available at www.sciencedirect.com

SCIENCE DIRECT®

www.elsevier.com/locate/brainres

BRAIN RESEARCH

Research Report

Anisomycin activates p38 MAP kinase to induce LTD in mouse primary visual cortex

Wei Xiong[a,*], *Ljubomir Z. Kojic*[a], *Lanjing Zhang*[c], *Shiv S. Prasad*[a], *Robert Douglas*[b], *Yutian Wang*[a], *Max S. Cynader*[a]

[a]*Brain Research Center, University of British Columbia, 2211 Wesbrook Mall, Brain Research Center, Vancouver, Canada BC V6T 2B5*
[b]*Ophthalmology Research Lab, 2550 Willow Street, Vancouver, Canada BC V5Z 3N9*
[c]*Department of Internal Medicine, University of Michigan Medical School, Ann Arbor, MI 48109, USA*

ARTICLE INFO

Article history:
Accepted 7 February 2006
Available online 11 April 2006

Keywords:
Mouse
Primary visual cortex
Anisomycin
Long-term depression
p38 MAP kinase
AMPA receptor

ABSTRACT

Anisomycin is both a well-established protein synthesis inhibitor and a potent activator of the p38/JNK MAPK pathway. It has been used to block the late phase of long-term potentiation (LTP) and long-term depression (LTD) in hippocampus. In this study, we have found that anisomycin produces a time-dependent decline in the magnitude of the field EPSP (fEPSP) in acute brain slices of mouse primary visual cortex. This anisomycin-mediated fEPSP depression occludes NMDA receptor-dependent LTD induced by low-frequency stimulation (LFS). In contrast, two other protein synthesis inhibitors, emetine and cycloheximide, have no effect either on baseline synaptic transmission or on LTD. Moreover, the decline of the fEPSP caused by anisomycin can be rescued by the application of the p38 inhibitor SB203580 but not by the JNK inhibitor SP600125. These results indicate that activation of p38 MAPK by anisomycin induces LTD and subsequently occludes electrically induced LTD. Also, the occlusion of LFS-LTD by anisomycin suggests that common mechanisms may be shared between the two forms of synaptic depression. Consistent with this view, bath application of a membrane permeant peptide derived from the carboxyl tail of GluR2 subunit of AMPA receptor, which specifically blocks regulated AMPA receptor endocytosis, thereby preventing the expression of LFS-induced LTD, significantly reduced the anisomycin-induced decline of the fEPSP. In conclusion, our results indicate that anisomycin produces long-lasting depression of AMPA receptor-mediated synaptic transmission by activating p38 MAPK-mediated endocytosis of APMA receptors in mouse primary visual cortex.

1. Introduction

Long-term depression (LTD) refers to a lasting decrease in the amplitude of a synaptic response which follows low-frequency stimulation (LFS) of a presynaptic pathway. The early phase of LTD is postulated to be the result of the internalization of postsynaptic receptors; and the maintenance of LTD involves a series of mechanisms, with posttranslational events typically underlying the early maintenance phase, and transcription- and translation-dependent events underling the late (>3 h) maintenance phase (Bennett, 2000; Huang et al., 1994; Luscher et al., 2000).

* *Corresponding author*. Fax: +1 604 8227229.
E-mail address: weixiong@interchange.ubc.ca (W. Xiong).

0006-8993/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.brainres.2006.02.015

Intensive studies have been performed to explore the protein synthesis-dependant phase of LTD using protein synthesis inhibitors. Among the protein synthesis inhibitors, anisomycin has been widely used to study the late phase of LTP and LTD (Kauderer and Kandel, 2000; Steward and Worley, 2001). It binds to the 60 S ribosomal subunit in eukaryotic cells and inhibits the peptidyltransferase reaction (Jimenez et al., 1975; Middlebrook and Leatherman, 1989). In hippocampus and cerebellum, anisomycin had no impact on basic synaptic transmission and did not influence the early phase of LFS-induced LTD (Huber et al., 2000; Linden, 1996; Sajikumar and Frey, 2003). In addition, anisomycin at 10–30 μM had no effect on voltage-gated calcium channels and mGluR1 receptors in the cerebellum (Linden, 1996).

In addition to its protein synthesis inhibition effect, anisomycin is a strong activator of the p38 and C-jun N-terminal kinase (JNK) MAPK pathway (Shifrin and Anderson, 1999). P38 MAPK was initially characterized owing to its role in the response of cells to various adverse stimuli such as heat shock and bacterial endotoxin linked to apoptosis. Once activated, it phosphorylates a variety of proteins and relay signals downstream. P38 MAPK is highly expressed in brain areas including cerebral cortex, hippocampus, cerebellum, and few nuclei of the brainstem (Zhu et al., 2002). Recently, emerging evidence suggests that p38 MAPK is also a key player in the regulation of synaptic plasticity. Provocative new results indicate a requirement for p38 activation in a different form of hippocampal LTD that depends on NMDAR activation (Schwarze et al., 1999). Moreover, p38 also appears to be crucial for metabotropic glutamate receptor (mGluR)-dependent LTD since perfusion of an active form of p38 can mimic and occlude this mGluR-dependent LTD form of plasticity (Bolshakov et al., 2000).

