Available online at www.sciencedirect.com
ScienceDirect
ELSEVIER
Neuroscience Letters 434 (2008) 23–28
Neuroscience Letters
www.elsevier.com/locate/neulet

The effect of MK-801 on mTOR/p70S6K and translation-related proteins in rat frontal cortex

Se Chang Yoon [a,1], Myoung Suk Seo [b,1], Se Hyun Kim [c], Won Je Jeon [b], Yong Min Ahn [c], Ung Gu Kang [c], Yong Sik Kim [c,*]

[a] Department of Psychiatry, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Republic of Korea
[b] Clinical Research Institute, Seoul National University Hospital, Seoul, Republic of Korea
[c] Department of Psychiatry and Behavioral Science and Institute of Human Behavioral Medicine, Seoul National University College of Medicine, Seoul, Republic of Korea

Received 18 September 2007; received in revised form 26 December 2007; accepted 8 January 2008

Abstract

In experimental animals, including rats, MK-801 produces characteristic behavioural changes that model schizophrenia. It has been hypothesized that these changes accompany long-term synaptic changes, which require protein neosynthesis. We observed the effect of MK-801 on the "mammalian target of rapamycin" (mTOR)/70-kDa ribosomal protein S6 kinase (p70S6K) pathway that regulates protein synthesis in the rat frontal cortex. A single injection of MK-801 (0.5, 1, or 2 mg/kg) induced an acute increase in the phosphorylation of Akt (Ser-473) eIF4E-binding protein (4E-BP1) (Thr-37/46) and p70S6K (Thr-389). In contrast, after repeated treatment with MK-801 (1 mg/kg for 5 or 10 days), the phosphorylation of Akt (Ser-473), mTOR (Ser-2481), 4E-BP1 (Thr-37/46), p70S6K (Thr-389), and S6 (Ser-240/244) increased. Thus, proteins in the mTOR/p70S6K pathway are modulated in chronic MK-801 animal models. These findings may suggest that repeated MK-801 treatment activates the signal transduction pathways involved in the initiation of protein synthesis in the rat frontal cortex.
© 2008 Elsevier Ireland Ltd. All rights reserved.

Keywords: NMDA receptor antagonist; Protein translation pathway; Psychotomimetic agent

MK-801 is a non-competitive and selective *N*-methyl-D-aspartate (NMDA) receptor antagonist known to have psychotomimetic effects. Acute treatment with MK-801 induces increased locomotor activity in rodents [3], and this locomotor effect is subject to sensitization on repeated administration [8,12,34]. The behavioural sensitization induced by repeated exposure to an NMDA receptor antagonist has been suggested as an appropriate animal model of schizophrenia [16]. This can be considered an adaptive response to prolonged exposure to NMDA receptor antagonists, which may involve long-term synaptic changes.

The role of neuronal activity-dependent protein synthesis is an important issue in the study of synaptic plasticity, especially in understanding the mechanism of long-term synaptic changes [18]. Long-term potentiation (LTP) is a canonical plasticity phenomenon, closely related to glutamatergic transmission [21]. LTP is inhibited by the protein synthesis inhibitor cycloheximide [30], suggesting that glutamatergic transmission activates translational signals in neurons. Recently, Gong et al. [13] reported that activation of the NMDA receptor resulted in synaptic activity-induced dendritic cell protein synthesis through the "mammalian target of rapamycin" (mTOR) signal pathway regulating protein translation in primary cultures of hippocampal neurons. Thus, NMDA receptor antagonists that inhibit glutamatergic transmission may reduce protein synthesis in the brain. Actually, phencyclidine (PCP), an NMDA receptor antagonist, was reported to inhibit protein synthesis in a brain homogenate [11]. However, treatment with NMDA receptor antagonists can enhance long-term plasticity-related changes. Systemic administration of MK-801 induced an initial suppression of LTP 4 h after treatment, but an enhancement of LTP ensued 24 h after MK-801 treatment in the hippocampal brain slice [7]. Moreover, as mentioned above, NMDA receptor antagonists themselves can induce locomotor sensitization after repeated exposure

* Corresponding author at: Department of Psychiatry and Behavioral Science, Seoul National University College of Medicine, 28 Yongon-Dong, Chongno-Gu, Seoul 110-744, Republic of Korea. Tel.: +82 2 2072 2204; fax: +82 2 744 7241.
E-mail address: kys@snu.ac.kr (Y.S. Kim).

[1] These authors contributed equally to this work as first authors.

0304-3940/$ – see front matter © 2008 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.neulet.2008.01.020

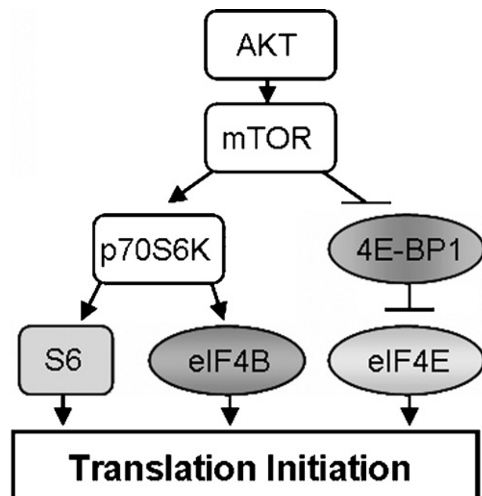

Fig. 1. Schematic diagram illustrating the Akt-mTOR-related protein translation initiation pathway. Akt activates mTOR, inducing p70S6K phosphorylation followed by S6 and eIF4B phosphorylation. In addition, mTOR inactivates the translation repressor 4E-BP, which releases eIF4E to facilitate translation initiation [15,23,27,28,31].

