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Research Report

Effects of spinal cord injury on c-fos expression in hypothalamic paraventricular nucleus and supraoptic nucleus in rats

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ARTICLE INFO

Article history:
Accepted 1 March 2006
Available online 13 April 2006

Keywords:
Neuronal activation
Area-specific effect
Immunocytochemistry
The weight-drop model
Wistar rat

ABSTRACT

The effects of spinal cord injury (SCI) on c-fos expression in hypothalamic paraventricular nucleus (PVN) and supraoptic nucleus (SON) in rats were investigated. As hypothesized, SCI has a significant effect on neuronal responses in the PVN and SON. A significant increase in c-fos in the PVN was found at 1, 6, 12 and 24 h following SCI, implying that the neurons in the PVN can be activated soon after SCI and persist for at least 24 h. However, in contrast to the PVN, SCI did not induce a significant increase in c-fos expression in the SON until 12 h following SCI. The highest expression of c-fos in the SON was found at the end point of this study (24 h) following SCI. The data demonstrated that SCI can significantly activate neurons in the PVN and SON. The activated neurons might involve in the initiation of a variety biochemical, ischemic and other injury processes. The area-specific effects of SCI on the PVN and SON suggest that these nuclei might play their roles in different stages in the prolonged time course following SCI.

1. Introduction

Spinal cord injury (SCI) occurs mostly as a result of motor vehicle accidents, sports accidents or falls and produces numerous functional defects or alterations distal to the site of injury. In the United States, about 1 million people with SCI are hospitalized, and there are approximately 250,000 new quadriplegics and paraplegics every year (Voda et al., 2005). SCI is a dynamic, complex event involving multiple pathophysiologic processes and has two phases: the primary and the secondary injury (Crowe et al., 2003; Ye et al., 2004). The primary injury is immediate and irreversible, and the secondary injury is the subsequent activation of cell death cascades mediating delayed tissue damage, neuronal atrophy and loss and retrograde cell death (Lemke and Faden, 1990; Taoka and Okajima, 1998; Tetzlaff et al., 1994). It is postulated that the primary mechanical injury to the spinal cord initiates a variety of biochemical, ischemic and other injury processes that

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doi:10.1016/j.brainres.2006.03.003

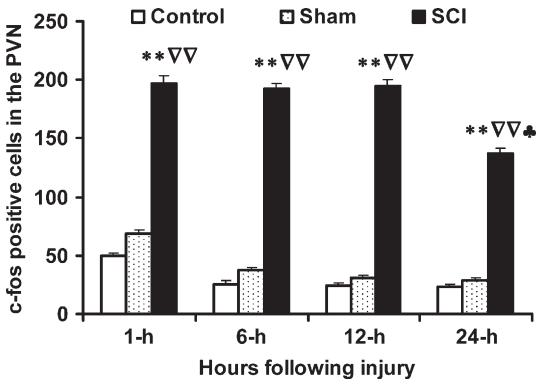

Fig. 1 – Effect of spinal cord injury on the expression of c-fos in the hypothalamic paraventricular nucleus (PVN). The changes of c-fos expression in the hypothalamic PVN of SCI (n = 5), Sham (n = 5) and Control (n = 4) rats were determined at 1, 6, 12 and 24 h following spinal cord injury using immunocytochemical method as described in Experimental procedures. Data are means ± SEM. $^{**}P < 0.01$ vs. the control of the same time point; $\nabla\nabla P < 0.01$ vs. Sham of the same time point; $\clubsuit P < 0.05$ vs. the SCI at 1 h.

increase or decrease the severity of pathology over the secondary injury (Anthes et al., 1995; Blight, 1993; Crowe et al., 2003; Schwab and Bartholdi, 1996). However, the relevant mechanisms have not been completely described.

A detailed understanding of the relationships between SCI and the responses of neurons in the brain would advance our knowledge of the pathophysiology of this disorder as well as the mechanisms involved in the initiation of a variety of biochemical, ischemic and other injury processes that are associated with the severity of the secondary injury (Nicholls and Saunders, 1996; Schwab and Bartholdi, 1996). In this study, we investigated the effects of SCI on the c-fos expression in the hypothalamic paraventricular nucleus (PVN) and the supraoptic nucleus (SON) in rats. The c-fos protein represents the product of the immediate-early gene, which is rapidly expressed in neurons following various noxious stimulations (Hunt et al., 1987; Morgan and Curran, 1991; Orendacova et al., 2001; Tolle et al., 1990). It was therefore used as an indicator of neuronal activity or neuronal response to SCI in this study.

