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Research report

Carnosine decreased neuronal cell death through targeting glutamate system and astrocyte mitochondrial bioenergetics in cultured neuron/astrocyte exposed to OGD/recovery

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ABSTRACT

Previously, we showed that carnosine upregulated the expression level of glutamate transporter 1 (GLT-1), which has been recognized as an important participant in the astrocyte-neuron lactate shuttle (ANLS), with ischemic model in vitro and in vivo. This study was designed to investigate the protective effect of carnosine on neuron/astrocyte co-cultures exposed to OGD/recovery, and to explore whether the ANLS or any other mechanism contributes to carnosine-induced neuroprotection on neuron/astrocyte. Co-cultures were treated with carnosine and exposed to OGD/recovery. Cell death and the extracellular levels of glutamate and GABA were measured. The mitochondrial respiration and glycolysis were detected by Seahorse Bioscience XF96 Extracellular Flux Analyzer. Results showed that carnosine decreased neuronal cell death, increased extracellular GABA level, and abolished the increase in extracellular glutamate and reversed the mitochondrial energy metabolism disorder induced by OGD/recovery. Carnosine also upregulated the mRNA level of neuronal glutamate transporter EAAC1 at 2 h after OGD. Dihydrokainate, a specific inhibitor of GLT-1, decreased glycolysis but it did not affect mitochondrial respiration of the cells, and it could not reverse the increase in mitochondrial OXPHOS induced by carnosine in the co-cultures. The levels of mRNAs for monocarboxylate transporter1, 4 (MCT1, 4), which were expressed in astrocytes, and MCT2, the main neuronal MCT, were significantly increased at the early stage of recovery. Carnosine only partly reversed the increased expression of astrocytic MCT1 and MCT4. These results suggest that regulating astrocytic energy metabolism and extracellular glutamate and GABA levels but not the ANLS are involved in the carnosine-induced neuroprotection.

1. Introduction

Cerebral ischemia causes severe brain damage and is a leading cause of death and long-term disability worldwide. Disrupted balance between the excitatory and inhibitory neurotransmitters and energy metabolism disorder are two of the hallmarks of ischemic stroke. Glutamate is the most abundant excitatory neurotransmitter in the brain. A high extracellular level of glutamate plays an important role in neuronal death. The extracellular glutamate level mainly depends on the activities of two subtypes of glutamate transporter, GLT-1 and EAAC1, which are localized predominantly in astrocytes and neurons, respectively (Zhao et al., 2012). Besides glutamate excitotoxicity, the extent of reduction of cerebral blood flow also determines the severity of ischemia and results in long lasting abnormality (van der Zijden et al., 2008). Thus, it seems that to reduce glutamate excitotoxicity and promote the recovery of brain energy metabolism may provide a gateway for therapeutic strategies directed at improvement of functional recovery after stroke.

Astrocyte-neuron lactate shuttle (ANLS) hypothesisis is one of the research hotspots in the neuroscience field. This hypothesis maintains that astrocytes are the primary sites of glucose uptake, glycolytic utilization, and export of lactate to neurons especially upon brain activation (Carpenter et al., 2015; Dienel, 2014; Mangia et al., 2011; Pellerin et al., 2007). Several studies also showed

