Indonesian Journal of Pharmacy

Vol 19 No 3, 2008

Table of Contents

Articles

Indonesian J Pharm indexed by:

This journal has been published by faculty of pharmacy Universitas Gadjah Mada in colaboration with IAI

Majalah Farmasi Indonesia, 19(3), 157 - 164, 2008

Peran *reelin signaling pathway* pada perkembangan toleransi antinyeri morfin

Pivotal role reelin signaling pathway in the development of tolerance to morphine-induced antinociception

Bambang Subakti Zulkarnain *) **dan Junaidi Khotib**
Department of Clinical Pharmacy, Faculty of Pharmacy Airlangga University, Surabaya

Abstract

Reelin merupakan protein endogen besar yang bertanggung jawab untuk mengontrol migrasi dan pertumbuhan dendrit pada neuron yang sedang berkembang. Akhir-akhir ini, reelin signaling pathway dipandang dapat memodulasi plastisitas sinaps pada otak tikus dewasa. Penelitian ini ditujukan untuk membuktikan peran penting reelin signaling pathway pada perkembangan toleransi terhadap respon antinyeri karena pemberian morfin. Ada bukti ilmiah bahwa pemberian berulang melalui injeksi intracerebroventikular yaitu antibodi monoklonal reelin, inhibitor kompetitif reelin – rekombinan apolipoprotein reseptor E2 dan disabled protein inhibitor (Dab1) – MG132 menyebabkan terjadinya hambatan pada perkembangan toleransi pada penggunaan morfin untuk antinyeri. Lebih lanjut, pemberian morfin in vivo secara kronik menyebabkan peningkatan secara bermakna pada immunoreaktifitas (IR) untuk Dab1 yang terfosforilasi di daerah thalamus. Data ini menunjukkan bahwa aktivasi reelin signaling pathway secara persisten karena pemberian kronik morfin mungkin merupakan faktor penyebab perkembangan toleransi pada pemakaian morfin sebagai antinyeri.
Kata Kunci: Toleransi morfin, Reelin, Dab1, MG132, Apolipoprotein reseptor E2

Abstract

The huge endogenous macromolecule protein responsible for controlling migration and dendritic growth of developing neurons, reelin, has recently been proposed that its signaling pathway modulates synaptic plasticity in the adult rodent brain. This study was carried out to investigate the pivotal role of the reelin signaling pathway in the development of tolerance to morphine-induced antinociception. There was evidence that repeated intracerebroventricular administration of reelin's monoclonal antibody, the competitive inhibitor to reelin – apolipoprotein receptor E2 recombinant, and disabled1 (Dab1) protein inhibitor – MG132, resulted in the inhibition to the development of antinociception tolerance to morphine administration. Furthermore, chronic in vivo administration with morphine caused significance increase of the immunoreactivity (IR) for phosphorylated-Dab1 in the thalamus. These data suggested that persistent activation of reelin signaling pathway due to chronic administration of morphine may be responsible for the development of tolerance to morphine-induced antinociception.
Key words: Morphine tolerance, Neuronal plasticity, Opioid receptor, Reelin signaling pathway

Introduction

It is well known that reelin plays an important role as a positioning regulator during the development of laminar structures of the cerebral cortex, hippocampus and cerebellum of mammalian brain. The current development

