Neurosci Bull February 1, 2008, 24(1): 29-33. http://www.neurosci.cn
DOI: 10.1007/s12264-008-1031-4

·Original Article·

Changes of 5-hydroxytryptamine and tryptophan hydroxylase expression in the ventral horn of spinal cord

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Abstract: Objective To investigate changes of 5-hydroxytryptamine (5-HT) and its synthesis rate-limiting enzyme tryptophan hydroxylase (TPH) in the ventral horn of spinal cord after exercise-induced fatigue, and to further discuss the mechanism of exercise-induced central fatigue at spinal level. **Methods** Sixteen healthy adult Wistar rats were randomly divided into 2 groups: exercise-induced fatigue group and control group. Immunohistochemical staining for 5-HT and TPH in the ventral horn were performed and analysized quantitatively. The mean optic densities of 5-HT and TPH positive fibers or terminals were measured by computerized image analyzer. **Results** Both 5-HT and TPH positive fibers/terminals decreased in the exercise-induced fatigue group. The immunohistochemical staining was weaker and the mean optic densities decreased obviously in the fatigue group compared with those in the control group ($P < 0.05$). **Conclusion** 5-HT and TPH in the ventral horn of spinal cord might be involved in exercise-induced fatigue.

Keywords: ventral horn; spinal cord; 5-hydroxytryptamine; tryptophan hydroxylase; exercise-induced fatigue; immunohistochemistry

1 Introduction

There are many reports on the role of 5-hydroxytryptamine (5-HT) in fatigue. Most studies indicates that there may be a positive relationship between 5-HT and fatigue, i. e. the increase of brain 5-HT may be a reason of fatigue. It is hypothesized that an increase of plasma free tryptophan enhanced brain serotonergic activity, and thus contributing to the onset of fatigue[1]. In the spinal cord, there is also a dense projection of serotonergic fibers and terminals from caudal raphe nuclei[2,3]. These serotonergic descending systems have potentially important functions in regulating somatomotor, sensorimotor and autonomic activity in the spinal cord. Evidences from electrophysiological and pharmacological studies have shown that one of the primary functions of 5-HT in the spinal cord is to regulate locomotor activity[4,5]. And the influence of 5-HT on the locomotion activity is regulated mainly by the activity of motoneurons in the spinal cord. However, the relationship between 5-HT in the ventral horn of spinal cord (the site of motoneurons located) and treadmill locomotion induced fatigue were not well known. In this work we investigated whether the fatigue induced by forced locomotion would affect the synthesis of 5-HT in the ventral horn of spinal cord in rats.

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Article ID:1673-7067(2008)01-0029-05
CLC number: R338
Document code: A
Received date: 2007-10-31

2 Materials and methods

2.1 Animals and treatments Adult male Wistar rats weighing (200±20) g were obtained from the animal center of the Academy of Military Medical Sciences (SCXK-Military 2002-001). Animals were housed under controlled temperature (20 °C-24 °C) and lighting (07:00-19:00) conditions with food and water available. The animals were randomly divided into two groups: exercise-induced fatigue group and control group ($n = 8$ for each group).

2.2 Treadmill exercise protocols A motor-driven treadmill was applied for measuring physical exercise load. Rats in the exercise group were familiarized to the motor-driven treadmill for 3 consecutive days before the formal exercise. The ani-

mals run on the treadmill for 5 min daily at a constant speed of 15 m/min and 0° inclination, which was used to show the running direction of these rats. The formal exercise load consisted of running at a speed of 9 m/min for 15 min, at 15 m/min for another 15 min, and at 20 m/min until exhaustion; once a day for 10 consecutive days. The time of exhaustion was defined as the time when experimental animals failed to keep up with the treadmill machine even being stimulated electrically for 3 times[6,7]. The control rats were left on the treadmill for rest until the exercise rats exhausted. On the last day (day 10), the rats were sacrificed at 3 h after the time of exhaustion,.

2.3 Tissue preparation Animals were fully anesthetized with urethane (1.5 g/kg, i.p.), followed by perfused with 200-250 mL of 0.9% sodium chloride and 4% formaldehyde solution resolved in 0.1 mol/L phosphate buffer (4 °C, pH 7.4). The perfusion pressure was maintained at 100 mmHg for 30 min. Next, the cervical enlargement of spinal cord (C3-C6) was removed and post-fixed in the same fixative for about 1 week. After conventional dehydration and clarification, tissues were paraffin embedded into blocks, and subsequently cut transversely into 5 μm sections on a paraffin microtome (Leica, RM2165). Two out of every ten sections were selected for 5-HT and TPH staining, respectively. Microtome sections at parallel levels from the two groups were processed and allowing the slide to dry until ready for use.

