Inhibition of the MAPK/ERK cascade: a potential transcription-dependent mechanism for the amnesic effect of anesthetic propofol

Eugene E. FIBUCH, John Q. WANG

Department of Anesthesiology, University of Missouri-Kansas City School of Medicine, Saint Luke's Hospital, Kansas City, Missouri 64108, USA

Abstract: Intravenous anesthetics are known to cause amnesia, but the underlying molecular mechanisms remain elusive. To identify a possible molecular mechanism, we recently turned our attention to a key intracellular signaling pathway organized by a family of mitogen-activated protein kinases (MAPKs). As a prominent synapse-to-nucleus superhighway, MAPKs couple surface glutamate receptors to nuclear transcriptional events essential for the development and/or maintenance of different forms of synaptic plasticity (long-term potentiation and long-term depression) and memory formation. To define the role of MAPK-dependent transcription in the amnesic property of anesthetics, we conducted a series of studies to examine the effect of a prototype intravenous anesthetic propofol on the MAPK response to *N*-methyl-D-aspartate receptor (NMDAR) stimulation in hippocampal neurons. Our results suggest that propofol possesses the ability to inhibit NMDAR-mediated activation of a classic subclass of MAPKs, extracellular signal-regulated protein kinase 1/2 (ERK1/2). Concurrent inhibition of transcriptional activity also occurs as a result of inhibited responses of ERK1/2 to NMDA. These findings provide first evidence for an inhibitory modulation of the NMDAR-MAPK pathway by an intravenous anesthetic and introduce a new avenue to elucidate a transcription-dependent mechanism processing the amnesic effect of anesthetics.

Keywords: glutamate; NMDA; long-term potentiation; Elk; cyclic AMP response element-binding protein; c-fos proteins; hippocampus; propofol

1 Introduction

Mitogen-activated protein kinases (MAPKs), a large family of cytosolic and nuclear serine/threonine kinases^[1], have been implicated in the regulation of a variety of cellular and synaptic activities in proliferative cells as well as matured neurons. In particular, MAPKs form central signaling pathways processing inducible gene expression in response to various forms of extracellular and intracellular inputs. Activation of glutamate receptors, such as *N*-methyl-D-aspartate receptor (NMDAR) subtype, results in a robust activation of the MAPK pathway and thus gene expression in hippocampal CA1 neurons. This stimulus-

transcription coupling has been demonstrated to be critical molecular steps toward the development and/or maintenance of two forms of synaptic plasticity: long-term potentiation (LTP) and long-term depression (LTD). It is generally accepted that the NMDAR-MAPK-LTP/LTD cascade constitutes an efficient transcription-dependent apparatus controlling the memory formation.

Intravenous anesthetics are known to produce amnesia, although potential molecular mechanism(s) underlying this adverse effect have long been puzzling. It has been noted that anesthetics can produce amnesia that long outlasts the duration of anesthesia. This suggests that there exists a relatively long-lasting biochemical process mediating the amnesic action. In searching for this responsible biochemical process, we recently hypothesized that anesthetic drugs may affect a transcription-dependent memory formation that results in amnesia. To experimentally explore this possibility, we conducted a series of pharmacological and molecular experiments in cultured rat hippocampal CA1 neurons. We

Corresponding author: John Q. WANG

Tel: 1-816-2351786

Fax: 1-816-2355574

E-mail: wangjq@umkc.edu

Article ID: 1673-7067(2007)002-0119-06

CLC number: R971⁺.3; R338.64

Document code: A

Received date: 2006-12-19

found that the intravenous anesthetic propofol displayed the ability to potently suppress the NMDAR function and NMDAR-mediated MAPK activation and gene expression. This provides evidence for an inhibitory influence of anesthetics over the sequential NMDAR-MAPK-transcription cascade and suggests a novel avenue to uncover molecular mechanisms for the amnesic effect of anesthetics.