in cellular and molecular biology showed that reelin signaling pathway is responsible for the axonal branching, synaptogenesis and synaptic plasticity in adult brain (Goffinet et al., 1984, 1995; D'Arcangelo et al., 1995; Rice and Curran, 2001; Quattrocchi et al., 2002; Kubasak et al., 2004, Chen Yet al., 2005). Initially, the process of reelin signaling pathway begin with binding to the very-low-density lipoprotein receptor (VLDLR) and the apolipoprotein receptor E2 (apoER2) which is then induces disabled-1 (Dab1) tyrosine phosphorylation. Following this, the phosphorylated Dab1 interacts with proteins known to be important for regulation of neuronal migration and synaptic plasticity including phosphatidylinositol 3-kinase (PI3K) and cyclin dependent kinase 5 (Cdk5) (D'Arcangelo et al., 1999, Bock et al., 2003; Bock et al., 2004; Beffert et al., 2004). Furthermore, the reelin signaling pathway has been shown to modulate directly on N-methyl-D-aspartate (NMDA) receptors and to be required for long-term potentiation induction (Chen et al., 2005; Sinagra et al., 2005). Cdk5 is a member of the cyclin-dependent kinase family of serine/threonine kinases. As for all members of the Cdk family, full activation of Cdk5 requires association with a regulatory subunit, three of which have been identified in brain: p35, p39 and p67 (Zhang et al., 2002). Substantial recent work has identified multiple diverse functions for Cdk5, including synaptogenesis, axonal targeting, development of neurodegenerative diseases and neuronal cytoskeletal dynamics (Ohshima et al., 1996; Zukerberg et al., 2000). Interestingly, it has been proposed that a functional relationship of reelin- and Cdk5-dependent signaling pathways shows some similarities to regulate neuronal migration and synaptic plasticity (Beffert et al., 2004).

The administration of morphine produces a powerful antinociception/analgesia (Besse et al., 1990 and prolonged exposure to morphine results in tolerance to morphine-induced antinociception (Narita et al., 1994, 2002; Smith et al., 2003). It has been well established that glutamate receptors, including NMDA receptors, are critical in the development and maintenance of opioid tolerance (Trujillo and Akil, 1991). In the present study, we therefore investigated whether the reelin signaling pathways in the adult brain could be involved in the development of the tolerance to morphine-induced antinociception.

Methodology

Animals

Male ICR mice were obtained from Tokyo Laboratory Animals Science Co. Ltd., Tokyo, Japan, weighing 23-25 g at the beginning of experiments. Animals were housed in groups of eight in a temperature-controlled room. They were maintained on a 12 hr light-dark cycle (light on 8:00 a.m. to 8:00 p.m.) and were allowed to adapt to this environment for a period of 1 week before the experiments. Food and water were available ad libitum.

Intracerebroventricular injection

Intracerebroventricular (i.c.v.) administration was performed as described previously (Haley and McCormick, 1957). Briefly, the injection was made with a 2-mm double-needle (Natsume Seisakusho, Tokyo) attached to a 25-μL Hamilton microsyringe. Solution was injected in a volume of 4 μL per mouse.

Antinociceptive Assessments

The development of antinociceptive tolerance to morphine was carried out by injecting mice with repeated administration of morphine (10 mg/kg) or saline (10 /kg) subcutaneously once a day for 7 consecutive days. The antinociceptive response following morphine injection was assessed by the hot plate test (55 ± 0.5 °C, Muromachi Kikai Co., Ltd., Tokyo, Japan) and the tail-flick test (Muromachi Kikai Co., LTD., Tokyo). The latencies of those methods were calculated 30 min after morphine or saline injection. The injection of reelin inhibitors or vehicle 30 min before every morphine injection to groups of mice was designed to assess the role of reelin signaling pathway to the development of tolerance to morphine treatment. Antinociception was calculated as percentage of the maximum possible effect (% MPE) according to the following formula:

$$\% \text{ MPE} = (\text{test latency} - \text{pre-drug latency}) / (\text{cut-off time} - \text{pre-drug latency}) \times 100.$$

The cut-off time was set at 30 sec for the hot-plate test or 10 sec for the tail-flick test to prevent tissue damage. Antinociceptive response is expressed as the mean with S.E.M. of % MPE.