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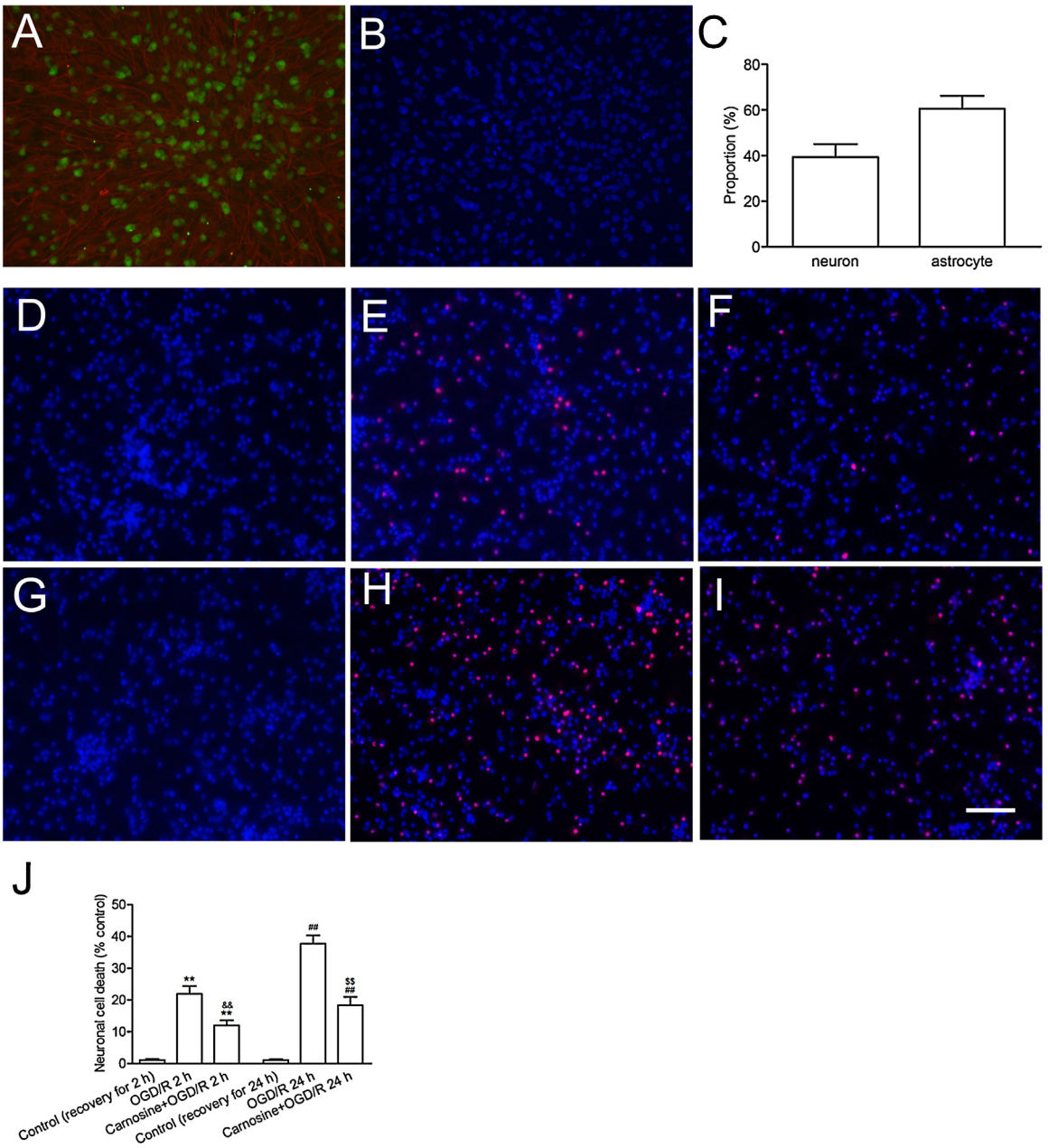

Fig. 1. Carnosine decreased neuronal death in neuron/astrocyte co-culture exposed to OGD/R. (A–C) Proportions of neurons and astrocytes in neuron/astrocyte co-culture by staining with (A) NeuN (green), GFAP (red) and (B) DAPI. (C) Quantitative analysis of proportions of neurons and astrocytes in the co-cultures. (D–J) Neuronal cell death under the influence of OGD/R and carnosine. The cell death was revealed by Hoechst 33342 (blue) and PI (red) staining, (D) control group (recovery for 2 h), (E) OGD/R for 2 h group, (F) carnosine + OGD/R for 2 h group, (G) control group (recovery for 24 h), (He) OGD/R for 24 h group, (I) carnosine + OGD/R for 24 h group, (J) the semi-quanitative results of D–I. Each value is expressed as mean ± S.E.M., n = 6. $^{**}P < 0.01$ *vs.* control (recovery for 2 h) group. $^{\&\&}P < 0.01$ *vs.* OGD/R 2 h group. $^{\#\#}P < 0.01$ *vs.* control (recovery for 24 h) group. $^{\$\$}P < 0.01$ *vs.* OGD/R 24 h group. Scale bar, 100 μm. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

that the ANLS is present in the glutamatergic activated conditions, such as cerebral ischemia: glutamate released in the extrasynaptic space is taken up by astrocytes via GLAST and GLT-1 (Pellerin et al., 2007). Glutamate uptake activates aerobic glycolysis in astrocytes and causes a large increase in cytosolic NADH that normalizes with the conversion of pyruvate into lactate and its release via monocarboxylate transporters (mainly MCT1 and 4) expressed on astrocytes. The released lactate was taken up by neurons via MCT2 and used as an important energy substrate. In addition, several lines of evidence showed that providing lactate immediately after restoring blood supply exerts a significant neuroprotective effect on the ischemic subjects (Baltan, 2015; Erlichman et al., 2008). Thus, it seems that the expression and activity of GLT-1 and GLAST has a closed relationship with astrocyte-neuron energy metabolism and brain function recovery under ischemic condition.

Carnosine (β-alanyl-L-histidine) is a naturally occurring dipeptide. It is mainly distributed in astrocytes in the central nervous system (CNS) in vertebrates. So far, not much is known about its physiological function but several putative roles have been considered, such as neurotransmitter, anti-inflammatory agent, free radical scavenger, mobile organic pH buffer and metal chelator (Bonfanti et al., 1999). Recently, a line of studies demonstrated that carnosine is neuroprotective in cerebral ischemia in mice and rats (Baek et al., 2014; Dobrota et al., 2005; Zhang et al., 2014). Our