2 The Ras-MAPK pathway and its role in transcription-dependent synaptic plasticity related to the memory formation

MAPKs are a superfamily of protein kinases that are densely expressed in postmitotic neurons of adult mammalian brain. As important kinases in several key signaling cascades, MAPKs are actively involved in the regulation of various cellular and synaptic activities^[1]. Activation of MAPKs involves a consecutive and sequential phosphorylation of four levels of signaling proteins: small GTPases (Ras and Rac proto-oncogenes), MAPK kinase kinases (Raf or MEKKs), MAPK kinases (MEKs), and MAPKs. In the case of extracellular signal-regulated protein kinase 1/2 (ERK1/2) subfamily of MAPKs, a prototype of MAPKs, they are activated via phosphorylation on Thr202 and Tyr204 through the upstream Ras-Raf-MEK1/2 pathway. Once activated, ERK1/2 translocate from the cytosol to the nucleus to activate specific transcription factors, leading to inducible gene expression^[2].

L-glutamate is among neurotransmitters that readily activate ERK1/2 in neurons (reviewed in Ref. 3). All glutamate receptors surveyed so far, including all ionotropic and metabotropic glutamate receptor subtypes, have been demonstrated to positively regulate ERK1/2 phosphorylation^[3]. The NMDAR is the most thoroughly characterized subtype in this event. In various brain regions, NMDARs consistently show the ability to upregulate ERK1/2 phosphorylation. The Ca²⁺-permeable NMDAR is believed to activate ERK1/2 via at least two Ca²⁺-dependent routes^[3]. First, Ca²⁺ signals derived from the Ca²⁺ influx through NMDARs activate several Ca²⁺-sensitive protein kinases to increase ERK1/2 phosphorylation^[4]. Ca²⁺/calmodulin-dependent protein kinases (CaMKs) and phosphatidylinositol (phosphoinositide) 3-kinases (PI3-kinases) are two important elements for forming a pathway to link NMDARs to ERK1/2. The second signaling route linking NMDARs to ERK1/2 involves a direct interaction between the NMDAR NR2B subunit and Ras-guanine nucleotide re-

leasing factors (RasGRFs), a neuron-specific activator of Ras. The direct binding of NR2B and RasGRFs creates a submembranous microdomain ensuring rapid transmission of Ca²⁺ signals to the Ras-ERK1/2 cascade^[5].

A noticeable role of active ERK1/2 is its strong regulation of gene expression. As an information superhighway between the surface receptor and the nucleus, ERK1/2 effectively link environmental inputs to genomic responses. In the cytoplasm, this action involves a rapid phosphorylation of ERK1/2 followed by dynamic translocation of phospho-ERK1/2 to the nucleus. In the nucleus, ERK1/2 activate specific sets of transcription factors including Elk-1 and cAMP response element-binding protein (CREB) by increasing their phosphorylation at Ser383 (Elk-1) and Ser133 (CREB). Active transcription factors in turn bind to specific promoter sites to facilitate target gene expression. A large number of genes are able to respond to this ERK1/2-dependent signaling pathway following NMDAR stimulation^[6,7]. Altered expression of genes, especially those perisynaptic proteins, are thought to be dynamic adaptive changes important for synaptic plasticity related to long-lasting changes in efficacy and strength of synapses.

A significant contribution of ERK1/2 to synaptic plasticity and memory formation in the adult brain has been established in several behavioral and cellular studies^[8,9]. This contribution is largely derived from the regulatory role of ERK1/2 in gene expression. Through regulating specific sets of transcriptional events, ERK1/2 modulate expression levels and thus functions of various key synaptic proteins. This process influences many forms of synaptic plasticity, including LTP and LTD, cellular models of learning and memory. In the hippocampal CA1 region, LTP can last for hours and requires *de novo* transcription and protein synthesis^[10]. Similarly, several forms of learning behaviors are mRNA- and protein synthesis-dependent^[11]. In the light of NMDAR-dependent forms of LTP, it has been shown that NMDAR-gated Ca²⁺ influx activates the ERK1/2 pathway, which in turn increases the Elk-1- and CREB-dependent transcription of a family of genes essential for the induction and/or maintenance of LTP^[8,9].

