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Research Report

Neuronal damage and gliosis in the somatosensory cortex induced by various durations of transient cerebral ischemia in gerbils

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ABSTRACT

Although many studies regarding ischemic brain damage in the gerbil have been reported, studies on neuronal damage according to various durations of ischemia–reperfusion (I–R) have been limited. In this study, we examined neuronal damage/death and glial changes in the somatosensory cortex 4 days after 5, 10 and 15 min of transient cerebral ischemia using the gerbil. To examine neuronal damage, we used Fluoro-Jade B (F-J B, a marker for neuronal degeneration) histofluorescence staining as well as cresyl violet (CV) staining and neuronal nuclei (NeuN, neuronal marker) immunohistochemistry. In the somatosensory cortex, some CV and NeuN positive ($^+$) neurons were slightly decreased only in layers III and VI in the 5 min ischemia-group, and the number of CV$^+$ and NeuN$^+$ neurons were decreased with longer ischemic time. The F-J B histofluorescence staining showed a clear neuronal damage in layers III and VI, and the number of F-J B$^+$ neurons was increased with time of ischemia–reperfusion: in the 15 min ischemia-group, the number of F-J B$^+$ neurons was much higher in layer III than in layer VI. In addition, we immunohistochemically examined gliosis of astrocytes and microglia using anti-glial fibrillary acidic protein (GFAP) and anti-ionized calcium-binding adapter molecule 1 (Iba-1) antibody, respectively. In the

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5 min ischemia-group, GFAP$^+$ astrocytes and Iba-1$^+$ microglia were distinctively increased in number, and their immunoreactivity was stronger than that in the sham-group. In the 10 and 15 min ischemia-groups, numbers of GFAP$^+$ and Iba-1$^+$ glial cells were much more increased with time of ischemia–reperfusion; in the 15 min ischemia-group, their distribution patterns of GFAP$^+$ and Iba-1$^+$ glial cells were similar to those in the 10 min ischemia-group. Our fining indicates that neuronal death/damage and gliosis of astrocytes and microglia were apparently increased with longer time of ischemia–reperfusion.

1. Introduction

In comparison with other tissues, the brain is very vulnerable to ischemia because of its high metabolic rate, low oxygen stores and an insufficient reserve of high-energy carbohydrates (Sims and Zaidan, 1995). During global cerebral ischemia, the reduction of blood supply to the brain triggers a number of neuro-pathophysiological processes that result in irreversible neuronal damage, and several regions, such as the hippocampus, of the brain are especially sensitive to transient cerebral ischemia (Kirino, 1982). Selective neuronal death has been shown in the human brain after cardiocirculatory arrest and subsequent cardiopulmonary resuscitation, although the most common clinical cause of the selective neuronal death is ischemia and reperfusion injury (Horn and Schlote, 1992; Petito et al., 1987).

In a gerbil model of transient cerebral ischemia, sensitive regions in the brain include the cerebral cortex, striatum and CA1 region of the hippocampus (Hwang et al., 2006, 2007, 2008; Lin et al., 1990; Ohk et al., 2012). In the hippocampus, the vulnerability differs from each hippocampal subregion: The CA1 region is the most vulnerable to ischemia, whereas the CA3 region and dentate gyrus are the most resistant to ischemic insults (Schmidt-Kastner and Freund, 1991; Yu et al., 2012). Especially, neuronal death in the CA1 region is called “delayed neuronal death (DND)” due to that it occurs very slowly (Kirino and Sano, 1984).

On the other hand, the somatosensory cortex may play a role in neural rehabilitation by influencing motor function in patients with brain lesions (Conforto et al., 2002; Wu et al., 2005). Neuronal damage in the somatosensory cortex, neurons in which are heterogeneous compared to those in the hippocampal CA1 region, is moderate and observed mainly in layers III and VI of the gerbil and rat somatosensory cortices after ischemia–reperfusion (Hwang et al., 2008; Lin et al., 1990). These brain structures play important roles in the control of different types of sensory or motor behavior, therefore, ischemia–reperfusion injury in the structures is a major cause of neurologic abnormalities (Li et al., 2004; Meno et al., 2003). Although deficits in sensorimotor function are common in humans undergoing hypoxic/ischemic episodes, little is known of selective neuronal death/damage in the somatosensory cortex induced by transient ischemia.

The Mongolian gerbil has been used as a good animal model to investigate mechanisms of selective neuronal death following transient global cerebral ischemia (Bian et al., 2007; Lorrio et al., 2009; Zhang et al., 2009), because about 90% of gerbils lack the communicating vessels between the carotid and vertebral circulations. Thus, the bilateral occlusion of the common carotid arteries essentially completely eliminates blood flow to the forebrain while completely sparing the vegetative centers of the brain stem. Although there has been a great deal of information on ischemic damage in the gerbil brain (Fukuchi et al., 1998; Janac et al., 2006), studies regarding neuronal damage/death in the somatosensory cortex according to the duration of ischemia–reperfusion have been limited. Therefore, the present study was undertaken in order to identify the degree of neuronal injury after various durations of transient cerebral ischemia using cresyl violet staining, NeuN immunohistochemistry and Fluoro-Jade B (F-J B) histofluorescence. F-J B is a very useful maker for neuronal degeneration (Schmued and Hopkins, 2000). In addition, we examined changes of astrocytes and microglia in the gerbil somatosensory cortex after ischemia–reperfusion.

2. Results

2.1. *Spontaneous locomotor activity*

To investigate change in motor behavior after ischemic damage, spontaneous motor activity was examined 24 h after ischemia–reperfusion in all the groups (Fig. 1). The spontaneous locomotor activity was significantly increased in the 5 min ischemia-group (mean±S.E.M., 570±61) after ischemia–reperfusion compared to that in the sham-group (298±48). In the 10 min and 15 ischemia-group, the activity was more increased (760±54 vs 1100±40) than the 5 min ischemia-group.

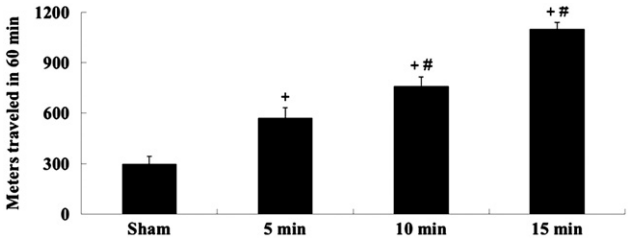

Fig. 1 – Locomotor activity of the sham- and ischemia-groups (5, 10 and 15 min). The spontaneous locomotor activity is evaluated in terms of entire distance (meters) traveled for 60 min ($n=10$ per group; $+P<0.05$, significantly different from the sham group; $\#P<0.05$, significantly different from the preceding-group). The bars indicate the means±SEM.