Immunohistochemical Study

Mice were repeatedly injected with morphine (10 mg/kg, s.c.) or saline (10 /kg, s.c.) once a day for 7 days. Twenty-four hr after the last injection, mice were deeply anesthetized with isoflurane and perfusion-fixed with 4 % paraformaldehyde (pH 7.4.). The spinal cords and several brain regions were quickly removed and post-fixed in 4 % paraformaldehyde for 2 hr and were prepared as described previously (Narita et al., 2004). Sections were cut transversely at a thickness of 8-10 μm on a cryostat (Leica CM1510, Leica Microsystems, Heidelberg, Germany). The sections were blocked in 10 % normal goat serum (NGS) in 0.01 M phosphate-buffered saline (PBS) for 1 hr at room temperature. Each primary antibody was diluted in 0.01 M PBS containing 10 % NGS [1:100 reelin (Chemicon International Inc., CA, USA) and 1:100 phosphorylated disabled-1 (p-Dab1, Abcam Ltd, Cambridgeshire, UK)] and incubated for 48 hr at 4 °C. The antibodies were then rinsed and incubated with each secondary antibodies conjugated Alexa 488 and Alexa 546 for 2 hr at room temperature. The slides were then coverslipped with PermaFluor Aqueous mounting medium (Immunon, Pittsburgh, PA, USA). All sections were observed with a light microscope (Olympus BX-80) and photographed with a digital camera (CoolSNAP HQ; Olympus).

Drugs

Apolipoprotein E2 was purchased from Sigma Chemical Co. (St. Louis, MO, USA). MG132 (N-[(phenylmethoxy) carbonyl]-L-leucyl-N-[(1S)-1-formyl-3- methylbutyl]-L-leucinamide) was obtained from Tocris Cookson Ltd. (Ballwin, MO). Apolipoprotein E2 was dissolved in normal saline while MG132 was dissolved in 30 % DMSO for in vivo experiments.

Statistical Analysis

Statistical analysis of significance differences between groups was carried out using a two-way ANOVA followed by by Bonferroni/Dunn test.

Results and Discussions

Reelin-like immunoreactivity in the mouse brain and spinal cord

To examine the expression and distribution of reelin in the brain and spinal cord of mice, we carried out an immunohistochemical study using the monoclonal antibody to reelin. Immunoreactivity (IR) for reelin was observed in the lamina I-VI of the dorsal horn of the spinal cord (Fig. 1). Furthermore, reelin-like-IR was also prominently detected in several brain regions of mice, such as the thalamus, periaqueductal gray (PAG) and cerebral cortex (Figure 2).

Effect of monoclonal antibody to reelin on the development of tolerance to morphine-induced antinociception

The effect of pretreatment with the monoclonal antibody to reelin on the development of tolerance to morphine-induced antinociception was assessed by the hot-plate or tail-flick tests. At first, we confirmed whether pretreatment with monoclonal antibody to reelin could affect acute morphine-induced antinociception. A single i.c.v. injection of monoclonal antibody to reelin with concentrations from 1:100 until 1:1000 dilution in saline had no effect on the acute morphine-induced antinociception and basal hot-plate or tail-flick latencies (data not shown).

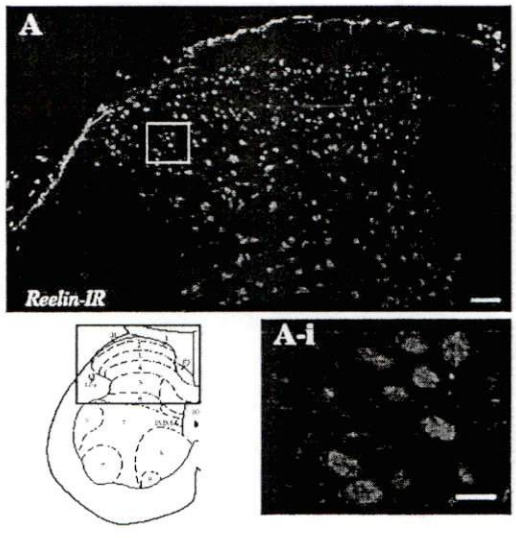

Figure 1. Reelin-IR in dorsal horn

Repeated s.c. administration of morphine (10 mg/kg) once a day for 7 consecutives days produced a time-dependent decline in antinociceptive effect of morphine, indicating the development of tolerance to morphine-induced antinociception (Fig. 3A and 3B). Interestingly, using the tail-flick method, repeated i.c.v. pretreatment with monoclonal antibody to reelin 1:100, 1:300 and 1:1000 diluted in saline completely inhibited the development of antinociceptive tolerance to morphine (Fig. 3A, $F_{(1,14)}=320.1$, $p<0.001$;

