Neuroscience Letters 636 (2017) 196–204

Contents lists available at ScienceDirect

Neuroscience Letters

journal homepage: www.elsevier.com/locate/neulet

Research article

In vitro detection of oxygen and glucose deprivation-induced neurodegeneration and pharmacological neuroprotection based on hippocampal *stratum pyramidale* width

Pınar Öz [a,b], Hale Saybaşılı [b,*]

[a] *Neuropsychopharmacology Application and Research Center, Üsküdar University Central Campus, Altunizade Mah. Haluk Türksoy Sk. No:14 34662, Istanbul, Turkey*
[b] *Institute of Biomedical Engineering, Boğaziçi Üniversity Kandilli Campus, Kandilli Mah., 34684 Istanbul, Turkey*

HIGHLIGHTS

- CA1 stratum pyramidale width measurements display the level of neurodegeneration.
- (L)-Carnitine is protective against ischemia-related neurodegeneration.
- Hypothermia is as effective as memantine, baclofen and L-carnitine against ischemia.

ARTICLE INFO

Article history:
Received 14 September 2016
Received in revised form 23 October 2016
Accepted 10 November 2016
Available online 11 November 2016

Keywords:
Ischemia
Neurodegeneration
Memantine
Baclofen
L-Carnitine
Hypothermia

ABSTRACT

Ischemia is one of the most prominent risk factors of neurodegenerative diseases such as Alzheimer's disease. The effects of oxygen and glucose depletion in hippocampal tissue due to ischemia can be mimicked *in vitro* using the oxygen and glucose deprivation (OGD) model. In this study, we applied OGD on acute rat hippocampal slices in order to design an elementary yet quantitative histological technique that compares the neuroprotective effects of (L)-carnitine to known neuroprotectors, such as the *N*-methyl-D-aspartate (NMDA) receptor antagonist memantine and the gamma-aminobutyric acid (GABA)-B receptor agonist baclofen. The level of neurodegeneration and the efficiency of pharmacological applications were estimated *via stratum pyramidale* width measurements in CA1 and CA3 regions of Nissl-stained 200-μm thick hippocampal slices. We demonstrated that (L)-carnitine is an effective pharmacological target against the neurodegeneration induced by *in vitro* ischemia in a narrow range of concentrations. Even though the effect of chemical neuroprotection was significant, full recovery was not achieved in the dose interval of 5–100 μM. In addition to chemical applications, hypothermia was used as a physical neuroprotection against ischemia-related neurodegeneration. Our results showed that incubation of slices for 60 min at 4 °C provided the same level of neuroprotection as the most effective doses of memantine, baclofen, and (L)-carnitine.

© 2016 Elsevier Ireland Ltd. All rights reserved.

1. Introduction

Ischemia-related neurodegeneration contributes to the neuronal cell and functional losses that occur in many neurodegenerative diseases such as Alzheimer's disease. Oxygen and glucose deprivation (OGD) can trigger neuronal apoptotic cascades within minutes due to the decrease of cellular adenosine triphosphate (ATP) levels, the release of excitatory amino acids, and the hyperexcitability collapse of the intracellular ion gradients that are caused by sustained depolarization [1]. In the CNS, hippocampus exhibits the characteristic neuropathological markers of ischemic and Alzheimer's brain and is one of the brain regions with most severe neurodegeneration under ischemic conditions [2–9]. Its implication in learning and memory processes and the differential vulnerability of subregions under ischemic conditions lends the hippocampus a central position in studies focusing on developing neuropharmacological targets against ischemic neurodegeneration.

Hippocampal subregions are known to be differentially vulnerable to ischemia-related neurodegeneration. The cause of

* Corresponding author.
E-mail address: saybasil@boun.edu.tr (H. Saybaşılı).

http://dx.doi.org/10.1016/j.neulet.2016.11.027
0304-3940/© 2016 Elsevier Ireland Ltd. All rights reserved.

selective vulnerability of CA1of the hippocampus, compared to CA3 and the dentate gyrus, has been suggested to be due to differential phospholipid distribution and/or metabolism [2,3], the age of the animal [4], insulin signaling [5], high expression of neurodegeneration-related genes in the CA1 [6], developmental decreases of astrocytic processes in the CA1 [7], and selective dysfunction of CA1 astrocytes [8]. Another important mechanism that may underlie this difference in vulnerability may be differential *N*-methyl-D-aspartate (NMDA)-receptor-dependent calcium loading and calcium-overload-dependent mitochondrial dysfunction in the CA1 compared to the CA3 [9]. Previous studies have indicated that NMDA-related excitotoxicity coupled with the hypoxic-ischemic neuronal injury may exacerbate damage [10]. In support of this, uncompetitive NMDA receptor antagonists, such as memantine, have been shown to be effective against OGD-related neuronal damage both *in vitro* [11–16] and *in vivo* [14,17].

In addition to excitatory coupling with OGD, inhibitory cascades may also be involved. OGD affects gamma-aminobutyric acid (GABA)-B receptor activity by attenuating expression of the GABA-B2 subunit in hippocampal tissue [18]. Treatment with baclofen, a GABA-B receptor agonist, attenuated cell death in the CA1 after OGD *in vitro* [18,19], and this effect was significant for a large dose range, both for CA1 and CA3 [18]. The neuroprotective effect of baclofen against NMDA-induced excitotoxicity occurs through the down-regulation of NMDA receptor function by increasing the tyrosine phosphorylation of the NR2A subunit [19]; this effect has been observed with baclofen treatment both before and during NMDA incubation [18]. Additionally, GABA-A receptor activation by an agonist such as muscimol has been shown to decrease NMDA-related excitotoxicity, however, it also increased neuronal death induced by OGD [20].

Exploration of the use of (L)-carnitine, a nutritional supplement, as a neuroprotective agent against OGD-related damage is rather recent. The neuroprotective effect of (L)-carnitine against OGD-induced neurodegeneration has been shown in both *in vitro* and *in vivo* conditions [21–25]. However, the molecular mechanism of this effect has yet to be fully discovered. One possible mechanism has been suggested to be through the regulation of key enzymes in carnitine homeostasis. For example, *in vitro* OGD has been shown to alter carnitine homeostasis in rat hippocampal slice cultures [24], and supplementing (L)-carnitine in the culture resulted in preserved structural integrity and synaptic transmission in these slices.

In the current study, we designed an *in vitro* ischemia model by applying OGD on thick acute hippocampal slices to provide insight on neurodegeneration and pharmacological protection based on hippocampal subregion morphology. Our results support the known neuroprotective effects of memantine, baclofen, and (L)-carnitine, and unveil their effects on CA1 morphology. In addition to chemical treatments, we also showed that hypothermia might provide sufficient protection against OGD-related neurodegeneration on hippocampal slices.

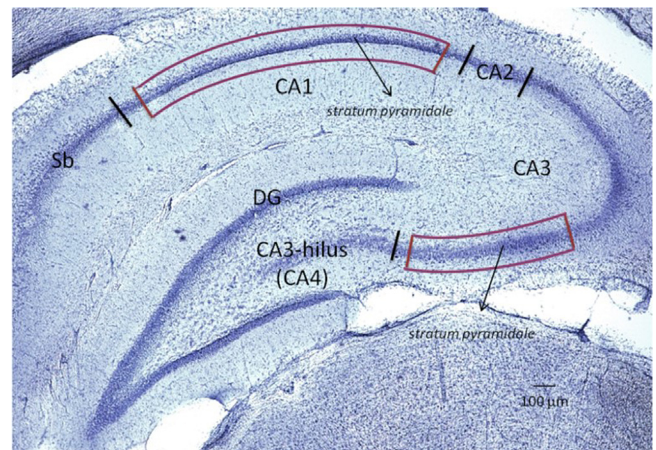

Fig. 1. Selected regional borders of CA1 and CA3 for histological analysis. For details, please refer to the text.

2. Materials and methods

2.1. Experimental design

The experiment was carried out on 22 healthy adult male Wistar rats (25–35 days old). Animals were held at the animal facility in a cage with a 12/12 light-dark cycle, controlled temperature and humidity, with access to food and water ad libitum. The Boğaziçi University Animal Experiments Local Ethical Committee in İstanbul, Turkey approved this experimental study. The maximum number of acute hippocampal slices that could be obtained from a single animal was 10. The slices in each group were obtained from 2 to 3 animals, with 2–3 slices from each animal.

The study consists of six experiment sets. (1) The relation between the incubation duration and the hippocampal subregion morphology was observed by incubating freshly obtained slices for 15 min, 30 min, or 60 min in artificial cerebrospinal fluid (ACSF). (2) The relation between the duration of OGD and the severity of neurodegeneration was analyzed in four experimental groups with 5 min, 15 min, 30 min, or 60 min incubation in OGD medium together with a control group incubated in ACSF for 60 min. (3,4,5) The chemical neuroprotection by baclofen, memantine, and L-carnitine against OGD-related neurodegeneration was analyzed in three experiment sets as explained in detail in the following sections (see Material and Methods 2.2 and Results 3.1–3.6). All groups were compared to the respective control groups and 60 min OGD-ACSF incubation. (6) The physical neuroprotection by hypothermia against OGD-related neurodegeneration was analyzed by 60 min incubation in OGD-ACSF at 4 °C. The control group was incubated in ACSF for 60 min at room temperature. The details of the experiments are given in detail in the following sections.

2.2. Brain slice preparation and incubation

Acute hippocampal brain slices were obtained using a Vibroslice (Campden Instruments, London, UK). After the rats were decapitated, their brains were quickly removed and placed into ice-cold ACSF (125 mM NaCl, 3.75 mM KCl, 1.2 mM NaH_2PO_4, 1.3 mM $MgCl_2{\cdot}6H_2O$, 2 mM $CaCl_2$, 10 mM Glucose, 26 mM $NaHCO_3$) saturated with carbogen and were immediately transferred to an incubation chamber that contained carbogen aerated ACSF at room temperature.

2.2.1. Oxygen and glucose deprivation model

To model an ischemic environment *in vitro*, OGD-ACSF solution was prepared without glucose and aeration, both for the preparation and for the incubation steps. To test the neurodegenerative effect of OGD, hippocampal slices were placed in the incubation chambers that contained OGD-ACSF for 5 min, 15 min, 30 min or 60 min following a 60 min ACSF incubation. The control group was incubated in ACSF for 60 min.

2.2.2. Pharmacological treatments

Baclofen (Sigma-Aldrich, St. Louis, MO, USA), Memantine-HCl (Sigma-Aldrich, St. Louis, MO, USA), and (L)-carnitine-HCl (Sigma-Aldrich, St. Louis, MO, USA) were used as neuroprotective agents against the neurodegenerative effects of OGD. For this purpose,

Download English Version:

https://daneshyari.com/en/article/5738740

Download Persian Version:

https://daneshyari.com/article/5738740

Daneshyari.com