2.4 Immunohistochemistry for 5-HT and TPH Tissue slides were deparaffinized and rehydrated. After washing in PBS for three times, the slides were incubated in 3% hydrogen peroxide (H_2O_2) and Triton x-100 for 10 min to reduce background staining and enhance penetration, and then rinsed with distilled water and incubated in PBS for 5 min. Following microwave antigen retrieval at 95 °C for 10 min and washing with PBS for 3 times, the sections were incubated overnight with mouse anti-5-HT antibody (Abcam Ab16007, 1:100) or with sheep anti-TPH antibody (Abcam Ab3907, 1:1 000). Next, the slides were incubated with biotinylated anti-mouse or anti-sheep secondary antibody (Zymed laboratories) for 45 min, and subsequently incubated with avidin-biotin-peroxidase complex (Haoyang Biotechnology Co. Ltd, Tianjin, China) for 45 min at room temperature. The immunocomplexes were localized by incubating the sections in 3.3'-diaminobenzidine (DAB) for approximately 3 min. Finally, the sections were mounted on gelatin-coated glass slides.

2.5 Data analysis The sections selected from the same region and equivalent area of the ventral horn of spinal cord were examined under a light microscope for evaluation of the immunolabelling. The mean optic density of 5-HT and TPH positive fibers/terminals was measure by Image-Pro-Plus 5.0 computerized image analyzer system. Data are expressed as mean±SD and analyzed by student's *t*-test. $P < 0.05$ was considered statistical significant.

3 Results

3.1 Immunoreactivity of 5-HT positive fibers/terminals in the ventral horn of spinal cord The 5-HT and TPH positive fibers/terminals in the ventral horn presented brown staining. The sections in the control group showed moderate immunoreaction (Fig. 1 C). A higher-power view of the ventral horn in the tissue sections showed brown immunoreactive particles in presumptive motoneurons. Note that the brown 5-HT granules appeared contacting with a presumptive motoneuron (Fig. 1 D). The density of labeled fibers/terminals in the ventral horn was lower in the fatigue group than in the control group (Fig.1 A and B). The mean optical density of 5-HT positive fibers/terminals of ventral horn in the fatigue group was considerably lower than that in the control group as well (0.199±0.01 *vs* 0.359±0.032, $P < 0.05$; Fig. 3).

3.2 Immunoreactivity of TPH-positive fibers/terminals in the ventral horn of spinal cord Photographs of TPH positive fibers/terminals in the ventral horn of spinal cord were presented in Fig. 2. Fibers/terminals containing TPH were stained light brown. Unlike 5-HT immunoreaction, the TPH-positive brown products were mainly present in the terminals adjacent to the large multipolar neurons (presumptive motorneurons) and their dendritic processes, but the positive-fibers were only stained lightly. The mean optical density of TPH in the ventral horn of rats in the fatigue group decreased dramatically compared with that of control group (0.249±0.019 *vs* 0.315±0.011, $P < 0.05$; Fig. 3).

4 Discussion

The results in the present study showed that the levels of 5-HT and TPH in the ventral horn of spinal cord decreased following exercise-induced fatigue, suggesting that the synthesis of 5-HT in the ventral horn of spinal cord was not the same as that in the brain during exercise. The different re-