3 Amnesic effect of anesthetic drugs and a potential transcription-dependent mechanism involving the ERK1/2 pathway

One of adverse consequences after clinical use of anesthetic drugs is the occurrence of amnesia or the inability

to remember events. This type of loss of memory is particularly sensitive to general anesthetics because amnesia occurs at concentrations well below those that cause sedation and immobility. Commonly used inhaled and intravenous anesthetics all cause amnesia usually at concentrations several fold lower than those required for immobility. Noticeably, such amnesia can last much longer than the duration of anesthesia, indicating an involvement of a relatively long-lasting biochemical process in this event. While detailed molecular mechanisms underlying the amnesic effect of anesthetics are unclear, the transcription-dependent learning and memory mechanism has drawn attention. A tempting scenario is that anesthetic drugs may suppress NMDAR- and ERK1/2-dependent LTP, which in turn results in an impaired memory. To test this possibility, a complete set of *in vitro* experiments has recently been conducted in this laboratory^[12] and the results from these experiments seem to support the involvement of NMDAR and ERK1/2 in amnesic effects of anesthetics.

We have chosen propofol (2,6-diisopropylphenol), a highly effective intravenous anesthetic, as our prototype anesthetic agent for study. This drug is currently widely used for general anesthesia or for sedation with local anesthesia and in intensive care units. Like other intravenous anesthetics, propofol produces amnesia and its suppression of memory function can persist for several hours after propofol administration^[13]. The amnesic effect of propofol has already been linked to its known inhibition of LTP at Schaffer collateral-commissural pathway to CA1 pyramidal cell synapses in the hippocampus^[14-16], although, at present, molecular mechanisms underlying the propofol inhibition of hippocampal LTP are poorly understood. To unravel possible molecular mechanisms for the propofol inhibition of LTP, we initiated an effort to test the effect of propofol on the ERK1/2 response to NMDAR inputs in cultured rat hippocampal CA1 neurons. We found that propofol consistently inhibited the NMDAR mediated activation of the ERK1/2 pathway. This for the first time reveals that propofol possesses the ability to inhibit the ERK1/2 response to NMDAR activation. Noticeably, the propofol effect was achieved at a low concentration range (1–10 $\mu\text{mol/L}$) which is thus believed to be clinically relevant because propofol at blood concentrations (approximately 3.5–3.7 $\mu\text{mol/L}$) produced 50% loss of memory^[17,18].

We then set out to elucidate how propofol suppresses the ERK1/2 response. We speculated that propofol may

achieve its effect by inhibiting NMDARs or any component of a signaling pathway linking NMDAR to Ras-ERK1/2. NMDAR NR1 subunits undergo a tight phosphorylation at two distinct sites (Ser897 and Ser896) of their intracellular C-termini^[19]. Such phosphorylation represents a major posttranslational modification of NMDAR function^[20]. It is possible that propofol may affect NMDAR phosphorylation and thus function to modulate NMDAR-dependent ERK1/2 phosphorylation. To determine whether propofol affects NR1 phosphorylation, the effect of propofol on NR1 phosphorylation at Ser897 and Ser896 was tested in hippocampal neurons using a phospho- and site-specific antibody. It was found that propofol reduced Ser897 and Ser896 phosphorylation^[21], indicating the ability of propofol to regulate NR1 phosphorylation in an inhibitory fashion. To determine whether the reduced NR1 phosphorylation occurs in parallel with any change of NMDAR function, we examined the effect of propofol on NMDAR-mediated Ca^{2+} influx under the same experimental conditions. We found that Ca^{2+} rises in response to NMDA stimulation was reduced in neurons exposed to propofol. Thus, NMDAR function in terms of Ca^{2+} influx was as sensitive as NMDAR phosphorylation in response to propofol application, although the direct causal relation between the two events was not defined in this study. Nevertheless, propofol was demonstrated to suppress NMDAR phosphorylation and NMDAR-mediated Ca^{2+} entry simultaneously. Since NMDAR-regulated ERK1/2 phosphorylation is Ca^{2+} -sensitive, the inhibitory effect of propofol on Ca^{2+} responses to NMDA may at least in part account for the propofol-induced reduction of ERK1/2 responses to NMDA.

