available at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/brainres

BRAIN RESEARCH

Research Report

Changes in NMDA receptor-induced cyclic nucleotide synthesis regulate the age-dependent increase in PDE4A expression in primary cortical cultures

Hassan Hajjhussein[a], *Neesha U. Suvarna*[a], *Carmen Gremillion*[b], *L. Judson Chandler*[c], *James M. O'Donnell*[d,*]

[a]*Department of Pharmacology, University of Tennessee Health Science Center, Memphis, TN 38105, USA*
[b]*Department of Pharmacology, Louisiana State University Health Sciences Center, Shreveport, LA 71130, USA*
[c]*Departments of Physiology/Neuroscience and Psychiatry, Medical University of South Carolina, Charleston, SC 29425, USA*
[d]*Departments of Behavioral Medicine and Psychiatry and Neurobiology and Anatomy, West Virginia University Health Sciences Center, P.O. Box 9104, Morgantown, WV 26506-9104, USA*

ARTICLE INFO

Article history:
Accepted 21 February 2007
Available online 12 March 2007

Keywords:
NMDA receptor
MK-801
Bicuculline
EHNA
Rolipram
Phosphodiesterase
PDE4
PDE2
Protein kinase G

ABSTRACT

NMDA receptor-induced cAMP and cGMP are selectively hydrolyzed by PDE4 and PDE2, respectively, in rat primary cerebral cortical and hippocampal cultures. Because cAMP levels regulate the expression of PDE4 in rat primary cortical cultures, we examined the manner in which NMDA receptor activity regulates the age-dependent increase in the expression of PDE4A observed *in vivo* and *in vitro*. Inhibiting the activity of NR2B subunit with ifenprodil blocked NMDA receptor-induced cGMP synthesis and increased NMDA receptor-induced cAMP levels in a manner that reduced PDE4 activity. Therefore, NR1/NR2B receptor-induced cGMP signaling is involved in an acute cross-talk regulation of NR1/NR2A receptor-induced cAMP levels, mediated by PDE4. Chronic inhibition of NMDA receptor activity with MK-801 reduced PDE4A1 and PDE4A5 expression and activity in a time-dependent manner; this effect was reversed by adding the PKA activator dbr-cAMP. Inhibiting GABA receptors with bicuculline increased NMDA receptor-induced cAMP synthesis and PDE4A expression in cultures treated between DIV 16 and DIV 21 but not in cultures treated between DIV 8 and DIV 13. This effect was due to a high tone of NMDA receptor-induced cGMP in younger cultures, which negatively regulated the expression of PDE4A by a PKG-mediated process. The present results are consistent with behavioral data showing that both PDE4 and PDE2 are involved in NMDA receptor-mediated memory processes.

* *Corresponding author*. Fax: +1 304 293 7038.
E-mail address: jodonnell@hsc.wvu.edu (J.M. O'Donnell).

Abbreviations: AC, adenylyl cyclase; Bic, bicuculline; cAMP, cyclic adenosine monophosphate; cGMP, cyclic guanosine monophosphate; DIV, days *in vitro*; EHNA, *erythro*-9-(2-hydroxy-3-nonyl)-adenine; MK-801, dizocilpine hydrogen maleate; IFN, ifenprodil; TTX, tetrodotoxin; NMDA, *N*-methyl-D-aspartate; PDE, phosphodiesterase; Rol, rolipram

0006-8993/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.brainres.2007.02.090

1. Introduction

Learning and memory are regulated by changes in neuronal activity that take place in the cerebral cortex and hippocampus (Flexner et al., 1964; Heilbrun, 1960; Scoville et al., 1957; Thompson et al., 1961). Excitatory neurotransmission in the central nervous system that is mediated by NMDA type glutamatergic receptors are of particular importance in these processes (Collingridge, 1987; Kauer et al., 1988; Michaelis, 1998; Ozawa et al., 1998; Vianna et al., 2000). Rats treated with the selective NMDA receptor antagonist MK-801 exhibit memory deficits that are reversed by inhibiting phosphodiesterase-4 (PDE4) with rolipram (Zhang et al., 2000) or phosphodiesterase-2 (PDE2) with Bay 60-7550 (Boess et al., 2004), suggesting a functional role for PDE4 and PDE2 in NMDA receptor-mediated learning and memory processes. Consistent with these behavioral data, it has been found that PDE4 and PDE2 mediate the hydrolysis of NMDA receptor-induced cAMP and cGMP, respectively, in rat primary cerebral cortical and hippocampal cultures (Suvarna and O'Donnell, 2002). Pharmacological characterization did not suggest the involvement of any of the other PDE families in hydrolyzing NMDA receptor-induced cAMP and cGMP (Suvarna and O'Donnell, 2002).

The PDE4 family is comprised of four genes designated PDE4A, B, C, and D, of which only PDE4A, B, and D are expressed appreciably in the cerebral cortex and hippocampus (Cherry and Davis, 1999). The expression of PDE4A in cerebral cortical neurons increases in an age-dependent manner, with early detection at day *in vitro* (DIV) 10, which increases gradually to DIV 21 (Ye et al., 2001). Treating primary cortical neuronal cultures with the voltage-gated sodium channel blocker tetrodotoxin reduces the expression of PDE4A, indicating that the expression of this subtype is activity-dependent (Ye et al., 2001). Because the majority of excitatory signaling occurs at glutamatergic synapses (for review, see Michaelis, 1998; Ozawa et al., 1998) and because tetrodotoxin reduced PDE4A expression, we speculated that glutamatergic activity played a role in regulating the age-dependent increase in cortical PDE4A expression.

