Available online at www.sciencedirect.com
SCIENCE DIRECT
Neuropharmacology 50 (2006) 917–923
NEURO PHARMACOLOGY
www.elsevier.com/locate/neuropharm

Acetyl-L-carnitine protects striatal neurons against in vitro ischemia: The role of endogenous acetylcholine

Barbara Picconi [a], Ilaria Barone [a], Antonio Pisani [b], Raffaella Nicolai [c], Paola Benatti [c], Giorgio Bernardi [a,b], Menotti Calvani [c], Paolo Calabresi [a,d,*]

[a] *Laboratorio di Neurofisiologia, Fondazione Santa Lucia, IRCCS, Rome, Italy*
[b] *Clinica Neurologica, Dip. Neuroscienze, Università di Roma ''Tor Vergata'', Rome, Italy*
[c] *Sigma-tau Industrie Farmaceutiche Riunite S.p.A., Pomezia, Rome, Italy*
[d] *Clinica Neurologica, Dip. Specialità Medico Chirurgiche e Sanità Pubblica, Università di Perugia, Ospedale Silvestrini, S. Andrea delle Fratte, 06156 Perugia, Italy*

Received 18 October 2005; received in revised form 3 January 2006; accepted 4 January 2006

Abstract

The neuronal death after ischemia is closely linked to the essential role of mitochondrial metabolism. Inhibition of mitochondrial respiratory chain reduces ATP generation leading to a dysregulation of ion metabolism. Acetyl-L-carnitine (ALC) influences the maintenance of key mitochondrial proteins for maximum energy production and it may play a neuroprotective role in some pathological conditions. In this study we have analyzed ALC-mediated neuroprotection on an in vitro model of brain ischemia. Field potential recordings were obtained from a rat corticostriatal slice preparation. In vitro ischemia (oxygen and glucose deprivation) was delivered by switching to a solution in which glucose was omitted and oxygen was replaced with N_2. Ten minutes of in vitro ischemia caused an irreversible loss of the field potential amplitude. Pretreatment with ALC produced a progressive and dose-dependent recovery of the field potential amplitude following in vitro ischemia. The neuroprotective effect of ALC was stereospecific since the pretreatment with two different carnitine-related compounds did not cause neuroprotection. The choline transporter inhibitor hemicholinium-3 blocked the neuroprotective effect of ALC. ALC-mediated neuroprotection was also prevented either by the non-selective muscarinic antagonist scopolamine, or by the putative M2-like receptor antagonist methoctramine. Conversely, the effect of ALC was not altered by the M1-like receptor antagonist pirenzepine. These findings show that ALC exert a neuroprotective action against in vitro ischemia. This neuroprotective effect requires the activity of choline uptake system and the activation of M2 muscarinic receptors.

Keywords: Ischemia; Electrophysiology; Mitochondrial metabolism; Neuroprotection; Field potentials; Striatum

1. Introduction

The neuronal death after ischemia is closely linked to the essential role of mitochondrial metabolism. Cerebral ischemia leads to mitochondrial dysfunction due to lack of oxygen, leaving the glycolytic metabolism as a main pathway for ATP production. Inhibition of mitochondrial respiratory chain triggers generation of lactate and hydrogen ions and dramatically reduces ATP generation leading to a dysregulation of cellular ion metabolism with subsequent intracellular calcium accumulation (Beal, 1992; Duchen, 2004; Greene and Greenamyre, 1996; Kristian, 2004).

Carnitine and its acetyl ester, acetyl-L-carnitine (ALC), plays an essential regulatory role in fatty acid oxidation and ALC also influences the maintenance of key mitochondrial proteins for maximum energy production (Bieber, 1988;

* Corresponding author. Clinica Neurologica, Dip. Specialità Medico Chirurgiche e Sanità Pubblica, Università di Perugia, Ospedale Silvestrini, S. Andrea delle Fratte, 06156 Perugia, Italy. Tel.: +39 075 578 4230; fax: +39 075 578 4229.
E-mail address: calabre@unipg.it (P. Calabresi).

0028-3908/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.neuropharm.2006.01.002

Dhitavat et al., 2002; Fariello et al., 1988; Liu et al., 2002b). ALC and its synthesizing enzyme, carnitine-acetyl-transferase, are widely distributed in the mammalian brain (Shug et al., 1982). In the brain ALC is involved in the regulation of carbohydrate, lipid, and protein metabolism (Rapoport, 1999). Exogenous ALC is actively transported through the blood-brain barrier and accumulates in the brain (Burlina et al., 1989). Administration of ALC increases high-affinity choline uptake, acetylcholine (ACh) synthesis and ACh release in synaptosomes preparation and in the striatum and hippocampus of freely moving rats (Ando et al., 2001; Imperato et al., 1989).

Several studies have suggested that ALC may play a neuroprotective role in some pathological conditions of the brain. ALC has been reported to restore age-related mitochondrial deficits (Hagen et al., 1998; Liu et al., 2002b; Paradies et al., 1994), to delay cognitive decline in Alzheimer's disease (Dhitavat et al., 2002; Spagnoli et al., 1991), and to attenuate the neurological damage following brain ischemia and reperfusion (Aureli et al., 1994; Calvani and Arrigoni-Martelli, 1999; Lolic et al., 1997; Rosenthal et al., 1992; Shuaib et al., 1995). However, the mechanisms underlying these neuroprotective effects have never been characterized at cellular and electrophysiological level.