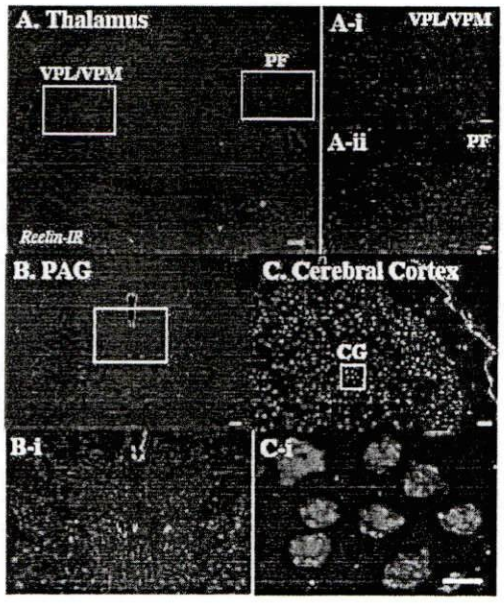

Fig. 2. Reelin-IR in other brain areas

$F_{(1,14)}=150.6$, $p<0.001$ and $F_{(1,15)}=7.2$, $p<0.001$, respectively). Furthermore, in hot-plate test, the development of tolerance to morphine-induced antinociception was suppressed by pretreatment with anti-reelin antibody at concentrations, 1:100 and 1:300 in saline (Fig. 3B, $F_{(1,14)}=19.0$, $p<0.01$ and $F_{(1,14)}=9.8$, $p<0.01$, respectively), whereas pretreatment with anti-reelin antibody at concentration 1:1000 in saline has no effect on the development of antinociceptive tolerance to morphine ($F_{(1,15)}=0.9$, $p<0.36$).

Effect of a competitive inhibitor of reelin apolipoprotein E2 (Apo E2) recombinant on the development of tolerance to morphine-induced antinociception

Reelin binds to the very low-density lipoprotein receptor (VLDLR) and the aporolipoprotein E2 (Apo E2) receptor. The next study was then undertaken to examine the effect of a competitive inhibitor of reelin, Apo E2 recombinant, on the development of tolerance to morphine-induced antinociception.