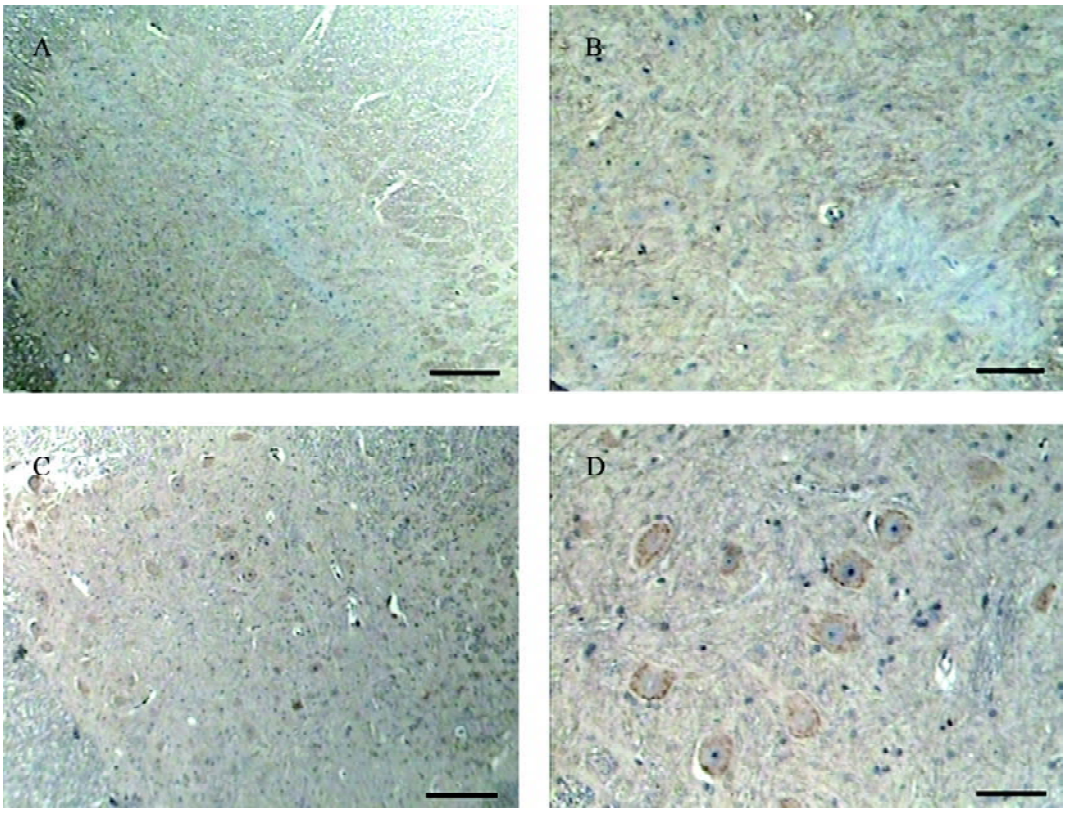

Fig. 1 Changes of the distribution of 5-HT fibers and terminals in the ventral horn of spinal cord after exercise-induced fatigue detcted by immunohistochemistry. A, B: exercise group; C, D: control group. Scale bar, 250 μm in A, C; 100 μm in B, D.