To characterize these neuroprotective effects we focused our study on the functional activity of striatal neurons. The striatum, in fact, is a brain structure particularly vulnerable to ischemic insult, and its neuronal damage is expressed as an alteration of both intrinsic membrane properties and synaptic function of the recorded striatal neurons (Calabresi et al., 1998, 2002, 2003a,b).

The aim of the present study is to analyze the neuroprotective effects of ALC on a well-established in vitro model of ischemia (Calabresi et al., 2000a,c, 2002) and to examine its cellular site of action by using electrophysiological recordings from corticostriatal rat slices.

2. Materials and methods

2.1. In vitro slice preparation

Preparation and maintenance of rat corticostriatal slices have been previously described (Calabresi et al., 1995, 2000c, 2003a,c; Costa et al., 2004). Briefly, corticostriatal coronal slices were prepared from 2-month-old Wistar rats (thickness, 270 μm/L). All the experiments were conducted in conformity with the European Communities Council Directive (86/609/ECC). Slices were kept in artificial Krebs, whose composition was as follows (in mmol/L): 126 NaCl, 2.5 KCl, 1.2 $MgCl_2$, 1.2 NaH_2PO_4, 2.4 $CaCl_2$, 11 glucose, and 25 $NaHCO_3$. Artificial cerebrospinal fluid (Krebs) temperature was maintained at 33–34 °C and was gassed with 95% O_2 and 5% CO_2. In vitro ischemia was delivered by switching for 10 min to an artificial Krebs solution in which sucrose replaced glucose, gassed with 95% N_2 and 5% CO_2. Ischemic and drug-containing solutions entered the recording chamber no later that 30 s after a 3-way tap was turned.

2.2. Electrophysiological recordings

Electrophysiological electrodes for extracellular recording (15–20 MΩ) were filled with 2 mol/L NaCl. The field potential amplitude was defined as the average of the amplitude from the peak of the early positivity to the peak negativity and the amplitude from the peak negativity to peak late positivity (Costa et al., 2004). Quantitative data on modifications induced by ischemia are expressed as a percentage of the control values, the latter representing the mean of responses recorded during a stable period (15–20 min) before the ischemic phase (Costa et al., 2004).

Field potential recordings were obtained with an Axon Instruments Axoclamp 2B amplifier and controlled by a computer running pClamp (v. 9) with a DigiData 1322A interface (Axon Instruments, Foster City, CA).

2.3. Statistical methods

For data presented as mean ± S.E.M., statistical analysis was performed with the use of Student's *t*-test for unpaired data. The significance level was established at $P < 0.05$.

2.4. Drugs

Drugs were applied by dissolving them to the desired final concentration in saline solution. Hemicolinium-3 (HC-3), methoctramine, pirenzepine and scopolamine were from Sigma (Italy). Acetyl-L-carnitine (ALC), acetyl-D-carnitine (ADC), and L-carnitine were kindly donated by Sigma-tau, Pomezia, Rome, Italy.

3. Results

3.1. Neuroprotective effect of acetyl-L-carnitine during in vitro ischemia

In corticostriatal slices field potentials of 1.08 ± 0.26 mV in amplitude and 1.93 ± 0.86 ms in duration were obtained following electrical stimulation of the white matter between cortex and striatum. After 10–15 min of stable baseline recording, ischemia was delivered for 10 min. As previously described a brief period (5 min) of in vitro ischemia produced a reversible inhibition of the field potential amplitude (data not shown) (Calabresi et al., 2000c, 2003c; Costa et al., 2004). Conversely, 10 min of ischemia progressively reduced field potential amplitude until their complete and irreversible suppression ($P < 0.001$ pre vs. post ischemia, $n = 16$; Fig. 1A, open circles).

Preincubation of the slices with increasing doses of ALC produced a progressive recovery of the field potential amplitude beginning from 10 μmol/L to 100 μmol/L doses. ALC had the maximal neuroprotective effect at 100 μmol/L ($P < 0.001$ compared to control at 30 min after ischemia, $n = 15$; Fig. 1A–C, filled circles). As shown in Fig. 1B, the EC_{50} of the neuroprotective effect of ALC was 11 μmol/L. Conversely, when ALC was administered for a period of 30 min following the ischemic insult it was unable to induce the recovery of the field potential ($P < 0.001$ pre vs. post ischemia, $n = 6$; Fig. 2A). ALC did not produce per se any significant change of the field potential amplitude ($n = 16$, data not shown).

The neuroprotective effect of ALC was stereospecific, in fact, the administration of two different carnitine-related compounds such as ADC, and L-carnitine did not produce any recovery of field potential amplitude following in vitro ischemia (ns, $P > 0.05$ compared to control at 30 min after ischemia, $n = 5$ for each experimental group; Fig. 2B).