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ACETYL-L-CARNITINE RESTORES SYNAPTIC TRANSMISSION AND ENHANCES THE INDUCIBILITY OF STABLE LTP AFTER OXYGEN–GLUCOSE DEPRIVATION

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Abstract—Hypoxic circumstances result in functional and structural impairments of the brain. Oxygen–glucose deprivation (OGD) on hippocampal slices is a technique widely used to investigate the consequences of ischemic stroke and the potential neuroprotective effects of different drugs. Acetyl-L-carnitine (ALC) is a naturally occurring substance in the body, and it can therefore be administered safely even in relatively high doses. In previous experiments, ALC pretreatment proved to be effective against global hypoperfusion. In the present study, we investigated whether ALC can be protective in an OGD model. We are not aware of any earlier study in which the long-term potentiation (LTP) function on hippocampal slices was measured after OGD. Therefore, we set out to determine whether an effective ALC concentration has an effect on synaptic plasticity after OGD in the hippocampal CA1 subfield of rats. A further aim was to investigate the mechanism underlying the protective effect of this compound. The experiments revealed that ALC is neuroprotective against OGD in a dose-dependent manner, which is manifested not only in the regeneration of the impaired synaptic transmission after the OGD, but also in the inducibility and stability of the LTP. In the case of the most effective concentration of ALC (500 μM), use of a phosphoinositide 3-kinase (PI3K) inhibitor (LY294002) revealed that the PI3K/Akt signaling pathway has a key role in the restoration of the synaptic transmission and plasticity reached by ALC treatment. © 2016 IBRO. Published by Elsevier Ltd. All rights reserved.

Key words: oxygen–glucose deprivation, acetyl-L-carnitine, long-term potentiation, PI3K/Akt, neuroprotection, ischemia.

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Abbreviations: aCSF, artificial cerebrospinal fluid; Akt, protein kinase B; ALC, acetyl-l-carnitine; fEPSPs, field excitatory postsynaptic potentials; LTP, long-term potentiation; OGD, oxygen–glucose deprivation; PI3K, phosphoinositide 3-kinase; TBS, theta burst stimulation.

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INTRODUCTION

Cerebral ischemia results in failure of the bioenergetic processes. The key element of this phenomenon is the breakdown of the mitochondria, which leads to the failure of ATP production and the excessive release of Ca^{2+}. It causes not only excitotoxicity, but also the generation of reactive oxygen species, the release of proapoptotic signal proteins and the subsequent neuronal death (Ten and Starkov, 2012). It has been well established that the hippocampus, and especially the CA1 subfield, is extremely sensitive to hypoxic–hypoglycemic conditions (Kirino, 1982; Pulsinelli et al., 1982), which can occur, for example, during ischemic stroke or cardiac arrest. Hippocampal slices are widely used to investigate the injury induced by ischemic events and to measure the effects of different pharmacological interventions against the neuronal damage (Picconi et al., 2006; Molz et al., 2015). Oxygen–glucose deprivation (OGD) mimicking brain ischemia can result in the reversible or irreversible depression of neurotransmission, depending on the duration of this insult. Electrophysiological recordings from slice preparations allow continuous monitoring of the changes in the evoked electrical responses which can occur as a result of the ischemic event or potential neuroprotective agents (Picconi et al., 2006; Nistico et al., 2008). Furthermore, the hippocampus is a suitable and generally used model system for the study of synaptic plasticity, and especially long-term potentiation (LTP), since it is involved in learning and memory (Ho et al., 2011). As a result of the vulnerability to hypoxic–hypoglycemic circumstances of this structure, the effects of ischemia are manifested in functional and morphological damages, e.g. LTP impairment and the loss of dendritic spines (Kocsis et al., 2014), or in irreversible harmful processes ending in cell death.

Acetyl-L-carnitine (ALC) is an endogenous compound, which is a short-chain acetyl ester of L-carnitine (Bremer, 1983; Bieber, 1988) synthesized in the human brain, liver and kidney by the enzyme ALC transferase. This molecule affects the overall energy metabolism and cell functions, including the regulation of the lipid, carbohydrate and protein metabolism (Rapoport, 1999), it boosts mitochondrial ATP production and it protects the mitochondria against oxidative stress (Zanelli et al., 2005). ALC is actively transported across the blood–brain barrier to the brain (Burlina et al., 1989), where it has a relatively high concentration (Shug et al., 1982). Several studies have demonstrated the neuroprotective effect of ALC

against different neurodegenerative diseases, such as Alzheimer's disease, ischemia or neuropathic pain (Di Cesare Mannelli et al., 2009; Zhou et al., 2011; Xu et al., 2015). We previously investigated the neuroprotective effect of ALC against global hypoperfusion in a 2-vessel occlusion (2VO) model (Kocsis et al., 2014, 2015). The effects of ALC were also tested on striatal slices in an *in vitro* ischemia model, where it prevented the loss of the recorded field excitatory postsynaptic potentials (fEPSPs) through the activation of M2 muscarinic receptors and the choline uptake system (Picconi et al., 2006). Moreover, the mechanisms underlying the neuroprotective effect of ALC exhibit a great deal of variety, since it provides a substrate reservoir for cellular energy production, facilitates the uptake of acetyl-CoA into the mitochondria during fatty acid oxidation, enhances different synthesis processes (Fiskum, 2004; Di Cesare Mannelli et al., 2010), has antioxidant and anti-apoptotic properties (Zanelli et al., 2005), and induces nerve regeneration by increasing the production and binding of the nerve growth factor (Foreman et al., 1995).