As aforementioned, active ERK1/2 phosphorylate nuclear transcription factors and facilitate gene expression. We then attempted to determine whether the impaired ERK1/2 response to NMDA by propofol has any effect on inducible gene expression. We tested Elk-1 and CREB, two specific factors downstream to the ERK1/2 pathway^[22-24], for their responses to NMDA stimulation in the presence of propofol. In addition, an immediate early gene c-Fos was tested as a reporter of inducible gene expression downstream to Elk-1 and CREB to determine a final output of gene expression. We found that the NMDA-stimulated phosphorylation of Elk-1 and CREB and c-Fos expression were blocked by propofol. The same blocking effect was also seen when the ERK1/2 pathway was inhibited by a MEK selective inhibitor. Thus, propofol is capable of sup-

pressing Elk-1/CREB-sensitive gene expression likely through the inhibition of NMDAR-dependent activation of the ERK1/2 pathway. Since the Elk-1/CREB-facilitated gene expression is implicated in the development and/or maintenance of LTP, the suppression of Elk-1/CREB-sensitive gene expression by propofol may contribute to the propofol inhibition of transcriptionally dependent LTP. Furthermore, these results provide evidence for an attractive notion that propofol produces its amnesic effect through inhibiting the NMDAR/ERK1/2- and transcription-dependent LTP. Further electrophysiological and behavioral experiments will be needed to define the role of the inhibited ERK1/2-transcription pathway by propofol in regulating NMDAR-dependent LTP and memory behaviors, respectively.

4 Conclusions

An initial effort has been made to unravel a molecular

mechanism responsible for the amnesic effect of intravenous anesthetics. Available data support a hypothesis that intravenous anesthetics such as propofol may produce amnesia through inhibiting NMDAR/ERK1/2-dependent LTP. As illustrated in Fig. 1, cytosolic Ca^{2+} signals derived from Ca^{2+} entry through NMDAR channels activate the Ras-ERK1/2 pathway, which in turn phosphates transcription factors (such as Elk-1 and CREB) to facilitate gene expression. The genomic responses then constitute a transcription-dependent mechanism for inducing and/or maintaining LTP. By suppressing this NMDAR/ERK1/2-dependent LTP, anesthetic drugs may exert their adverse effect on memory. It should be pointed out that the study of this kind is still in its infant stage. Future molecular, electrophysiological, and behavioral studies will be needed to accurately define the role of the ERK1/2 pathway in processing the amnesic effect of anesthetics.

Fig. 1 Schematic illustration of propofol inhibition of LTP through inhibiting NMDAR dependent activation of MAPK/ERK1/2. Sequential events underlying the NMDAR/ERK-dependent LTP are initiated with an increase in Ca^{2+} influx through activated NMDAR channels. Ca^{2+} signals then activate the Ras-ERK1/2 cascade probably through the direct interaction with RasGRFs or through CaMK/PI3-Kinases. Active ERK1/2 proteins translocate into the nucleus. With the aid of RSK/MSK1, they activate their transcription factor targets (CREB and Elk-1), leading to the facilitation of inducible gene expression implicated in the maintenance of longlasting LTP. Propofol inhibits NMDARs and thus blocks the NMDAR-mediated activation of the ERK1/2 pathway and impairs the NMDAR/ERK1/2-dependent LTP. The inhibitory effect of propofol may result from its inhibition of phosphorylation of NMDAR NR1 subunits at serine residues 897 and 896. Abbreviations: Glu, L-glutamate; MSK1, mitogen- and stress-activated protein kinase-1; RSK, ribosomal S6 kinase. Also see text for other abbreviations.

Acknowledgements: This work was supported by a grant from the Saint Luke's Hospital Foundation (Kansas City, MO, USA) and grants (No. R01-DA010355 and No. R01-MH061469) from the National Institutes of Health (Bethesda, MD, USA).