Effects of SCI on the neuronal activity in the hypothalamic PVN and SON are unknown. However, the hypothalamic PVN and SON constitute the major integrative center for many neuroendocrine processes (Benavides et al., 2003, 2005) and function as a regulator of the systemic stress response (Cullinan et al., 1995; Herman and Cullinan, 1997). Previous studies have shown that a number of stressors, such as surgical interventions (Stenberg et al., 2005), chronic social stress (Matsuda et al., 1996), peripheral stimulation (Bullitt, 1989) and other types of stresses (Ceccatelli et al., 1989; Imaki et al., 1992), can significantly affect the c-fos expression in the PVN and SON. We hypothesized that SCI might also have a significant effect on neurons in the hypothalamic PVN and SON. The experiments in this study were therefore designed to investigate this possibility by monitoring the expression of c-fos. The findings showed that SCI can significantly activate neurons in the PVN and SON.

2. Results

2.1. *Effect of spinal cord injury on animal behavior*

After the experimental procedure, the behavior of rats was evaluated for 5 min using the Tarlov/Bohlman scale. The evaluation showed no evidence of spinal cord injury due to the procedure in animals of the Sham or Control group, with scores of 4 or 5 on the Tarlov/Bohlman scale in both cases. The rats in the SCI group showed partial paraplegia, and all animals scored 2 on the Tarlov/Bohlman scale.

2.2. *Effect of spinal cord injury on expression of c-fos in the hypothalamic paraventricular nucleus*

To determine the effect of spinal cord injury on the expression of c-fos in the PVN, we investigated the changes in c-fos expression in the hypothalamic PVN of the SCI (n = 5), Sham (n = 5) and Control (n = 4) rats at 1, 6, 12 and 24 h following SCI using immunocytochemical method. At 1 h following SCI, a significant increase in the expression of c-fos in the hypothalamic PVN was found in the SCI rats (Fig. 1), compared with that in the Sham or Control animals ($P < 0.01$). The c-fos positive cells in the SCI rats increased about 3-fold above that of the Sham or Control animals (Fig. 1) (both $P < 0.01$). The significantly increased expression of c-fos in the PVN was also found in the rats at 6 and 12 h following SCI ($P < 0.01$ vs. Sham or Control rats). There was no difference in the magnitude of the increased c-Fos expression in the SCI rats at 1, 6 and 12 h following SCI. The expression of c-fos was significantly lower in the SCI rats at 24 h than at 1, 6 and 12 h following SCI ($P < 0.05$). However, it was still markedly higher than that of the corresponding Sham or Control rats ($P < 0.05$). The finding showed that spinal cord injury can lead to a significant increase in c-fos expression in the PVN for at least 24 h (Fig. 1).

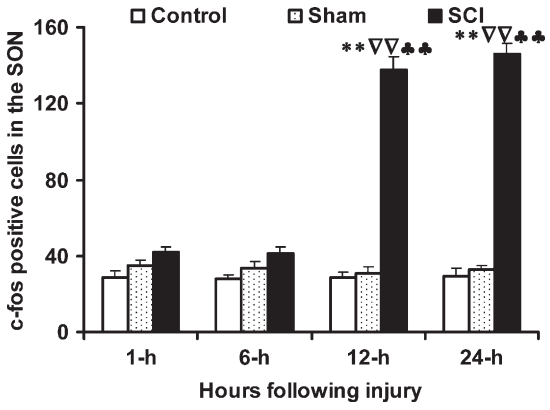

Fig. 2 – Effect of spinal cord injury on the expression of c-fos in the supraoptic nucleus (SON). The changes of c-fos expression in the hypothalamic SON of SCI (n = 5), Sham (n = 5) and Control (n = 4) rats were determined at 1, 6, 12 and 24 h following spinal cord injury using immunocytochemical method as described in the Experimental procedures. Data are means ± SEM. $^{**}P < 0.01$ vs. the Control of the same time point; $\nabla\nabla P < 0.01$ vs. Sham of the same time point; $\clubsuit\clubsuit P < 0.01$ vs. the SCI at 1 h.