[8,12,34], which may require long-term synaptic changes. Thus, the long-term effect of NMDA receptor antagonists on the intracellular mechanism related to protein synthesis may be different from the acute response.

To examine these possibilities, we studied the protein translation-related signaling system, including mTOR, in the rat brain after a single and repeated treatment with the NMDA receptor antagonist MK-801. Initiation is the rate-limiting step in translation [15]. The involvement of the mTOR/70-kDa ribosomal protein S6 kinase (p70S6K) pathway in translation initiation has been well characterized (Fig. 1). Activated mTOR induces the phosphorylation of p70S6K followed by p70S6K-induced phosphorylation of the small ribosomal protein 6 (S6) and the eukaryotic translation initiation factor 4B (eIF4B), which promotes the initiation of protein translation [23,27,31]. The eIF4E protein plays a rate-limiting role in translation initiation, and the eIF4E-binding protein (4E-BP) prevents eIF4E from engaging in the active translation initiation process [15,23,31]. mTOR also phosphorylates 4E-BP, reducing its affinity for eIF4E and releasing eIF4E to facilitate translation initiation [23,31]. Akt is located upstream from mTOR [27]. Acute treatment with 0.5 or 1 mg/kg MK-801 results in the increased phosphorylation of Akt in the rat frontal cortex [2]. Repeated injections of MK-801 for 5 or 10 days also increased the phosphorylation of Akt [29]. In addition, repeated injections of MK-801 increases the phosphorylation of extracellular signal-regulated kinase 1/2 (ERK1/2) [29], which is also involved in translation initiation [17,23].

Thus, we hypothesized that the mTOR/p70S6K signaling pathway could be affected by MK-801 treatment in the rat brain. As the influence of prefrontal glutamate is essential for the process of sensitization in accumbal dopaminergic neurons [33], we focused on the prefrontal cortex. We examined the phosphorylation of mTOR (Ser-2448 and Ser-2481), 4E-BP1 (Thr-37/46), p70S6K (Thr-389), S6 (Ser-240/244), eIF4B (Ser-422), and eIF2α (Ser-51) in the rat frontal cortex after single and repeated intraperitoneal (i.p.) injections of MK-801.

All animal treatments were consistent with the protocols described in the NIH Guide for the Care and Use of Laboratory Animals. Formal approval to conduct this experiment has been obtained from the animal subjects review board of Seoul National University Hospital. The animals and drug treatments were described in our previous reports [1,2,29]. Male Sprague–Dawley rats (200–250 g) were given i.p. injections of MK-801 (Tocris, Ellisville, MO, USA) dissolved in normal saline, whereas vehicle control animals received injections containing an equivalent volume of normal saline. Sham animals (normal controls) were treated in the same way, but did not receive injections.

To examine the dose–response relationship, we observed the level of phosphorylation 60 min after MK-801 injection. In our previous experiments with acute treatment, different doses of MK-801 induced different levels of locomotor activation and phosphorylation in the rat frontal cortex. Treatment with ≥4 mg/kg MK-801 seemed to inhibit various signal transduction pathways in a non-specific manner [1,2]. Thus, we examined the effects of acute treatment with 0.5, 1, or 2 mg/kg MK-801.

For the repeated treatments, we examined the effects of once-daily injections for 5 or 10 consecutive days. The animals were sacrificed by decapitation 24 h after the final injection. We used 1 mg/kg MK-801 in this experiment because our previous experiments showed similar results in the level of phosphorylation of ERK1/2 and Akt with 0.5, 1, and 2 mg/kg MK-801 in the rat frontal cortex after repeated treatment with MK-801 [29].

The frontal cortices were homogenized immediately in a glass–Teflon homogenizer in 10 v/w of ice-cold RIPA(+) buffer (50 mM Tris, pH 7.4, 150 mM NaCl, 1.0% Triton, 1.0% sodium deoxycholate, 0.1% SDS) containing 1 mM DTT, 1% protease inhibitor cocktail (Sigma–Aldrich, St. Louis, MO, USA), and 1 mM PMSF (Sigma–Aldrich). After centrifugation (20,000 × *g*, 10 min), the supernatants were boiled in Laemmli sample buffer. The protein concentrations in the homogenates were determined using a Bradford protein assay kit (Bio-Rad Laboratories, Hercules, CA, USA). Equal amounts of protein were separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) and transferred to nitrocellulose membranes. The membranes were blocked with 5% skim milk in TBS-T (0.1% Tween-20 in TBS) at room temperature for 1 h and then incubated with specific antibodies at 4 °C overnight: anti-mTOR, anti-p70S6K, anti-4E-BP1, anti-S6, anti-eIF4B, anti-eIF2α, anti-phospho-Ser-473-Akt, anti-phospho-Ser-2448 and Ser-2481-mTOR, anti-phospho-Thr-389-p70S6K, anti-phospho-Thr-37/46-4E-BP1, anti-phospho-Ser-240/244-S6, anti-phospho-Ser-422-eIF4B, anti-phospho-Ser-51-eIF2α (Cell Signaling Technology, Beverly, MA, USA), anti-Akt (Santa Cruz Biotechnology, Santa Cruz, CA, USA), and anti-actin (Sigma–Aldrich). The membranes were then incubated with anti-rabbit and anti-mouse IgG antibodies conjugated to horseradish peroxidase (Santa Cruz Biotechnology) and developed using the enhanced chemiluminescence system (SuperSignal; WestPico, Pierce, Rockford, IL, USA). The

Download English Version:

https://daneshyari.com/en/article/4348651

Download Persian Version:

https://daneshyari.com/article/4348651

Daneshyari.com