The PDE4 promoter contains CRE sequences that bind the cAMP-responsive element-binding protein (CREB) and increases the transcription of the enzyme (D'Sa et al., 2002; Le Jeune et al., 2002; Rena et al., 2001). Because NMDA receptor activity increases cyclic nucleotide synthesis, cyclic nucleotide-dependent protein kinase activity, and activates CREB (Chetkovich and Sweatt, 1993; Fedele and Raiteri, 1999; Nicoll and Malenka, 1999; Schwartz and Greenberg, 1987; Suvarna and O'Donnell, 2002; Roberson and Sweatt, 1996; Vianna et al., 2000), which increases the transcription of PDE4 (D'Sa et al., 2002; Houslay et al., 1998; Le Jeune et al., 2002; Rena et al., 2001), we investigated the effect of chronic NMDA receptor inhibition on the expression of PDE4 subtypes in maturing rat primary cortical cultures between DIV 8 and DIV 21.

2. Results

2.1. *Effect of NMDA receptor activity and PDE activity on cAMP and cGMP levels in maturing primary cortical neuronal cultures*

Acutely treating primary cortical neuronal cultures with NMDA (30 μM; 15 min) increased cGMP in DIV 8, 13, and 21 cultures (Fig. 1A, 744±55, 332±50, and 255±32% of control, respectively). The effect of acute NMDA treatment on cGMP was age-dependent with younger cultures exhibiting a greater cGMP response to NMDA treatment than older cultures. However, increasing endogenous glutamate release by inhibiting $GABA_A$ receptor activity with acute bicuculline (Bic; 30 μM, 15 min) did not increase cGMP signaling in primary cortical cultures. Selectively inhibiting PDE2-hydrolytic activity with EHNA (20 μM; 25 min) significantly increased cGMP levels at DIV 8, 13, and 21 (Fig. 1A, 820±55, 388±44, and 242±32% of control, respectively) without affecting cAMP levels in these cultures. EHNA accentuated the effect of NMDA on cGMP in DIV 8, 13, and 21 cultures (Fig. 1A, 5651±343, 2035±154, and 1152±140% of control, respectively).

Acute treatment with NMDA (30 μM), bicuculline (30 μM), or rolipram (Rol) alone had no effect on cAMP levels in DIV 8, 13, and 21 cultures (Fig. 1B). However, NMDA or bicuculline in the presence of rolipram (20 μM; 25 min) increased cAMP levels

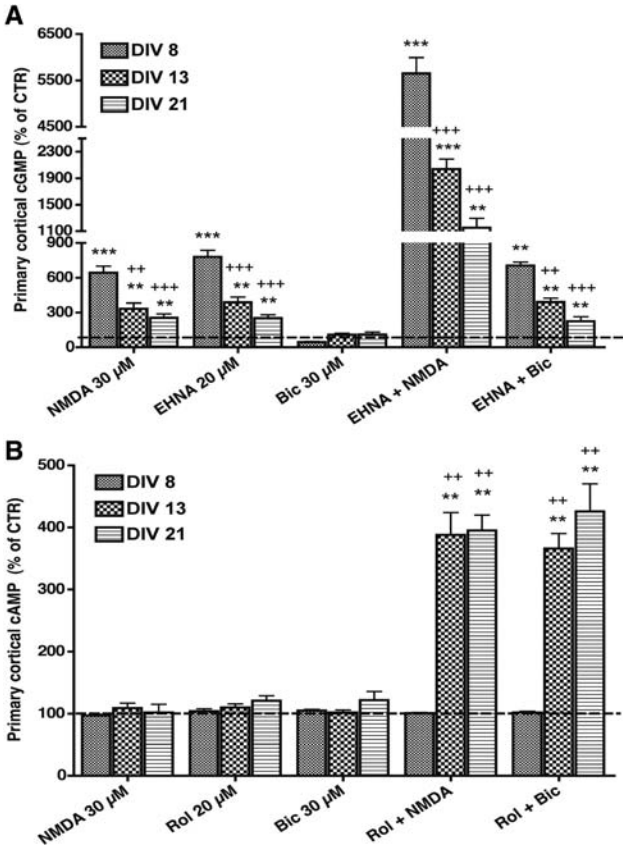

Fig. 1 – Effect of acute NMDA receptor activation and PDE inhibition on cAMP and cGMP levels. Primary cortical neuronal cultures were acutely treated at DIV 8, 13, or 21 with the PDE2 inhibitor EHNA (20 μM; 25 min), the PDE4 inhibitor rolipram (Rol; 20 μM; 25 min), the $GABA_A$ receptor antagonist bicuculline (Bic; 30 μM; 15 min), NMDA (30 μM; 15 min), or a combination of these drugs. Cyclic GMP (A) and cAMP (B) levels were determined by RIA. The dotted line represents basal control levels. Values shown are means±S.E. expressed as a percentage of control samples from four independent experiments. $^{**}p<0.01$ and $^{***}p<0.001$ vs. control; $^{++}p<0.01$ and $^{+++}p<0.001$ vs. DIV 8 from the same group.