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ACETYL-L-CARNITINE NORMALIZES THE IMPAIRED LONG-TERM POTENTIATION AND SPINE DENSITY IN A RAT MODEL OF GLOBAL ISCHEMIA

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Abstract—As a consequence of an ischemic episode, energy production is disturbed, leading to neuronal cell death. Despite intensive research, the quest for promising neuroprotective drugs has largely failed, not only because of ineffectiveness, but also because of serious side-effects and dosing difficulties. Acetyl-L-carnitine (ALC) is an essential nutrient which plays a key role in energy metabolism by transporting fatty acids into mitochondria for β-oxidation. It is an endogenous compound and can be used at high dose without toxicity in research into ischemia. Its neuroprotective properties have been reported in many studies, but its potential action on long-term potentiation (LTP) and dendritic spine density has not been described to date. The aim of the present study was an evaluation of the possible protective effect of ALC after ischemic insults inflicted on hippocampal synaptic plasticity in a 2-vessel occlusion (2VO) model in rats. For electrophysiological measurements, LTP was tested on hippocampal slices. The Golgi-Cox staining technique was used to determine spine density. 2VO resulted in a decreased, unstable LTP and a significant loss of dendritic spines. ALC administered after 2VO was not protective, but as pretreatment prior to 2VO it restored LTP nearly to the control level. This finding paralleled the histological analysis: ALC pretreatment resulted in the reappearance of dendritic spines on the CA1 pyramidal cells. Our data demonstrate that ALC administration can restore hippocampal function and spine density. ALC probably acts by enhancing the aerobic metabolic pathway, which is inhibited during and following ischemic attacks.

Key words: brain ischemia, hippocampus, acetyl-L-carnitine, LTP, dendritic spine.

INTRODUCTION

Although ischemic stroke is a leading cause of death (Lozano et al., 2012), there is at present hardly any effective therapy for patients. The application of intravenous recombinant tissue plasminogen activator, which is the only neuroprotective intervention against stroke (Adams et al., 2007), is limited because victims do not meet the entry criteria of pivotal clinical trials (because of severe hypertension, an age above 80, a history of previous stroke, etc.) or they often reach the Emergency Department too late (Alderazi et al., 2012). Ischemic stroke also results in lifelong disabilities, which is due to neuronal cell death, a consequence of excitotoxicity, mitochondrial dysfunction and disturbed energy production (Beal, 1992).

The quest for neuroprotective drugs for humans has largely been a failure, not only because of ineffectiveness, but also because of severe side-effects or dosing difficulties. Researchers have therefore started to focus on human endogenous compounds which can be administered at higher doses without toxicity.

L-Carnitine (trimethylamino-β-hydroxybutyrate), an essential nutrient in all mammalian species, exists either as free carnitine or as acylcarnitines. The acyl groups can vary considerably in length, as illustrated by the shorter acyl chain in acetyl-L-carnitine (ALC) and the longer chain in palmitoylcarnitine. The primary function of carnitine and ALC is the transportation of long- and medium-chain fatty acids into mitochondria for β-oxidation (Virmani and Binienda, 2004). Exogenous carnitine derivatives contribute to the modulation of cholinergic neurotransmission and the nerve growth factor, to the improvement of the mitochondrial energetics and antioxidant activity, and to the modulation of protein and gene expression (reviewed by Jones et al., 2010). ALC can be synthesized in the brain by carnitine acetyltransferase (Bird et al., 1985), and can readily cross the blood–brain barrier in a Na^+-dependent manner through the organic cation/carnitine transporter (Inano et al., 2003), which offers promising therapeutic applications.

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Abbreviations: 2VO, 2-vessel occlusion; aCSF, artificial cerebrospinal fluid; ALC, acetyl-L-carnitine; CCAs, common carotid arteries; fEPSPs, field excitatory postsynaptic potentials; LTP, long-term potentiation; TBS, theta burst stimulation.

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ALC has been shown to exhibit neuroprotective effects after cerebral ischemia in animal models, and also in cultured neurons and on brain slice preparations. ALC treatment prior to transient forebrain ischemia prevented neuronal damage in the cortex, hippocampus and striatum of gerbils (Shuaib et al., 1995). ALC pretreatment was also found to reduce infarct size significantly after middle cerebral artery occlusion in rats (Jalal et al., 2010). In an electrophysiological study, ALC proved to protect striatal neurons against *in vitro* ischemia involving oxygen-glucose deprivation (Picconi et al., 2006).

