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BRAIN RESEARCH

Research Report

Delta opioid agonist [D-Ala2, D-Leu5] enkephalin (DADLE) reduced oxygen–glucose deprivation caused neuronal injury through the MAPK pathway

Sun Ke[1], *Su Dian-san*[1], *Wang Xiang-rui*[*]

Department of Anesthesiology, RenJi Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai, China

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ABSTRACT

It has been demonstrated that [D-Ala2, D-Leu5] enkephalin (DADLE), a delta opioid agonist, protected neuron from hypoxic neuronal injury by activating the delta opioid receptor (DOR). However, whether DADLE can prevent neuronal injury induced by severe hypoxia like oxygen–glucose deprivation (OGD) is not clear. Here, we investigated whether DADLE has a protective effect against neuronal injury induced by oxygen–glucose deprivation. Neuron viability was measured by MTT and neuron injury was assessed by lactate dehydrogenase (LDH) release. Protein expression was examined by Western blot. The results showed that DADLE protected the cortical neuron in a dose-dependent way from OGD injury. And this neuroprotective effect could be completely blocked by delta2 opioid antagonist Naltrindole. DADLE increased phosphorylation of ERK and prevented OGD-induced p38 phosphorylation. Neither DADLE nor Naltrindole had any appreciable effect on phosphorylation of JNK. One of the protective mechanisms of DADLE on OGD neurons may be due to the dynamic balance between the activation of ERK and the p38.

1. Introduction

Neuronal death as a result of neuronal injuries such as stroke, is an irreversible process that leads to long term neurological deficit. The prevention of neuronal loss is therefore critical in rescuing the brain from neurological disaster. However, clinical strategies that mitigate hypoxic/ischemic injury induced neuronal loss are still very limited.

Recent data indicated that DADLE, can protect against ischemia–reperfusion-induced brain damage after transient middle cerebral artery occlusion (MCAO) in rats (Su, 2000). In our laboratory, Su Dian-san et al. (2007) also reported that intracerebroventricular treatment with DADLE attenuated the neuronal death and behavior retardation induced by forebrain ischemia in rats. However, the detailed mechanisms of its neuroprotection were not totally clarified.

Opioid receptors belong to the family of G protein-coupled receptors (GPCRs) (Lopez-Ilasaca et al., 1997; Polakiewicz et al., 1998) and their activation regulates multiple cellular processes, including activation of Mitogen-activated protein kinase (MAPK) (Burt et al., 1996; Fukuda et al., 1996; Wilson et al., 1997; Zhang et al., 1999). There are three members in MAP kinases, including extracellular signal-regulated kinases (ERK1/2), p38 kinase, and c-Jun N-terminal kinase (JNK). It has been reported that oxidative stress stimulates MAPK cascades through opioid receptors in neurons (Borlongan et al., 2000; Xia et al., 1995). However, the role of these MAPKs in neuronal oxidative injury is still unclear. In this study, we investigated

[*] *Corresponding author*. Fax: +86 21 50903239.
E-mail address: mdpaperwang@yahoo.cn (W. Xiang-rui).
[1] Contributed equally to this work.

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the effects of delta opioid agonist DADLE on the expression of these three MAPK proteins as well as the cell viability and lactate dehydrogenase (LDH) release in the OGD-treated cortical neurons.

2. Results

2.1. LDH level in culture medium was increased along with duration of OGD

We first investigated the effects of OGD on Medium LDH level by exposing the cortical neurons in OGD conditions at different times (0, 0.5, 1, 2, 4 and 6 h) at 37 °C. The results, as shown in Fig. 1, indicated that the cell injury was increased with an increase in the strength of OGD. A dramatic increment of the LDH activity was found at 2, 4 and 6 h of ischemia. The differences in the LDH activity between 4 and 6 h of OGD are greater than that between 2 and 4 h (Fig. 1). Therefore, 4 h of OGD was used in the following experiment.