In the primary visual cortex, LTD has been postulated to play a crucial role in the experience-dependent visual plasticity during the critical period (Heynen et al., 2003). In this study, we initially intended to study the late phase of LTD in primary visual cortex. Surprisingly, we found that anisomycin administered prior to LFS induced prolonged depression of basal synaptic transmission that occluded subsequent LFS-LTD. We were able to demonstrate that this anisomycin LTD was primarily mediated via the p38 MAPK signaling pathway and, likewise LFS-induced LTD, required GluR2-dependent clathrin-mediated endocytosis of postsynaptic AMPA receptors.

2. Results

2.1. *Effect of anisomycin on synaptic transmission and long-term depression in the primary visual cortex*

The initial aim of our study was to test the effect of anisomycin on the late phase of LTD in mouse primary visual cortex. It has been shown that anisomycin has no effect on baseline fEPSP and short term LTD induced by LFS in hippocampus and cerebellum (Huber et al., 2000; Linden, 1996). In the first experiment, we started recording fEPSP 60 min after incubating slices containing primary visual cortex in 20 μM anisomycin. LFS was delivered after minimal 15-min stable baseline. In this preparation, surprisingly, LTD could not be induced in the slices pretreated with anisomycin (95 ± 3% of the baseline recorded in the last 5 min prior to LFS; $n = 31$; $P < 0.01$ compared to DMSO-treated slices), while normal LTD was induced in sliced pretreated with 0.1% DMSO as a vesicle (85 ± 1%; $n = 10$) (Fig. 1A). Although previous reports have suggested that anisomycin at 20 μM does not interfere with synaptic function other than with protein synthesis in the hippocampus (Iordanov et al., 1997) and the cerebellum (Linden, 1996), we decided to look at the basal synaptic transmission during anisomycin treatment. After achieving 15-min baseline, we applied 20 μM anisomycin through bath perfusion. A gradual decline in the basal fEPSP was observed. The normalized fEPSP magnitude was 88 ± 3% and 80 ± 3% of baseline, 30 min and 60 min after the beginning of anisomycin perfusion respectively ($n = 11$; $P < 0.01$ compared to original baseline) (Fig. 1B). Consistent with the initial finding, in the same slices, subsequent LFS-induced LTD was significantly reduced 30 min after the end of LFS (92 ± 3% of the baseline recorded in the last 5 min prior to LFS; $n = 11$; $P < 0.05$ compared to slices treated with DMSO) (Fig. 1B). In contrast, slices treated only with 0.1% DMSO as a vesicle did not have a decline in the baseline synaptic transmission (96 ± 3% of baseline; $n = 3$; $P < 0.05$ compared with slices treated with anisomycin) and showed a normal magnitude of LTD (83 ± 2% of baseline recorded 5 min prior to LFS; $n = 3$; $P > 0.05$ compared with control LTD; $P < 0.05$ compared with slices treated with anisomycin). These results suggest that anisomycin is able to induce LTD-like phenomenon that occludes the LTD induced by LFS in primary visual cortex.

To confirm that anisomycin has no effect on baseline fEPSP in hippocampal CA3-CA1 pathway, we applied anisomycin (20 μM) after obtaining a stable baseline. In hippocampus, in contrast to primary visual cortex, anisomycin did not change the amplitude of fEPSP (104 ± 2% and 100 ± 2% of baseline, 30 min and 60 min after the beginning of anisomycin perfusion respectively; $P > 0.05$ compared to original baseline; $n = 4$). In the same slices, a subsequent LFS, in the presence of anisomycin, induced LTD that was 84 ± 3% of the baseline ($P > 0.05$ compared with DMSO control group; $n = 4$) (Fig. 1C).

In addition, we confirmed that the LFS-induced LTD that was occluded by anisomycin was dependent on NMDA receptor activation since 50 μM D-APV blocked this LTD (97 ± 2%; $n = 6$; $P < 0.05$ compared to control LTD), while the control group showed a normal LTD (85 ± 1%; $n = 17$) (Fig. 1D). Interestingly, we found that the magnitude of fEPSP reduction after 1-h treatment with anisomycin was similar to the magnitude of the normal LTD induced by LFS without anisomycin ($P > 0.05$).

2.2. *Activation of p38 is required for anisomycin-induced decline of fEPSPs and LTD occlusion*

Anisomycin is a potent activator of p38/JNK MAPK. Treatment with anisomycin at 3.8 μM for 15 min is enough to activate p38/JNK MAPK pathway (Shifrin and Anderson, 1999). To determine whether the activation of p38/JNK was the cause of the anisomycin LTD or whether it resulted from a more