end of all nuclear-transcribed mRNAs possess a cap structure (m^7GpppN, in which "m" represents a methyl group and "N", any nucleotide) that is specifically recognized by eIF4E. Thus, eIF4E guides the ribosome to an mRNA 5′ end and facilitates its binding. On the other hand, eIF4B potentiates ribosome recruitment by stimulating the helicase activity of the eukaryotic initiation factor 4A (eIF4A), to unwind mRNA secondary structure for efficient translation (Gingras et al., 1999; Hay and Sonenberg, 2004; Holz et al., 2005; Rogers et al., 2002). Thus, mTOR controls the efficiency of protein translation within cells *via* its critical downstream targets (Fig. 1).

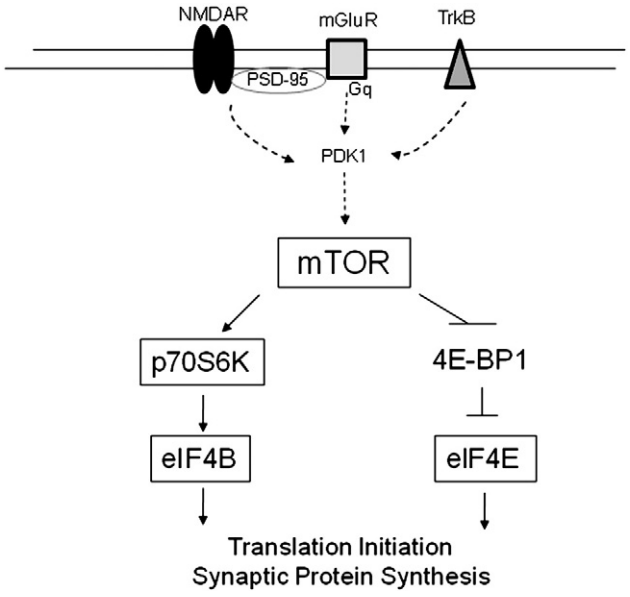

Fig. 1. Simplified diagram illustrating the mTOR signaling pathway. Neuronal receptors (NMDAR, mGluR, and TrkB) activate downstream signaling pathways, including PDK1, leading to mTOR activation. Activated mTOR phosphorylates p70S6K followed by p70S6K-induced phosphorylation of eIF4B which promotes the initiation of protein translation. mTOR also phosphorylates and inactivates eukaryotic 4E-BP1 reducing its affinity for eIF4E and releasing eIF4E to facilitate translation initiation. Abbreviations: NMDAR, N-methyl-D-aspartate receptor; mGluR, metabotropic glutamate receptor; PSD-95, postsynaptic density-95 kDa; TrkB, tyrosine kinase B receptor; PDK1, phosphoinositide-dependent kinase 1; mTOR, mammalian target of rapamycin; p70S6K, p70 kDa ribosomal protein S6 kinase; eIF4B, eukaryotic initiation factor 4B; 4E-BP1, eukaryotic initiation factor 4E binding protein 1; eIF4E, eukaryotic initiation factor 4E.
Diagram drawn using information from Hoeffer and Klann (2010) and Klann et al. (2004).

There is abundant evidence linking mTOR signaling to synaptic plasticity, memory, neurological disorders, and cancer (Gong et al., 2006; Hay and Sonenberg, 2004; Hoeffer and Klann, 2010). To date there are no studies that implicate the mTOR signaling pathway in the pathology of MDD. We hypothesize that deficits in the mTOR-dependent translation initiation pathway contribute to the molecular pathology seen in the PFC in MDD. Therefore, the goal of this study is to examine MDD-related changes in the protein level of mTOR and its downstream signaling targets: p70S6K, eIF4E, eIF4B in cortical tissue (PFC BA10) from the same MDD subjects as those used in our previous postmortem studies (Deschwanden et al., 2011; Feyissa et al., 2009). Additionally, levels of eIF4E phosphorylated at serine 209 (p-eIF4E Ser209) and eIF4B phosphorylated at serine 504 (p-eIF4B Ser504) were examined.

2. Methods

2.1. Human subjects

Postmortem brain samples were collected at autopsy at the Cuyahoga County Coroner's Office in Cleveland, OH. Informed written consent was obtained from the legal next-of-kin of all subjects. Next-of-kin were interviewed and retrospective psychiatric assessments were conducted in accordance with Institutional Review Board policies at Case Western Reserve University and the University of Mississippi Medical Center. A trained interviewer administered the Schedule for Affective Disorders and Schizophrenia: lifetime version (SADS-L) or the Structured Clinical Interview for DSM-IV Psychiatric Disorders (SCID-IV) to knowledgeable next-of-kin to subjects in the study approximately three months after death to determine current and lifetime Axis I psychopathology (First et al., 1996; Spitzer and Endicott, 1978). Diagnoses for Axis I disorders were assessed independently by a clinical psychologist and a psychiatrist. Consensus diagnosis was reached in conference, using information from knowledgeable informants, The Cuyahoga County Coroner's Office, and all available inpatient and outpatient medical records. Twelve subjects met criteria for major depressive disorder and twelve subjects did not meet criteria for an Axis I disorder (termed psychiatrically healthy controls) except for nicotine and alcohol dependence based on the Diagnostic and Statistic Manual of Mental Disorders—Revised DSM-IV (Table 1). Among the twelve depressed subjects, 10 were suicide victims. Blood and urine samples from all subjects were examined by the coroner's office for psychotropic medications and substances of abuse, including ethanol (Tables 1 and 2). The average duration of depression was 9.6 (±3.6) years. Depressed subjects and psychiatrically healthy controls were matched as closely as possible for age, gender, postmortem interval (PMI), tissue pH, and storage time in freezer (Tables 1 and 2).

2.2. Immunoblotting

Tissue samples were dissected from the anterior region of the prefrontal cortex (PFC) containing Brodmann's area 10 (BA10). Frozen blocks were cut into 50 μm-thick sections and tissue punches containing all six cortical layers of the gray matter were collected and used. Western blot experiments were performed as described previously (Deschwanden et al., 2011; Feyissa et al., 2009; Feyissa et al., 2010). Immunoreactivities of mTOR, p70S6K, eIF4E, p-eIF4E (Ser209), eIF4B, and p-eIF4B (Ser504) were investigated in twelve depressed subjects and twelve psychiatrically healthy controls. Immunoblots of six pairs of subjects were on the same gel with