Available online at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/brainres

Brain Research

Research Report

Neuroprotective effects of okadaic acid following oxidative injury in organotypic hippocampal slice culture

Un Jeng Kim[a], *Ran Won*[b], *Kyung Hee Lee*[c,*]

[a]*Department of Physiology, Yonsei University College of Medicine, Seoul 120-752, Republic of Korea*
[b]*Department of Biomedical Laboratory Science, Division of Health Science, Dongseo University, Busan 617-716, Republic of Korea*
[c]*Department of Dental Hygiene, Division of Health Science, Dongseo University, Busan 617-716, Republic of Korea*

ARTICLE INFO

Article history:
Accepted 29 May 2015
Available online 9 June 2015

Keywords:
Hippocampal slice culture
Okadaic acid
Oxidative injury
Optical imaging
Synaptic plasticity

ABSTRACT

Oxidative stress produces neurotoxicity often related with various CNS disorders. A phosphatase inhibitor enhances the actions of the signaling kinases. Protein kinases mediated-action shows the neural protection in brain injury. Phosphatase inhibitor, okadaic acid (OA), may enhance the protection effect and benefit to improve neuronal plasticity in post-injury. Thus, we investigated that the protein prophatase inhibitor affects neuroprotective signaling and neuroplastic changes in hippocampus after oxidative injury. Electrophysiological and biochemical assays were used to observe changes in synaptic efficacy following electrical and/or pharmacological manipulation of synaptic function. Neuronal cell death, as assessed by propidium iodide (PI) uptake, was reduced by OA treatment (24 and 48 h) compared with KA treatment. The pattern of DCFH-DA fluorescence in hippocampal slices corresponded well with PI uptake. The phospho-AKT/AKT ratio showed that the level of phospho-AKT was significantly increased in the OA-treated group. Furthermore, the OA-treated group exhibited significantly increased expression of SOD2 compared with the KA-only group. Optical imaging revealed that KA treatment tended to delay the latency of electrical stimulation and decrease the amplitude of optical signals of synaptic activity. These results suggest that OA may protect hippocampal neurons against oxidative stress and the survived neurons may functional to synaptic plasticity changes.

1. Introduction

Oxidative stress and mitochondrial dysfunction are known to be primary factors in the progression of various neurodegenerative disorders (Won et al., 1999). Several studies have described the mechanisms by which kainic acid (KA) contributes to brain damage (Atkinson et al., 2009; Barancik et al., 1999; Berven et al., 2001). On one of the study, it is reported that KA can excite neuronal pathways, possibly causing direct excitotoxic brain damage (Wang et al., 2005). In the case of KA-treated rats, the results revealed an up-regulation of malondialdehyde acid (MDA), nitric oxide (NO),

*Corresponding author. Fax: +82 513202732.
E-mail address: kyhee@gdsu.dongseo.ac.kr (K.H. Lee).

http://dx.doi.org/10.1016/j.brainres.2015.05.039
0006-8993/

and N-acetylaspartate (NAA), all of which are indicators of oxidative stress in the brain. A significant increase in both caspase-3 and nuclear factor-kappa B (NF-κB) activity was also observed (Costa et al., 2013), indicating the occurrence of apoptotic cell death.

Conflicting data have been obtained regarding the potentially neuroprotective effects of okadaic acid (OA), a selective and potent protein phosphatase 2A (PP2A) inhibitor (Ahn et al., 2009; Atkinson et al., 2009; Matias et al., 1999). OA has been shown to induce apoptotic cell death in immortalized cell lines (Matias et al., 1999; Schmidt et al., 1995). However, PP2A inhibitors have also been demonstrated to inhibit apoptogenesis caused by various factors such as antisomycin, bistratene A, cisplatin, etoposide, gamma-radiation, and tetrandrine (Chatfield and Eastman, 2004; Morana et al., 1996; Song et al., 1992) in the brain. OA enhances the phosphorylation of signaling kinases (Andjelković et al., 1996; Chatfield and Eastman, 2004). These complicated results demonstrate that the precise molecular mechanisms and effects of OA remain to be determined.