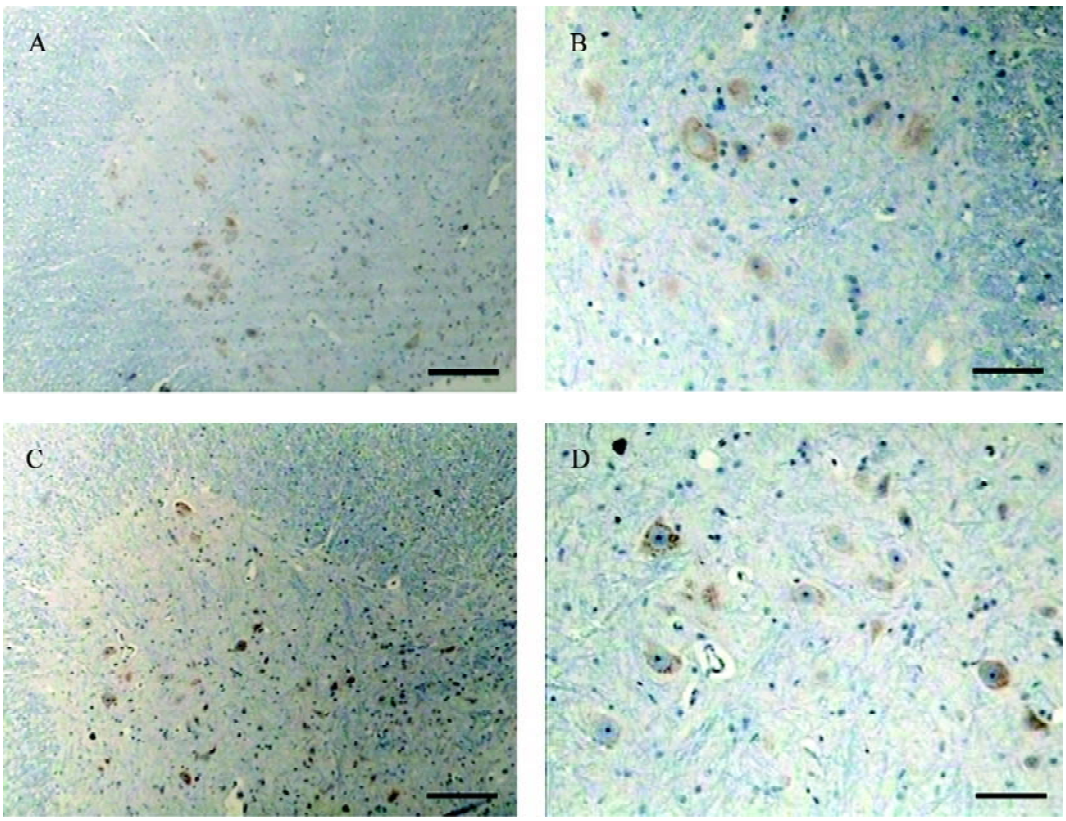

Fig. 2 Changes of TPH-positive fibers and terminals in the ventral horn of spinal cord after exercise-induced fatigue detected by immunohistochemistry. A, B: exercise group; C, D: control group. Scale bar, 250 μm in A, C; 100 μm in B, D.

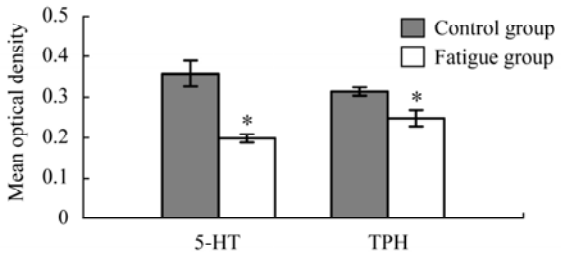

Fig. 3 Changes of mean optical density of 5-HT and TPH in the ventral horn of spinal cord after exercise-induced fatigue.*$P < 0.05$ *vs* control group.

sponses of 5-HT during fatigue between brain and spinal cord might be due to their different 5-HT sources from the brainstem. The serotonergic fibers and terminals in the ventral horn of spinal cord are mainly derived from the 5-HT cell bodies in nucleus raphe pallidus (NRP) and nucleus raphe obscurus (NRO), but less in nucleus raphe magnus (NRM). While the cell bodies of the serotonergic fibers in the brain are sourced from dorsal raphe nucleus (DRN)[8,9]. The activity of NRP/NRO neurons is totally different from that of DRN neurons during fatigue. 5-HT is synthesize/released in actively firing neurons, hence during exercise, only 5-HT neurons that are firing could increase the production/release of 5-HT[10]. Recently, by using electrophysiological technique Fornal's laboratory[11] found that with the development of fatigue, the mean discharge rates of a group of presumed serotonergic neurons decreased in the NRO/NRP but not in the DRN, which send their projections mainly to the ventral horn of spinal cord and forebrain, respectively. They suggested that the activity of serotonergic neurons may be inversely related to fatigue, that is, muscle fatigue was associated with suppression rather than promotion of serotonergic neuronal activity. Our results was in accordance with another report from a rat microdialysis study, which found a slight decrease in extracellular concentrations of 5-HT during locomotion and a much larger decrease during the post-exercise period in the ventral horn of the spinal cord[12,13].

On the other hand, the unparallel level of 5-HT between spinal cord and brain might be associated with their different roles during exercise. In the brain, 5-HT acts as an inhibitory neurotransmitter. Elevation of 5-HT synthesis in the brain during fatigue may induce lethargy and loss of central drive and/or motivation[14]. However, the primary role of 5-HT in the ventral horn of spinal cord is to increase the excitability of motoneurons[15] by depolorizing the membrane potential[16] and promote plateau potentials, which act to driven action potential firing[17]. Therefore, inhibition of 5-HT synthesis and TPH expression in the ventral horn of spinal cord disfacilitates motor output and thereby contributes to central fatigue.

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运动性疲劳后脊髓前角 5- 羟色胺和色胺酸羟化酶表达的变化

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摘要：目的 探讨运动性疲劳后脊髓前角 5- 羟色胺(5-hydroxytryptamine，5-HT)及其合成关键酶色胺酸羟化酶(tryptophan hydroxylase，TPH)表达的变化，阐释在脊髓水平运动性疲劳的发生机制。**方法** 健康成年雄性 Wistar 大鼠16只随机分为运动性疲劳组和对照组，采用免疫组化方法和计算机图像分析技术，检测大鼠脊髓前角的5-HT及 TPH 免疫反应阳性纤维及终末的平均积分光密度值。**结果** 运动性疲劳后，脊髓前角 5-HT 及 TPH 阳性反应纤维和终末减少，染色较浅，平均积分光密度值明显低于对照组($P < 0.05$)。**结论** 脊髓前角 5-HT 及 TPH 的含量在运动性疲劳后降低，提示脊髓前角 5-HT 与运动性疲劳的发生有关。

关键词：脊髓前角；5- 羟色胺；色胺酸羟化酶；运动性疲劳；免疫组化