References:

- [1] Peyssonnaud C, Eychene A. The Raf/MEK/ERK pathway: new concepts of activation. *Biol Cell* 2001, 93: 53-62.
- [2] Wang JQ, Tang Q, Samdani S, Liu Z, Parekar NK, Choe ES, Yang L, Mao L. Glutamate signaling to Ras-MAPK in striatal neurons: mechanisms for inducible gene expression and plasticity. *Mol Neurobiol* 2004, 29: 1-14.
- [3] Wang JQ, Fibuch EE, Mao L. Regulation of mitogen-activated protein kinases by glutamate receptors. *J Neurochem* 2007, 100: 1-11.
- [4] Perkinton MS, Ip JK, Wood GL, Crossthwaite AJ, Williams RJ. Phosphatidylinositol 3-kinase is a central mediator of NMDA receptor signalling to MAP kinase (Erk1/2), Akt/PKB and CREB in striatal neurons. *J Neurochem* 2002, 80: 239-254.
- [5] Krapivinsky G, Krapivinsky L, Manasian Y, Ivanov A, Tyzio R, Pellegrino C, Ben-Ari Y, Clapham DE, Medina I. The NMDA receptor is coupled to the ERK pathway by a direct interaction between NR2B and RasGRF1. *Neuron* 2003, 40: 775-784.
- [6] Sgambato V, Vanhoutte P, Pages C, Rogard M, Hipskind R, Besson MJ, Caboche J. *In vivo* expression and regulation of Elk-1, a target of the extracellular-regulated kinase signaling pathway, in the adult rat brain. *J Neurosci* 1998, 18: 214-226.
- [7] Mao L, Tang Q, Samdani S, Liu Z, Wang JQ. Regulation of MAPK/ERK phosphorylation via ionotropic glutamate receptors in cultured rat striatal neurons. *Eur J Neurosci* 2004, 19: 1207-1216.
- [8] Sweatt JD. Mitogen-activated protein kinases in synaptic plasticity and memory. *Curr Opin Neurobiol* 2004, 14: 311-317.
- [9] Thomas GM, Huganir RL. MAPK cascade signaling and synaptic plasticity. *Nature Rev Neurosci* 2004, 5: 173-183.
- [10] Malenka RC. The long-term potential of LTP. *Nat Rev Neurosci* 2003, 4: 923-926.
- [11] Yin JC, Wallach JS, Del Vecchio M, Wilder EL, Zhou H, Quinn WG, Tully T. Induction of a dominant negative CREB transgene specifically blocks long-term memory in *Drosophila*. *Cell* 1994, 79: 49-58.
- [12] Kozzin J, Mao L, Yang L, Arora A, Fibuch EE, Wang JQ. Inhibition of glutamatergic activation of extracellular signal-regulated protein kinases in hippocampal neurons by the intravenous anesthetic propofol. *Anesthesiology* 2006, 105: 1182-1191.
- [13] Sanou J, Ilboudo D, Goodall G, Bourdalle-Badie C, Erny P. Evaluation of cognitive functions after anesthesia with propofol. *Ann Fr Anesth Reanim* 1996, 15: 1155-1161.
- [14] Wei H, Xiong W, Yang S, Zhou Q, Liang C, Zeng BX, Xu L. Propofol facilitates the development of long-term depression (LTD) and impairs the maintenance of long-term potentiation (LTP) in the CA1 region of the hippocampus of anesthetized rats. *Neurosci Lett* 2002, 324: 181-184.
- [15] Takamatsu I, Sekiguchi M, Wada K, Sato T, Ozaki M. Propofol-mediated impairment of CA1 long-term potentiation in mouse hippocampal slices. *Neurosci Lett* 2005, 389: 129-132.
- [16] Nagashima K, Zorumski CF, Izumi Y. Propofol inhibits long-term potentiation but not long-term depression in rat hippocampal neurons. *Anesthesiology* 2005, 103: 318-326.
- [17] Leslie K, Sessler DI, Schroeder M, Walters K. Propofol blood concentration and the Bispectral Index predict suppression of learning during propofol/epidural anesthesia in volunteers. *Anesth Analg* 1995, 81: 1269-1274.
- [18] Veselis RA, Reinsel RA, Feshchenko VA, Wronski M. The comparative amnestic effects of midazolam, propofol, thiopental, and fentanyl at equisedative concentrations. *Anesthesiology* 1997, 87: 749-764.
- [19] Tingley WG, Ehlers MD, Kameyama K, Doherty C, Ptak JB, Riley CT, Huganir RL. Characterization of protein kinase A and protein kinase C phosphorylation of the *N*-methyl-D-aspartate receptor NR1 subunit using phosphorylation site-specific antibodies. *J Biol Chem* 1997, 272: 5157-5166.
- [20] Wang JQ, Liu X, Zhang G, Parekar NK, Arora A, Haines M, Fibuch EE, Mao L. Phosphorylation of glutamate receptors: a potential mechanism for the regulation of receptor function and psychostimulant action. *J Neurosci Res* 2006, 84: 1621-1629.
- [21] Kingston S, Mao L, Yang L, Arora A, Fibuch EE, Wang JQ. Propofol inhibits phosphorylation on *N*-methyl-D-aspartate receptor NR1 subunits in neurons. *Anesthesiology* 2006, 104: 763-769.
- [22] Vanhoutte P, Barnier JV, Guibert B, Pages C, Besson MJ, Hipskind RA, Caboche J. Glutamate induces phosphorylation of Elk-1 and CREB, along with *c-fos* activation, via an extracellular signal-regulated kinase-dependent pathway in brain slices. *Mol Cell Biol* 1999, 19: 136-146.
- [23] Rajadhyaksha A, Barczak A, Macias W, Leveque JC, Lewis SE, Konradi C. L-type Ca^{2+} channels are essential for glutamate-mediated CREB phosphorylation and *c-fos* gene expression in striatal neurons. *J Neurosci* 1999, 19: 6348-6359.
- [24] Yang L, Mao L, Tang Q, Samdani S, Liu Z, Wang JQ. A novel Ca^{2+} -independent signaling pathway to extracellular signal-regulated protein kinase by coactivation of NMDA receptors and metabotropic glutamate receptor 5 in neurons. *J Neurosci* 2004, 24: 10846-10857.