In the present study, our aim was to examine the potential neuroprotective effect of ALC against *in vitro* global ischemia delivered to hippocampal slices. To the best of our knowledge, there has not been any study so far in which the LTP function was measured on a hippocampal slice after OGD. We therefore set out to determine whether an effective ALC concentration has an effect on synaptic plasticity after OGD in the hippocampal CA1 subfield.

EXPERIMENTAL PROCEDURES

Animals and housing conditions

Male Wistar rats weighing 200–250 g ($N = 21$) supplied by Charles River Laboratories, were kept under constant environmental conditions (23 °C; humidity 55 ± 5%; a 12-h/12-h light/dark cycle) and were housed individually in standard plastic cages, where they had free access to food and water. Every effort was made to minimize the number of animals used and their suffering. The principles of animal care (NIH publication No. 85-23), and the protocol for animal care approved both by the Hungarian Health Committee (1998) and by the European Communities Council Directive (2010/63/EU) were followed. Before the experimental procedures, all the rats were in normal health and had no neurological deficits.

In vitro slice preparation

The preparation and maintenance of rat hippocampal slices were described previously (Kocsis et al., 2014, 2015). Briefly, the animals were decapitated and the middle parts of the hippocampi were placed in ice-cold artificial cerebrospinal fluid (aCSF) composed of (in mM): 130 NaCl, 3.5 KCl, 1 NaH_2PO_4, 24 $NaHCO_3$, 1 $CaCl_2$, 3 $MgSO_4$ and 10 D-glucose (all from Sigma, Schnelldorf, Germany), saturated with 95% O_2 + 5% CO_2. Coronal hippocampal slices (350 μm) were prepared with a vibratome (Leica VT1200S, Wetzlar, Germany). Slices were transferred to a holding chamber and allowed to recover in the solution used for recording (differing only in that it contained 3 mM $CaCl_2$ and 1.5 mM $MgSO_4$) for at least 1 h.

Electrophysiological recordings

For the electrophysiological experiments, slices were transferred to a Haas recording chamber, where the flow rate of the recording solution (34 °C) was 1.5–2 ml × min^{-1}. A bipolar concentric stainless steel electrode (Neuronelektrod Ltd, Budapest, Hungary) was placed in the stratum radiatum between the CA1 and CA2 regions of the hippocampal slices. The stimulus intensity was adjusted to between 30 and 60 μA (constant current, 0.2-ms pulses delivered at 0.05 Hz) to evoke the half-maximum response. fEPSPs were recorded with a 1.5–2.5 MΩ resistance glass micropipette filled with aCSF. The recordings were amplified with a neutralized, high input-impedance preamplifier and filtered (1 Hz–3 kHz). The fEPSPs were digitized (AIF-03, Experimetria Ltd., Budapest, Hungary), acquired at a sampling rate of 10 kHz, saved to a PC and analyzed off-line with Origin Pro 8 software (OriginLab Corporation, Northampton, MA, USA). The fEPSPs were monitored until the amplitudes were generally stable, and a 10-min-long baseline was then recorded, which was followed by a 15-min OGD. The fEPSPs were allowed to recover for 40 min after the OGD, and at the end of this period theta burst stimulation (TBS) was applied (bursts of four impulses at 100 Hz with a burst interval of 350 ms) for LTP measurements. After the TBS, changes in fEPSP amplitudes were recorded for a further 35 min (Fig. 1).

In vitro ischemia

In vitro ischemia was delivered by switching for 15 min to an OGD aCSF solution in which sucrose replaced glucose, and gassed with 95% N_2 + 5% CO_2. After the OGD, the slices were perfused with normal aCSF again until the end of the measurements. In the preliminary experiments, OGD was delivered for different periods (5, 8, 12, 15, 16, and 17 min) in order to determine the appropriate length of the ischemia for our study. After shorter terms of OGD, the fEPSPs returned, but the aim was to find the limit when the fEPSPs no longer displayed recovery. Electrical noise could not be excluded completely during the electrophysiological measurements, and it also was present after the elimination of the fEPSPs. This is the reason why the recorded amplitudes never reached the value of zero. Both fEPSP amplitudes and initial slopes were recorded and quantified in all of the measurements; however as no appreciable differences between these two parameters were observed, only the amplitudes are expressed in the figures.

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