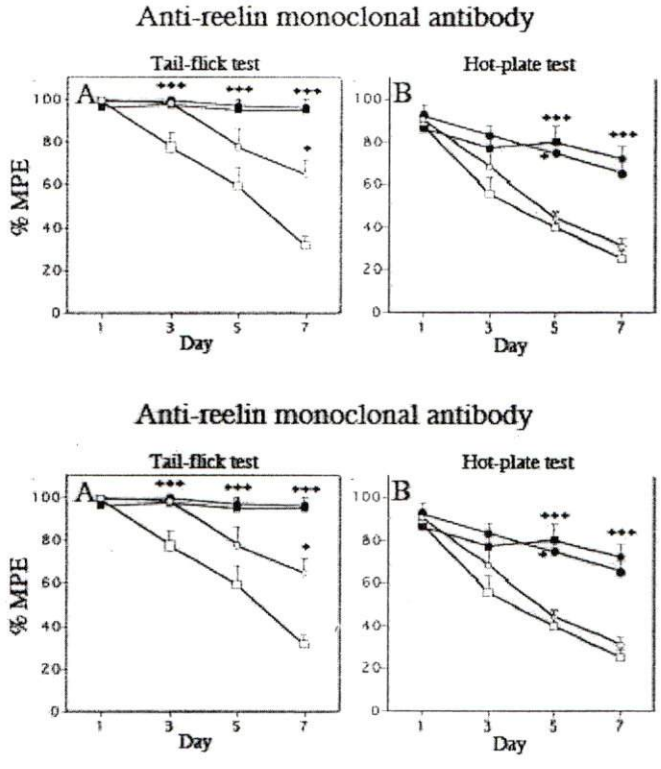

Figure 3. Tail-flick and Hot-plate test for reelin monoclonal antibody and apolipoprotein E2

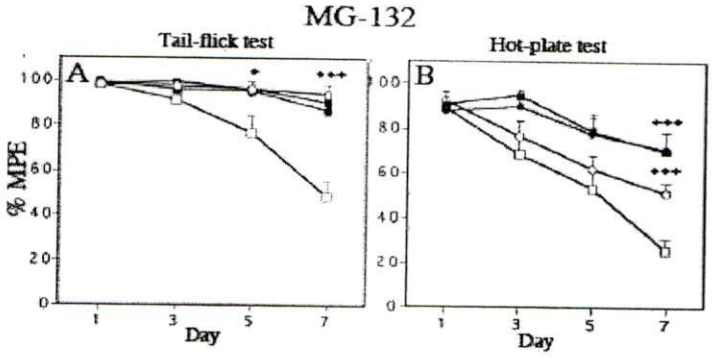

Figure 4. Tail-flick and Hot-plate test for inhibitor of Dab1, MG-132

As shown in Figs. 3C and 3D, the development of tolerance to morphine-induced antinociception was significantly inhibited by repeated pretreatment with Apo E2 recombinant at various concentrations 1:100; 1:300 and 1:1000 in saline (Fig. 3C; $F_{(1,14)}=82.3$, $p<0.001$; $F_{(1,12)}=69.2$, $p<0.001$ and $F_{(1,12)}=52.5$, $p<0.001$, Fig. 3D; $F_{(1,12)}=6.3$, $p<0.05$; $F_{(1,12)}=7.6$, $p<0.05$ and $F_{(1,12)}=4.9$, $p<0.05$, respectively).

Effect of a disabled-1 (Dab-1) protein inhibitor MG132 on the development of tolerance to morphine-induced antinociception

The cytoplasmic adaptor protein disabled 1 (Dab-1) is predominantly expressed in neurons and has been shown to function at the downstream of reelin. It may be possible that the binding of reelin to Apo E2 receptor induces tyrosine phosphorylation of Dab-1, which triggers an intracellular signaling cascade. Phosphorylated-Dab1-like immunoreactivity (p-Dab1-IR) was detected in the posterior complex (Po) of the thalamus in saline-treated mice. Interestingly, repeated treatment with morphine produced a marked increase in p-Dab1-IR in the Po of the thalamus compared with that observed in saline-treated mice (data not shown). The next study was to investigate the effect of pretreatment with a Dab-1 protein inhibitor MG 132 on the development of tolerance to morphine-induced antinociception. Repeated i.c.v. pretreatment with a series of the doses of MG132 (1, 10 and 20 nmol/mouse) completely blocked the development of tolerance to morphine. (Fig. 4A; $F_{(1,15)}=51.8$, $p<0.001$; $F_{(1,14)}=41.6$, $p<0.001$ and $F_{(1,15)}=22.5$, $p<0.01$, Fig. 4B; $F_{(1,15)}=4.6$, $p<0.05$; $F_{(1,14)}=21.0$, $p<0.001$ and $F_{(1,15)}=12.0$, $p<0.01$).

