Neuroscience 147 (2007) 294–303

PREVENTIVE EFFECTS OF INTRATHECAL METHYLPREDNISOLONE ADMINISTRATION ON SPINAL CORD ISCHEMIA IN RATS: THE ROLE OF EXCITATORY AMINO ACID METABOLIZING SYSTEMS

G.-J. WU,[a,b,c1] W.-F. CHEN,[d1] C.-S. SUNG,[e] Y.-H. JEAN,[f] C.-M. SHIH,[g] C.-Y. SHYU[h] AND Z.-H. WEN[i]*

[a]*Department of Anesthesiology, Shin Kong Wu Ho-Su Memorial Hospital, Taipei, Taiwan*

[b]*Department of Medicine, School of Medicine, Fu-Jen Catholic University, Taipei, Taiwan*

[c]*Graduate Institute of Medical Sciences, College of Medicine, Taipei Medical University, Taipei, Taiwan*

[d]*Department of Neurosurgery, Chang Gung Memorial Hospital, Chang Gung University College of Medicine, Kaohsiung, Taiwan*

[e]*Department of Anesthesiology, Veterans General Hospital, Taipei, Taiwan*

[f]*Section of Orthopedic Surgery, Pingtung Christian Hospital, #60 Da-Lan Road, Pingtung 900, Taiwan*

[g]*Department of Biochemistry, School of Medicine, Taipei Medical University, Taipei, Taiwan*

[h]*Cook College, Rutgers, The State University of New Jersey, New Brunswick, NJ, USA*

[i]*Department of Marine Biotechnology and Resources, National Sun Yat-Sen University, #70 Lien-Hai Rd, Kaohsiung 804, Taiwan*

Abstract—Spinal cord ischemic injury usually results in paraplegia, which is a major cause of morbidity after thoracic aorta operations. Ample evidence indicates that massive release of excitatory amino acids (EAAs; glutamate) plays an important role in the development of neuronal ischemic injuries. However, there is a lack of direct evidence to indicate the involvement of EAAs in the glutamate metabolizing system (including the glutamate transporter isoforms, i.e. the Glu-Asp transporter (GLAST), Glu transporter-1 (GLT-1), and excitatory amino acid carrier one (EAAC1); glutamine synthetase (GS); and glutamate dehydrogenase (GDH)) in spinal cord ischemia. In the present results, we found that methylprednisolone (MP; intrathecal (i.t.) injection, 200 μg twice daily administered for 3 days before ischemia), a synthetic glucocorticoid, is the therapeutic agent for the treatment of spinal injuries in humans, can significantly reduce the ischemia-induced motor function defect and down-regulate the glutamate metabolizing system (including GLAST, GLT-1, GS, and GDH) in male Wistar rats. The spinal cord ischemia-induced down-regulation of EAAC1 protein expression in the ventral portion of the lumbar spinal cord was partly inhibited by pretreatment with i.t. MP. However, MP did not affect the down-regulation of EAAC1 in the dorsal portion of the lumbar spinal cord after spinal cord ischemia. The i.t. injection of MP alone did not change the neurological functions and the expression of proteins of the glutamate metabolizing system in the spinal cord. Our results indicate that spinal cord ischemia-induced neurological deficits accompany the decrease in the expression of proteins of the glutamate metabolizing system in the lumbar portion of the spinal cord. The i.t. MP pretreatment significantly prevented these symptoms. These results support the observation that MP delivery through an i.t. injection, is beneficial for the treatment of spinal cord ischemic injuries.

Key words: spinal cord ischemia, glutamate metabolizing systems, methylprednisolone, intrathecal.

Spinal cord ischemia, which often occurs after the repair of thoracoabdominal aortic aneurysms or dissection, is an uncommon but devastating entity in clinical practice. The subsequent paraplegia that occurs can be a serious complication following aortic surgery (Kouchoukos, 1991; Svensson et al., 1993). Researchers have yet to find an effective cure to prevent it. The results of studies on the mechanisms and prevention of spinal cord ischemia are still unclear and require further investigation.