2.2. DADLE at 100 nM induced the best neuroprotective effect

We investigated the effects of different DADLE concentrations on the viabilities of ischemic cells. The cortical neurons were pre-incubated with different concentrations of DADLE for 1 h and then treated with ischemia (OGD) for 4 h. Cell viability was measured after the OGD treatment. It was found that cell viability increased progressively with the DADLE concentrations of added, it reached the highest value at 100 nM, and then decreased with the concentrations of DADLE. The value at 10 μM is lower than OGD (Fig. 2). Therefore, this dosage (100 nM) was used in the following experiments. DADLE exerts dual actions in the present study:

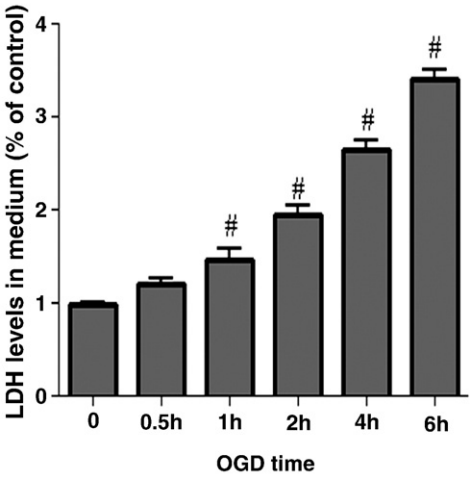

Fig. 1 – Time course of OGD-induced injury in cultured cortical neurons. Medium lactate dehydrogenase (LDH) activities were measured immediately after OGD. Values are shown as percentage to control levels from sister cultures maintained in normal condition. The data were presented as mean ± S.D. ($n=6$). $^{\#}p<0.05$ versus 0 h.

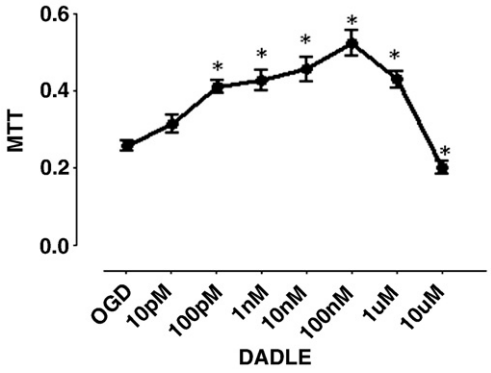

Fig. 2 – Effects of DADLE on the viabilities of ischemic cells. The OGD neurons were pre-incubated with different concentrations of DADLE (0 pM, 10 pM, 100 pM, 1 nM, 10 nM, 100 nM, 1 μM and 10 μM) for 1 h before treated with ischemia (OGD) for 4 h. The cell viabilities were then assayed. The data were mean ± S.D. ($n=10$). $^{*}p<0.05$ versus the OGD.

the protection at low concentrations and the cytotoxicity at high concentrations. DADLE at low concentration (nM) shows high affinity to delta opioid receptor, while at high concentration (μM), it also shows considerable affinity to mu-opioid receptor. Although activation of delta opioid receptor provides neuroprotection against hypoxia induced injury, activation of mu-opioid receptor exacerbates the injury (Hayashi et al., 2002). Thus, the reduction of MTT level by 10 μM DADLE after OGD treatment may result from activation of mu-opioid receptor.

2.3. DOR antagonist Naltrindole abolished the neuroprotective effect of DADLE

To test whether the neuroprotective effect of DADLE was through activation of delta opioid receptor, we use MTT and LDH assays to examine whether DOR antagonist Naltrindole could block DADLE-induced neuroprotection. Naltrindole and DADLE were co-administered to neuronal cultures for 1 h before treated with OGD. MTT and LDH assays revealed that 1 μM Naltrindole could completely abolish DADLE-induced neuroprotection of cortical neurons on OGD model (Fig. 3). Therefore, this dosage of Naltrindole (1 μM) was used in the following experiments.

2.4. DADLE had a reciprocal effect on phosphorylation of ERK and p38

To understand the potential mechanisms involved in the protective effects of DADLE against OGD injury, we investigated the effects of DADLE on the level of total and phosphorylated p38, ERK and JNK. As shown in Fig. 4A, OGD significantly increased the levels of phosphorylated p38 MAPK (pp38) (156 ± 21% versus 100% in normoxia; $p<0.01$).

DADLE blocked OGD-induced upregulation of phospho-P38, while Naltrindole treatment reversed the effect of DADLE on phospho-p38 level.

DADLE induced a significant increase in phospho-ERK levels in neurons during OGD (311 ± 20% in DA + OGD and