The importance of reelin for neuronal migration and cortical lamination during the embryonic phase of brain development has been extensively studied (Goffinet 1984; Rice et al., 1998; Hartfuss et al., 2003; Beffert et al., 2004). However, little has been known about the role of reelin in the adult brain. Here we show for the first time that reelin and the receptors to which it binds are likely to contribute to the development of tolerance to morphine-induced antinociception in mice. Reelin signaling requires binding to two members of the LDL receptor gene family, the VLDLR and the apoER2, on the surface of neurons (Mahley et al., 1998; D'Arcangelo et al., 1999). Further transmission of the signal is dependent upon the Dab1. Dab1 is a neuron-specific cytoplasmic protein that binds to the NPxY motif in the cytoplasmic tails of the VLDLR and the apoER2 (Trommsdorff et al., 1999). Clustering of VLDLR and/or apoER2 by reelin binding leads to tyrosine phosphorylation in the PI/PTB domain of Dab1 and activation of nonreceptor tyrosine kinases of the Src family through a feed forward mechanism (Ballif et al., 2003; Bock et al., 2004). In the present study, we demonstrated that the level of p-Dab1-IR in the thalamus was significantly increased by repeated in vivo treatment with morphine. Interestingly, the increased IR for p-Dab1 was colocalized with reelin-IR in the thalamus of morphine-treated mice. The treatment with monoclonal antibody to reelin will trap the

endogenous reelin, resulting in the blockade of the activation of downstream of reelin pathway (Lacor et al, 2000; Caruncho et al, 2004; Fatemi et al, 2005). Here we show that the pretreatment with the monoclonal antibody to reelin, the competitive inhibitor of reelin (Apo E2 recombinant) and a Dab1 protein inhibitor (MG 132) caused the apparent inhibition of the development of tolerance to morphine-induced antinociception. Taken together, these findings support the idea that the increased phosphorylation state of Dab1 related to activated reelin in the thalamus following repeated treatment with morphine may be responsible for the development of tolerance to morphine-induced antinociception. considerable evidence suggests that reelin- and Cdk5-dependent signals have been implicated in numerous aspects of both functional and structural plasticity through its regulation of signal transduction pathways (Ohshima and Mikoshiba, 2002; Beffert et al., 2004). Furthermore, both pathways are also involved in modulating synaptic neurotransmission through regulation of N-methyl-D-aspartate (NMDA) receptor activity (Beffert et al., 2004; Chen et al., 2005). Interestingly, change in function of NMDA receptor has been shown to affect the development of psychological dependence on and antinociceptive tolerance to morphine.

Conclusion

In conclusion, the present data indicate that repeated in vivo treatment with morphine induces the increase in Dab1 activity possibly related to activating reelin in the thalamus of mice. In addition, the development of tolerance to morphine-induced antinocicepton was suppressed by several kinds of inhibitors to modulate reelin signaling. These findings provide further evidence for the critical role of reelin signaling in the regulation of morphine tolerance.

Acknowledgement

We thank Prof. Dr. Tsutomu Suzuki and Assoc. Prof. Dr. Minoru Narita for funding, supervision, and discussion for this work.

References

Ballif B. A, Arnaud L, Cooper J. A., 2003, Tyrosine phosphorylation of Disabled-1 is essential for Reelin-stimulated activation of Akt and Src family kinases. Brain Res. Mol. Brain Res. 117:152-159.

Beffert U, Weeber E. J, Morfini G, Ko J, Brady S. T, Tsai L. H, Sweatt J. D, Herz J, 2004, Reelin and cyclin-dependent kinase 5-dependent signal cooperate in regulating neuronal migration and synaptic transmission. *J. Neurosci.* 24: 1897-1906.

Besse D, Lombard M. C, Zajac J. M, Roques B. P, Besson J. M., 1990, Pre- and postsynaptic distribution of μ, δ and κ opioid receptors in the superficial layers of the cervical dorsal horn of the rat spinal cord. Brain Res. 521, 15-22.