The excitatory amino acid (EAA) glutamate, which is a major excitatory neurotransmitter in the CNS, also plays an important role in the pathogenesis of neuronal injury. Pharmacological studies in rodents and recent clinical studies in humans have shown that excessively high extracellular concentrations of glutamate caused by ischemia can be toxic to neurons (Nishizawa, 2001). Marsala et al. (1994) and Rokkas et al. (1995) had demonstrated a strong positive relationship between the increase in glutamate concentrations and aortic cross-clamping induced by spinal cord ischemia. The presence of excess extracellular glutamate activates neuronal glutamate receptors; these then induce massive lethal Ca^{2+} influx and subsequently result in neurotoxicity (Arundine and Tymianski, 2003). To ensure a high signal-to-noise ratio during synaptic signaling and to protect the neurons, the extracellular concentration of glutamate in the synapse needs to be maintained at an appropriate level (<1 μM) (Danbolt, 2001). There is no evidence to indicate that extracellular metabolism of glutamate occurs since the concentration is basically maintained by plasma membrane transporters. Glutamate transporters (GTs) are membrane proteins that reuptake

[1] These authors contributed equally to this work.

*Corresponding author. Tel: +886-7-5252021; fax: +886-7-5255020.
E-mail address: wzh@mail.nsysu.edu.tw (Z.-H. Wen).

Abbreviations: ANOVA, analysis of variance; CSF, cerebrospinal fluid; EAA, excitatory amino acid; EAAC1, excitatory amino acid carrier 1; EAAT, excitatory amino acid transporter; GDH, glutamate dehydrogenase; GFAP, glial fibrillary acid protein; GLAST, Glu-Asp transporter; GLT-1, Glu transporter-1; GS, glutamine synthetase; GT, glutamate transporter; H&E, hematoxylin and eosin; i.t., intrathecal; MDI, motor deficit index; MP, methylprednisolone; SOD, superoxide dismutase; TTBS, 5% non-fat dry milk in 0.1% Tween 20 in 20 mM Tris–HCl, 137 mM NaCl, pH 7.4..

0306-4522/07$30.00+0.00
doi:10.1016/j.neuroscience.2007.04.040

glutamate into the nerve terminals or glial cells in order to remove it from the extracellular space around the neurons. Five related but distinct eukaryotic high-affinity GTs have been cloned: the neuronal transporter excitatory amino acid carrier 1 (EAAC1); excitatory amino acid transporter 4 (EAAT4); the glial transporter, i.e. Glu-Asp transporter (GLAST); Glu transporter-1 (GLT-1); and a recently identified retinal transporter EAAT5 (Danbolt, 2001). Homologs of GLAST (EAAT1), GLT-1 (EAAT2), and EAAC1 (EAAT3) have been identified in the mammalian CNS (Rothstein et al., 1994; Lehre et al., 1995). Glutamate removal is essential for maintaining functional communication between neurons and preventing the concentration of glutamate from reaching toxic levels. The purpose of the present study is to evaluate how spinal cord ischemia induces changes in the expression of GT subtypes in various rat spinal cord regions.

Glutamate is taken up by the glial cells and subsequently metabolized by glutamine synthetase (GS) and glutamate dehydrogenase (GDH) into neutral metabolites, such as glutamine, thus preventing the overexcitation of neurons and excitotoxicity. Martin and Waniewski (1996) reported that GS is important for converting glutamate into nontoxic glutamine in astrocytes; this glutamine is then used in the neuronal TCA cycle. GDH converts glutamate into 2-oxoglutarate and other related metabolites (malate, pyruvate, and lactate), which provide energy to the neuron (Nicklas, 1984; Kaneko et al., 1988). Thus, GS and GDH might play an important role in modulating extracellular glutamate levels. In this study, we also examine the expression of GS and GDH in the spinal cord during spinal cord ischemia.