Clinical studies have demonstrated the positive effects of ALC too. It enhanced cerebral blood flow (Postiglione et al., 1991), and improved energy levels, general functioning and well-being of patients (Malaguarnera et al., 2011).

The effects of ALC administration on focal *in vivo* and global *in vivo* and *in vitro* models have been investigated (Rosenthal et al., 1992; Shuaib et al., 1995; Picconi et al., 2006; Jalal et al., 2010; Zhang et al., 2012), but there have not yet been any reports of its effects on hippocampal long-term potentiation (LTP). We recently demonstrated a severe consequence of global hypoperfusion on the hippocampal LTP function, which can be detected by means of electrophysiological measurements and Golgi-Cox staining (Nagy et al., 2011). These methods are suitable for the investigation into the effects of potential neuroprotective agents on synaptic plasticity. We set out to determine whether ALC has a neuroprotective effect on synaptic plasticity after global hypoperfusion, and whether it influences dendritic spine genesis in the CA1 subfield of the hippocampus.

EXPERIMENTAL PROCEDURES

Animals and housing conditions

Male Wistar rats weighing 220 ± 20 g (N = 45) supplied by Charles River Laboratories, were kept under constant environmental conditions (23 °C; humidity 55 ± 5%; 12-h/12-h light/dark cycle) and were housed individually in standard plastic cages. Before and after all experimental procedures, all animals 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 (86/609/EEC) were followed. Before the experimental procedures, all the rats were in normal health and had no neurological deficits. The animals were randomly divided into six groups, as follows: sham-operated controls (N = 9); animals that underwent 2-vessel occlusion (2VO) (N = 10); a 2VO + ALC post-treatment group (N = 10); an ALC pretreatment + 2VO group (N = 10); an ALC pretreatment sham-operated group (N = 3); and a sham-operated ALC post-treatment group (N = 3).

Transient 2-vessel occlusion

Transient global hypoperfusion was effected by 2VO, described previously (Marosi et al., 2009). Anesthesia was induced with sodium pentobarbital (60 mg/ml; i.p.), and the body temperature of the animals was maintained at 37 ± 0.5 °C by means of an automatic heat controller (Supertech TMP-5a, Hungary). Through a midline neck incision, the common carotid arteries (CCAs) were dissected and exposed. To induce transient forebrain ischemia, the CCAs were clamped for 30-min with non-traumatic clips (Aesculap, B. Braun Medical Ltd., Hungary). After the 30-min period, the clips were released from the CCAs and blood flow was restarted. In the sham-operated groups, the CCAs were exposed without clamping.

Drug administration in the experimental groups

The effects of ALC (Sigma, Germany) on 2VO-operated and on sham-operated animals were measured pretreatment and post-treatment. ALC was dissolved in 0.9% saline (total volume 1 ml) and was administered i.p. The animals received the treatment once a day for 5 days either before (pretreatment) or after (post-treatment) the 2VO/sham operation. The animals in the pretreated and pretreated sham-operated groups received the last ALC injection 1 day before the 2VO or sham operation. The first ALC treatment was applied 1 h after the surgical intervention in the post-treated and post-treated sham-operated groups.

In vitro electrophysiology

The animals were decapitated 5 days after ischemic insult. 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, Germany), saturated with 95% O_2 + 5% CO_2. Coronal hippocampal slices (350 μm) were prepared with a vibratome (Campden Instruments, UK), transferred to a Haas recording chamber and incubated at room temperature for 1 h to allow the slices to recover in the solution used for recording (differing only in that it contained 3 mM $CaCl_2$ and 1.5 mM $MgSO_4$). The flow rate of the recording solution was 1.5–2 ml min^{-1} and the experiments were performed at a controlled chamber temperature of 34 °C.

For the electrophysiological experiments, a bipolar concentric stainless steel electrode (Neuronelektrod Ltd., 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. Field excitatory postsynaptic potentials (fEPSPs) were recorded with a 1.5–2-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., Hungary), acquired at a sampling rate of 10 kHz, saved to a PC and analyzed off-line with Origin 6.0 software (OriginLab Corporation, Northampton, USA). The fEPSPs were monitored until the amplitudes were generally stable, and a 10-min-long