Bock H. H, Jossin Y, May P, Berger O, Herz J, 2004, Apolipoprotein E receptor are required for reelin-induced proteasomal degradation of the neuronal adaptor protein disabled-1. *J. Biol. Chem.* 279: 33471-33479.

Caruncho H. J, Dopeso-reyes I. G, Loza M. I, Rodriquez M. A, 2004, GABA reelin, and the neurodevelopmental hypothesis of schizophrenia. Critical Rev. Neurobiol. 16: 25-32.

Chen Y, Beffert U, Ertunc M, Tang TS, Kavalali ET, Bezprozvanny I, Herz J., 2005, Reelin modulates NMDA receptor activity in cortical neurons. *J. Neurosci.* 25: 8209-8216.

D'Arcangelo G, Miao G, Chen SC, Soares H. D, Morgan J. I, Curran T, 1995, A protein related to extracelluler matrix proteins deleted in the mouse mutant reeler. Nature 374: 719-723.

D'Arcangelo G, Homayouni R, Keshvara L, Rice DS, Sheldon M, Curran T , 1999, Reelin is a ligand for lipoprotein receptors. Neuron 24: 471-479.

Fatemi S. H, Stary JM, Earle JA, Agaghi-Niknam M, Eagan E, 2005, GABAergic dysfunction in schizophrenia and mood disorder as reflected by decreased levels of glutamic acid

decarboxylase 65 and 67 kDa and reelin proteins in cerebellum. Schizophrenia Res. 72: 109-122

Goffinet A. M., 1984, Event governing organization of postmigratory neurons: study on brain development in normal and reeler mice. Brain Res. 319: 261-296

Goffinet AM., 1995, A real gene for reeler. Nature 374: 675-677.

Haley M. J, McCormick WG, 1957, Pharmacological effects produced by intracerebral injections of drugs in the conscious mouse. Br. *J. Pharmacol.* 12: 12-15.

Hartfuss E, Foster E, Bock HH, Hack MA, Leprince P, Luque JM, Herz J, Frostcher M, Gotz M ,2003, Reelin signaling directly affects radial glia morphology and biochemical maturation. Development 130: 4597-4608.

Kubasak M. D Brooks R, Chen S, Villeda S. A, Phelps P. E., 2004, Developmental distribution positive cell and their secreted product in the rodent spinal cord. *J. Comp. Neurol.* 468: 165-178.

Lacor P. N, Grayson DR, Auta J, Sugaya I, Costa E, Gidotti A., 2000, Reelin secretion from glutamatergic neurons in culture is dependent from neurotransmitter regulation. Proc. Nat. Acad. Sci. 97: 3556-3561.

Luque J. M., 2004, Integrin and the reelin-DAB1 pathway: a sticky affair? Dev. Brain Res. 152: 269-271.

Mahley R. W., 1998, Apolipoprotein E: cholesterol transport protein with expanding role in cell biology. Science 240: 622-630.

Morimura T, Hattori M, Ogawa M, Mikoshiba K., 2005, Disabled-1 regulates the intracellular trafficking of reelin receptor. *J. Biol. Chem.* (in press).

Narita M, Makimura M, Feng Y, Hoskins B, Ho I. K., 1994, Influence of chronic morphine treatment on protein kinase C activity: comparison with butorphanol and implication for opioid tolerance. Brain Res. 650, 175-179.

Narita M, Ioka M, Suzuki M, Narita M, Suzuki T., 2002, Effect of repeated administration of morphine on the activity of extracellular signal regulated kinase in the mouse brain. Neurosci. Lett. 324, 97-100.

Ohshima T, Ogawa M, Veeranna, Hirasawa M, Longenecker G, Ishiguro K, Pant H. C, Brady R. O, Kulkarni A. B, Mikoshiba K.,2001, Synergistic contributions of cyclin-dependant kinase 5/p35 and reelin/dab1 to the positioning of cortical neurons in the developing mouse brain. Proc. Nat. Acad. Sci. 98: 2764-2769.