MAPK/ERK 的抑制作用：丙泊酚导致失忆的一个可能的转录依赖性机制

Eugene E. FIBUCH, John Q. WANG

密苏里 - 堪萨斯大学医学院, 圣鲁克医院麻醉科, 堪萨斯, 密苏里 64108, 美国

摘要: 静脉麻醉剂可引起失忆, 但其潜在的分子机制尚未阐明。最近, 为了确定其可能的分子机制, 我们将注意力转向一条关键的细胞内信号通路, 即丝裂原活化蛋白激酶(MAPKs)信号通路。各种类型的突触塑型(长时程增强和长时程抑制)及记忆的形成和保持均离不开核内的转录事件。作为一条重要的突触至神经核的信号通路, MAPKs将细胞表面的谷氨酸受体与细胞核内的转录事件联系在一起。为确定 MAPK依赖的转录事件在麻醉剂致失忆特性中的作用, 我们在海马神经元细胞中做了一系列研究以检测静脉麻醉剂丙泊酚可否改变MAPK对 *N*-甲基-*D*-天门冬氨酸受体(NMDAR)信号的反应。结果提示丙泊酚可以抑制NMDAR激活经典MAPKs: 胞外信号调节蛋白激酶 1/2 (ERK1/2)。丙泊酚抑制ERK1/2激活的同时还降低其转录活性。这些结果证明了NMDAR-MAPK通路可受静脉麻醉剂的抑制, 此为麻醉剂致失忆效应的研究从转录机制上开辟了一条新途径。

关键词: 谷氨酸; NMDA; 长时程增强; Elk; cAMP 反应元件结合蛋白; c-fos 蛋白; 海马; 丙泊酚