Organotypic hippocampal slice cultures (OHSCs) have been used to investigate neuronal death induced by various treatments such as hypoxia/aglycemia, excitotoxins, neurotoxins, oxidative stress, and organic solvents (Vornov et al., 1991; Wilde et al., 1997). Although OHSCs are grown *in vitro*, they exhibit good preservation of connectivity, similar to that in the *in vivo* environment; thus, OHSCs are commonly used to study neuronal connectivity and plasticity (Mellentin et al., 2006; Stoppini et al., 1991). OHSCs have been grown on multielectrode arrays and used continuously for electrophysiological measurements (Shimono et al., 2002). Functional plasticity is one of the most prominent cellular models of activity-dependent long-lasting changes of synaptic transmission in the brain. Moreover, optical recording techniques with voltage-sensitive dyes (VSDs) are powerful tools for observing electrical activities of the brain (Tominaga et al., 2001). Optical recording has many advantages over conventional electrophysiological techniques, including its ability to display cell membrane potential changes directly and non-invasively, making it possible to monitor multiple sites simultaneously (Canepari et al., 2010; Nakagami et al., 1997; Padamsey and Emptage, 2011).

Therefore, the present study was conducted to determine if OA, a selective and potent protein prophatase 2A (PP2A) inhibitor, increases neuroprotective signaling and induces neuroplastic recovery in OHSC after KA-induced oxidative injury.

2. Results

2.1. Effects of OA on KA-induced neuronal death

To study the role of OA in KA-induced injury of OHSCs, neuronal viability was first examined by measuring the uptake of PI, a well-known marker of cell death (Fig. 1). Hippocampi treated with 5 μM KA for 18 h exhibited more progressive cell death in the CA3 area compared with the CA1 area. To assess the effect of OA-treatment on neuronal survival, OHSCs were maintained in culture medium with different doses of OA for 24 h before exposure to KA (pre) and for 24 h after exposure to KA (post), while the KA-only group (control) was cultured in medium without OA. Particularly in the CA3 area, OA treatment groups showed significantly reduced PI staining 24 and 48 h after the OA-treatment compared with the control ($p<0.05$, Fig. 1). This result indicates that OA protects hippocampal cells from KA-induced acute cell death, implying that OA exerts neuroprotective effects.

2.2. Formation of ROS in KA-induced toxicity

The DCFH-DA fluorescence dye was used to detect the reactive oxygen species (ROS) formation in the hippocampal tissues. Green fluorescence intensity which indicates the ROS formation level was detected evenly throughout most cell layers (see the first column (Normal) in Fig. 2(a)). The formation of ROS in KA-induced oxidative stress was shown in Fig. 2. The fluorescence in the tissues showed minute changes in before the KA-treatment (see the 2nd image on upper row (Pre) in Fig. 2(a)) and the pre-treatment of OA (see right 4 images on upper row in Fig. 2(a)). However, 18 h after the KA-treatment, the tissue generated significantly increased DCFH-DA fluorescence over normal ($p<0.05$) while the OA pre-treatment significantly reduced the DCFH-DA fluorescence as compared to groups ($p<0.05$, Fig. 2(b) Left). Thus, pretreatment with OA resulted in significantly decreased ROS production, indicating that OHSCs were protected from oxidative injury. At 24 h after the OA-treatment, DCFH-DA fluorescence tended to be lower compared with the KA-treated group ($p<0.05$, Fig. 2(b) Right).

2.3. Activation of survival signals by OA

To investigate the mechanisms underlying OA-mediated cell survival, the phosphorylation of AKT which is known as a factor in ROS-related cell signaling, was examined. The KA group exhibited significantly decreased phosphorylation of AKT (Fig. 3(a)). On the other hand, 24 h after OA-treatment, the level of AKT phosphorylation was significantly increased in the OA-treated group compared with the KA-only group ($p<0.05$, Fig. 3(a)). To further clarify the mechanism of neuronal cell survival, the production of SOD2, a key executor of cell survival, was analyzed after OA-treatment followed by KA-treatment (Fig. 3(b)). In the cultured slices, SOD2 was downregulated in the presence of KA; on the other hand, SOD2 was upregulated in the OA-treated group compared with the KA-treated group.

2.4. Electrophysiological study

To electrophysiologically measure the synaptic strength and plasticity of the surviving neurons in the KA-treated and OA-treated slices, optical imaging with VSD was performed (Fig. 4). Cellular activities which visualize the active area and the distribution of stimulus-induced activities were observed by optical imaging (Fig. 4(a)). A typical spatiotemporal change in the signal transmission was observed in the hippocampus after SC stimulation. A normal tissue exhibited strong activity accompanied by normal synaptic propagation