Ohshima T, Ogawa M, Takeuchi K, Takahashi S, Kulkarni A. B, Mikoshiba K., 2002, Cyclin-dependent kinase 5/p35 contributes synergistically with Reelin/Dab1 to the positioning of facial branchiomotor and inferior olive neurons in the developing mouse hindbrain. *J. Neurosci.* 22:4036-4044.

Ohshima T, Mikoshiba K., 2002, Reelin signaling and Cdk5 in the control of neuronal positioning. Mol. Neurobiol. 26:153-166.

Pramatarova A, Ochalski P. G, Chen K, Gropman A, Myers S, Min K. T, Howell B. W., 2003, Nck interacts with tyrosine-phosphorilated disabled 1 and redistributes in reelin-stimulated neurons. Mol. Cell. Biol. 23: 7210-7221.

Quattrocchi CC, Wannenes F, Persico A. M, Ciafre S. A, D'Arcangelo G, Farece M. G, Keller F., 2002, Reelin is a serine protease of the extracellular matrix. *J. Biol. Chem.* 277: 303-309.

Rice D. S, Curran T., 2001, Role of the reelin signaling pathway in central nerves system development. Annu. Rev. Neurosci. 24: 1005-1039.

Rice D. S., Sheldon M, D'Arngelo G, Nakajima K, Goldowitz D, Curran T., 1998, Disabled-1 acts downstream of reelin in a signaling pathway that control laminar organization in the mammalian brain. Development 125: 3719-3729.

Sanada K, Gupta A, Tsai L. H., 2004, Disabled-1-regulated adhesion of migrating neurons to radial glial fiber contributes to neuronal positioning during early corticogenesis. Neuron 42: 197-211.

Sinagra M, Verrier D, Frankova D, Korwek K. M, Blahos J, Weeber EJ, Manzoni O. J, Chavis P., 2005, Reelin, very-low-density lipoprotein receptor, and apolipoprotein E receptor 2 control somatic NMDA receptor composition during hippocampal maturation in vitro. *J. Neurosci.* 25: 6127-6136.

Smith F. L., Javed R. R., Elzey M. J. and Dewey W. L., 2003, The expression of a high level of morphine antinociceptive tolerance in mice involves both PKC and PKA. Brain Res. 985, 78-88.

Tesseur I, Van Dorpe J, Spittael K, Van den Haute C, Moechars D van Leuven, 2000, Expression of human apolipoprotein E4 in neurons causes hyperphosphorylation of protein tau in the brain of transgenic mice Am. *J. pathol.* 150: 951-964.

Trommsdorff M, Borg J. P, Margolis B, Her J., 1998, Internalization of cytosolic adaptor proteins with neuronal apolipoprotein E receptor and amyloid precursor protein. *J. Biol. Chem* 273: 33556-33560.

Trujillo K. A, Akil H., 1991, Inhibition of morphine tolerance and dependence by the NMDA receptor antagonist MK-801. Science 251: 85-87.

Zhang J, Krishnamurthy P. K, Johnson G. V., 2002, Cdk5 phosphorylates p53 and regulates its activity. *J Neurochem.* 81: 307-313.

Zukerberg L. R, Patrick G. N, Nikolic M, Humbert S, Wu C. L, Lanier L. M, Gertler F. B, Vidal M, Van Etten RA, Tsai L. H., 2000, Cables links Cdk5 and c-Abl and facilitates Cdk5 tyrosine phosphorylation, kinase upregulation, and neurite outgrowth. Neuron. 26: 633-646.

* Korespondensi : Bambang Subakti Zulkarnain, S.Si..Clin.Pharm(UQ)
Departement of Clinical Pharmacy Faculty of Pharmacy
Airlangga University Ph: 62-31-7051 6625
E-mail : bambang_sz@yahoo.com

Indonesian Journal of Pharmacy

Editorial Team

Editor in Chief

Editorial Board

Indonesian J Pharm indexed by: