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Neuropharmacology 49 (2005) 703–714
NEURO PHARMACOLOGY
www.elsevier.com/locate/neuropharm

Down-regulation of the glial glutamate transporter GLT-1 in rat hippocampus and striatum and its modulation by a group III metabotropic glutamate receptor antagonist following transient global forebrain ischemia

Jin-Chung Chen [a,*], Hong Hsu-Chou [a], Jin-Lan Lu [a], Yao-Chang Chiang [a], Hwa-Ming Huang [a], Hung-Li Wang [b], Tony Wu [c], Jui-Jung Liao [c], Tu-Shai Yeh [c]

[a] *Department of Pharmacology, Chang-Gung University, 259 Wen-Hwa 1st Road, Tao-Yuan, Kwei-Shan, Taiwan, ROC*
[b] *Department of Physiology, Chang-Gung University, 259 Wen-Hwa 1st Road, Tao-Yuan, Kwei-Shan, Taiwan, ROC*
[c] *Department of Neurology, Chang-Gung Memorial Hospital, Lin-Kao, Taiwan, ROC*

Accepted 6 May 2005

Abstract

Our goals were to identify biochemical markers for transient global ischemia-induced delayed neuronal death and test possible drug therapies against this neuronal damage. Four-vessel occlusion (4-VO) for 20 min was used as a rat model. The temporal expression profiles of three glutamate transporters (GLT-1, GLAST and EAAC1) were evaluated in the CA1 region of the hippocampus and the striatum. The protein levels of the GLT-1 were significantly down-regulated between 3 and 6 h after ischemia–reperfusion in the CA1 region and striatum, returned to the control (2-VO) levels 24 h after reperfusion and remained unchanged for up to 7 days. The levels of GLAST in the CA1 region and striatum, and EAAC1 in the CA1 region did not change after ischemia from 1 h to 7 days. Pretreatment with group III metabotropic glutamate receptor antagonist s-α-MCPA (20 μg/5 μl) 30 min prior to 4-VO significantly restored the GLT-1 levels in the CA1 region caused by global ischemia at both 3 and 6 h after reperfusion. The loss of pyramidal neurons in the CA1 region due to ischemia–reperfusion could also be prevented by intraventricular pretreatment with s-α-MCPA. The current findings pinpoint the significance of GLT-1 during ischemia/reperfusion and suggest a potential application of group III metabotropic glutamate receptor antagonist against ischemic/hypoxic neuronal damage.

Keywords: Transient global ischemia; Delayed neuronal death; Hippocampal CA1; Striatum; GLT-1; Metabotropic glutamate receptor

1. Introduction

Hypoxia or ischemia (global or focal) results in extensive and progressive brain dysfunction, particularly in the hippocampus, striatum and prefrontal cortex (Choi, 1994). Animal models of transient ischemic insult (with carotid and/or vertebral artery occlusion for 5–20 min) in rats or gerbils mimic human pathological condition, notably a significant delayed neuronal death occurs in the hippocampus after 3–7 days reperfusion (Jorgensen and Diemer, 1982; Walton et al., 2000). Although molecular mechanisms underlying the pathogenesis of delayed neuronal death are not yet understood, excitatory amino acids (glutamate and aspartate)-mediated excitotoxicity is thought to be essential (Banasiak et al., 2000; Siesjo et al., 1995). Transient ischemia leads to an immediate glutamate and

* Corresponding author. Tel.: +11 886 3 3283016x5282; fax: +11 886 3 3283031.
E-mail address: jinchen@mail.cgu.edu.tw (J.-C. Chen).

0028-3908/$ - see front matter © 2005 Published by Elsevier Ltd.
doi:10.1016/j.neuropharm.2005.05.002

aspartate efflux from the hippocampus after reperfusion, consequently over-stimulates the postsynaptic glutamate receptors, notably the NMDA and AMPA subtypes (Choi, 1994; Alicke and Schwartz-Bloom, 1995). Pharmacological manipulations with glutamate receptor antagonists were found to decrease neuronal death after global ischemia (Ishimaru et al., 1996, 1997). Since enhanced glutamate release subsides within an hour after the ischemia–reperfusion, it is plausible that other neuronal event(s) might be involved in hippocampal delayed neuronal death observed 3–7 days after ischemia (Pettmann and Henderson, 1998).

Extracellular levels of glutamate are controlled mainly by the non-vesicular glutamate transporters. Chronic inhibition of the glutamate transporters would lead to a slower clearance of synaptic glutamate and promotes postsynaptic neuronal damage (Nicholls and Attwell, 1990; Rothstein et al., 1993). To date, five sodium and potassium-dependent glutamate transporters have been cloned: GLT-1 (Pines et al., 1992), GLAST (Storck et al., 1992), EAAC1 (Kanai and Hediger, 1997), EAAT4 (Fairman et al., 1995) and EAAT5 (Arriza et al., 1994). In particular, GLT-1 and GLAST are expressed in astrocyte but play a crucial role in eliminating extracellular glutamate to protect neurons from glutamate excitotoxicity (Gegelashvili and Schousboe, 1997). Knockout of the glial transporter GLT-1 and GLAST, but not the neuronal transporter EAAC1, would lead to increased extracellular levels of glutamate and thus, cause profound neuronal damage (Tanaka et al., 1997). The finding ischemia that glial GLT-1 plays a role in the pathophysiology of neurodegenerative disorder amyotrophic lateral sclerosis (Fujita et al., 1999; Rao et al., 2000) implicates the significance of GLT-1 in excitatory amino acids-associated brain disorder(s).

Other than impact of uptake regulation, treatments with glutamate receptor antagonist against stroke or ischemia have been attempted (Allen et al., 1999; Bordi and Ugolini, 1999). Due to the profound side effects of ionotropic glutamate antagonist reported in the animal research, compounds that target on G-protein coupled metabotropic glutamate receptors (mGluRs) are considered to be an alternative approach against ischemia-induced neuronal damage (Olney, 1994). At present, eight subtypes of mGluRs with different splice isoforms are known, which can be subdivided into three groups based on sequence homology and second messenger systems (Pin and Duvoisin, 1995). In general, metabotropic group I (mGluR1 and 5) was known to increase phosphoinositide hydrolysis and calcium influx (Bordi and Ugolini, 1999), while group II (mGluR2 and 3) and group III receptors (mGluR4, 6, 7 and 8) are coupled negatively with adenylyl cyclase (Conn and Pin, 1997). Data on changes of mGluR subtype expression after transient global ischemia (Iversen et al., 1994; Sommer et al., 2000) implicate a potential role of particular subtypes responsible for neuronal death or survival of hippocampal CA1 neurons. It is in general believed that activation of presynaptic mGluRs has neuroprotective effect due to its ability to inhibit glutamate release (Nakanishi, 1994). On the other hand, drugs turn on postsynaptic mGluRs would evoke neurotoxicity. Due to a lack of selective drugs to differentiate specific mGluR subtypes, clinical application of mGluRs in stroke or hypoxia is limited.

The purpose of this study is to evaluate first, whether expression of glutamate transporters alters in the rat brain following transient global ischemia. The results indicate protein levels of GLT-1, but not GLAST and EAAC1, decreased after 3–6 h reperfusion in both hippocampal CA1 region and striatum. To test if manipulations of mGluRs would affect the levels of GLT-1 after ischemia–reperfusion, we treated the animals with various mGluR antagonists (groups I, II and III) individually and examined the amount of GLT-1 in hippocampal CA1 at various reperfusion times. We found both groups I and III mGluR antagonist (L-AP3 and s-α-MCPA, respectively) could restore the levels of GLT-1 in the hippocampal CA1 region 6 h after ischemia–reperfusion. Pretreatment of s-α-MCPA also prevents the delayed neuronal death in the hippocampal pyramidal layer, as accessed by Nissl stained brain sections.

2. Materials and methods

2.1. Subjects

Male Sprague–Dawley rats weighing 300–350 g (National Animal Breeding Center, Academic Sinica, Taipei, Taiwan) were used for the experiments. After arrival, they were acclimatized to a room with controlled ambient temperature (22 ± 2 °C), humidity (50 ± 10%) and a 12-h day–night cycle (lights on, 07:00–19:00 hours) for at least 7 days before experimentation. The rats were group-housed three per cage. They were given food (Western Lab Product 7001, Orange, CA) and water ad libitum. All animals were treated in strict accordance with the NIH Guide for the Care and Use of Laboratory Animals, and the procedures were approved by the Animal Care Committee at the Institute.

2.2. Transient global cerebral ischemia

Rats underwent transient forebrain ischemia using a procedure of four-vessel occlusion (4-VO) (Wang et al., 2002). The animals were anaesthetized with chlorohydrate (300 mg/kg, i.p.). One day before the experiment, the vertebral arteries were electrocauterized, in the meantime both common carotid arteries were exposed. Then, a small loop of elastic tube was placed