Methylprednisolone (MP), a synthetic glucocorticoid, has long been approved by the FDA, on the U.S. and worldwide markets and available for a variety of uses including acute SCI. The most significant characteristic of intrathecal (i.t.) injections is that the dosage of medication required and the side-effects caused by it are reduced. Therefore, i.t. MP administration is an effective mode of treatment for postherpetic neuralgia (Kotani et al., 2000). However, its effect is very limited since a technique for direct glucocorticoid delivery to the injury site in spinal cord ischemia is yet to be developed. The mechanisms responsible for the effect of MP on neuronal injuries remain to be investigated. Several studies have shown that the vascular anatomy of rats and humans is similar (Koyanagi et al., 1993; Scremin, 1995). These studies also provided a suitable animal model for studying spinal cord ischemia-induced neurobehavioral deficits and evaluating the neuroprotective effect of MP. Therefore, in this study, we have elucidated the role of glutamate metabolizing systems in the development of spinal ischemia. Moreover, we have investigated the effect of i.t. MP on neurobehavioral deficits as well as on the EAA metabolizing system in the spinal cord of ischemic rats. During the study, we have noted a correlation between the MP-induced protective effect and the changes in the expression of proteins of the glutamate metabolizing system in the spinal cord of ischemic rats. The present results suggest that down-regulation of the expression of GTs (GLSAT, GLT-1, and EAAC1), GDH, and GS might contribute to an increase in EAA-induced neurotoxicity in the spinal cord of ischemic rats. Both spinal cord ischemia-induced neurological dysfunction and the down-regulation of the expression of proteins of the glutamate metabolizing systems were significantly inhibited by i.t. MP injections.

EXPERIMENTAL PROCEDURES

Implantation of i.t. catheters

Male Wistar rats (400–450 g) were used in the experiments. As shown in our previous study (Wen et al., 2005), i.t. catheters (PE5 tubes 9 cm, 0.008 inch inner diameter, 0.014 inch outer diameter) were inserted via the atlantooccipital membrane into the i.t. space at the level of the lumbar enlargement of the spinal cord and externalized and fixed to the cranial aspect of the head. The rats were then returned to their home cages for a 4-day recovery period. Each rat was housed individually and lived on a 12-h light/dark daily cycle with food and water freely available. Rats were excluded from the study if they showed evidence of gross neurological injury or the presence of fresh blood in the cerebrospinal fluid (CSF). The use of animals conformed to the Guiding Principles in the Care and Use of Animals of the American Physiology Society and was approved by the National Sun-Sen University Animal Care and Use Committee. Every effort was made to minimize the number of animals used and their suffering.

The induction of spinal cord ischemia in rats

Male Wistar rats (400–420 g) implanted with one i.t. catheter were used. This rat spinal cord ischemia model was a modification of the one that was previously described by Taira and Marsala (1996). Briefly, animals were anesthetized in a plastic box with 4% isoflurane in room air. After induction, 2.5% isoflurane in an air/O_2 mixture was delivered to the rats through a mask. The tail artery was cannulated with a 22-gauge polytetrafluoroethylene catheter in order to monitor distal arterial pressure and to administer heparin intra-arterially. The left carotid was cannulated with a 20-gauge polytetrafluoroethylene catheter in order to collect the blood sample. In order to induce spinal cord ischemia, the left femoral artery was exposed. Aortic occlusion was induced by the inflation of a 2F Fogarty catheter placed into the thoracic aorta for 12 min, and the left carotid artery was also cannulated with a 20-gauge polytetrafluoroethylene catheter to collect blood and pump it into the peripheral stream during aortic occlusion. Immediately after the completion of arterial cannulations, all rats received 100 U of heparin (0.1 ml) through the tail artery. After the completion of all surgical procedures, 0.4 ml of protamine sulfate (4 mg) was administered intraperitoneally. Animals were then returned to their cages for recovery of motor function and finally killed for spinal sample collection.

Study groups and MP treatment

After i.t. catheter insertion, the rats were randomly divided into the following four groups: (1) the control group (C, $n=8$), in which an i.t. injection of saline was administered and a balloon catheter was placed in the thoracic aorta without inflation; (2) the ischemia-operated group (I, $n=10$), in which an i.t. injection of saline was given and a balloon catheter was placed in the thoracic aorta with inflation; (3) the ischemia plus MP group (I+M, $n=6$), in which an i.t. injection of MP (200 μg twice daily) was administered from 3 days prior to the induction of ischemia; and (4) the only MP-administered group (M, $n=6$), in which ischemia was not induced. Rats from each group were killed 48 h after ischemia had been induced and the behavioral test had been performed.