Firing activity of locus coeruleus noradrenergic neurons increases in a rodent model of Parkinsonism

Tao WANG¹, Qiao-Jun ZHANG², Jian LIU^{1,3}, Zhong-Heng WU², Shuang WANG¹

¹Department of Physiology and Pathophysiology, School of Medicine, Xi'an Jiaotong University, Xi'an 710061, China

²Department of Neurology, the Second Hospital, Xi'an Jiaotong University, Xi'an 710004, China

³Key Laboratory of Environment- and Genes- Related Diseases, Ministry of Education of China, Xi'an 710061, China

Abstract: Objective To investigate the changes in the firing activity of noradrenergic neurons in the locus coeruleus (LC) in a rat model of Parkinson disease (PD). **Methods** 2 and 4 weeks after unilateral lesion of the nigrostriatal pathway in the rat by local injection of 6-hydroxydopamine (6-OHDA) into the right substantia nigra pars compacta (SNc), the firing activity of noradrenergic neurons in LC was recorded by extracellular single unit recording. **Results** The firing rate of LC noradrenergic neurons increased significantly 2 and 4 weeks after 6-OHDA lesions compared to normal rats, respectively ($P < 0.05$). The percentage of irregularly firing neurons was obviously higher than that of normal rats during the fourth week after SNc lesion ($P < 0.05$). **Conclusion** LC noradrenergic neurons are overactive and more irregular in 6-OHDA-lesioned rats. These changes suggest an implication of the LC in the pathophysiological mechanism of PD.

Keywords: locus coeruleus; 6-hydroxydopamine; Parkinson disease; electrophysiology

1 Introduction

The locus coeruleus (LC) is the most important noradrenergic nucleus in the brain stem, which projects to the cerebral cortex, thalamus, basal ganglia, hippocampus, cerebellum and spinal cord. Thus, LC-noradrenergic neurotransmitter system consequently plays an important role in a wide variety of brain functions^[1]. In Parkinson disease (PD), the degeneration of dopaminergic neurons of the substantia nigra pars compacta (SNc) in the midbrain triggers a cascade of functional changes affecting the whole basal ganglia network. Low levels of dopamine in the striatum lead to symptoms such as slow movement (bradykinesia) and impaired motor

control (resting tremor, postural instability and rigidity). While widespread dopamine deficiency is the main feature of PD, other neurotransmitter systems degenerate or are altered by the degenerative process, such as the noradrenergic and serotonergic brainstem nuclei^[2]. Several studies have suggested the involvement of these neurotransmitters in the pathophysiology of PD. Neurochemical studies have found that the levels of noradrenaline (NA) decrease significantly in the striatum, substantia nigra, cerebral cortex, hypothalamus and hippocampus of PD patients^[1]. The reduction of NA is attributable to the loss of noradrenergic neurons in the LC. Furthermore, the degree of LC neuron loss, compared with age-matched controls, has been reported to be about 83% in PD postmortem studies^[3,4]. In fact, the neuronal loss was found to be greater in the LC than in the substantia nigra in subjects with PD^[4]. In addition, studies have observed that the number of α_1 and β_1 receptors increased in the prefrontal cortex of parkinsonian patients postmortem, particularly in demented parkinsonian patients, while α_2 receptors

Corresponding author: Jian LIU

Tel: 86-29-82655160

Fax: 86-29-82656364

E-mail: liujian@mail.xjtu.edu.cn

Article ID: 1673-7067(2009)01-0015-06

CLC: Q189

Document code: A

Received date: 2008-10-23

decreased^[5]. These studies demonstrate that the noradrenergic neurotransmitter system of the LC is severely affected in PD. However, at present, there are no *in vivo* electrophysiological studies that have investigated the firing activity of noradrenergic neurons in the LC after unilateral lesion of the nigrostriatal pathway in rats. To address this issue, we examined the changes in firing rate and firing pattern of noradrenergic neurons of the LC in a 6-hydroxydopamine (6-OHDA) rat model of PD using electrophysiological techniques.

2 Materials and methods

2.1 Animals Fifty-four adult male Sprague-Dawley rats, weighing 240-280 g, were used in this study. The experiments were performed according to the guideline of the Institutional Animal Care Committee of the University. All efforts were made to minimize the number of animals used and their suffering.

2.2 6-OHDA lesions Thirty minutes prior to the injection of 6-OHDA, rats were pretreated with desipramine (25 mg/kg, i.p.; Sigma) in order to protect noradrenergic neurons. Then they were anaesthetized with 4% chloral hydrate (300 mg/kg, i.p.) and received stereotaxic injection of 2 μ L 6-OHDA hydrochloride solution (4 μ g/ μ L in saline containing 0.01% ascorbic acid; Sigma) into the right SNc following the coordinates: AP 5.0-5.3 mm posterior to bregma, L 1.9-2.1 mm from the midline, D 7.2 mm from the dura^[6]. After each injection the micropipette was left in place for an additional 5 min and then withdrawn slowly. One week post-surgery, rats were given apomorphine (0.05 mg/kg, s.c.; Sigma) and those exhibiting more than 20 contralateral turns per 5 min were chosen for the electrophysiological investigation^[7].

2.3 Electrophysiological recordings Electrophysiological recordings were made 2 weeks and 4 weeks after 6-OHDA lesions of the SNc, respectively. Extracellular single unit recordings were performed in rats anaesthetized with chloral hydrate (300 mg/kg, i.p.). Glass microelectrodes (10-20 M Ω) filled with 2% Pontamine Sky Blue in 0.5 mol/L sodium acetate were stereotaxically directed to the right LC (AP 9.7-10.3 mm posterior to bregma, L 1.3-1.5 mm from the midline, D 5.3-6.2 mm from the dura)^[6]. Extracellular spikes were amplified, bandpass-filtered, displayed on an oscilloscope and stored

in a computer equipped with the Spike 2 analysis system (Cambridge Electronic Design, UK) for off-line analysis. Body temperature was maintained at 37-38 °C, heart rate and pupillary diameter were monitored throughout the experiment. At the end of each experiment, the recording site was marked by the ejection of Pontamine Sky Blue (-20 μ A, 15 min).

2.4 Histology The rat was given an overdose of urethane, and perfused with saline followed by 4% paraformaldehyde. The brain was removed, frozen, sectioned at 40 μ m and stained with cresyl violet for histological verification and reconstruction of recording sites. To determine the extent of nigral dopaminergic degeneration, sections of the SNc from rats receiving 6-OHDA injection were examined for immunocytochemical staining of tyrosine hydroxylase (TH), as previously described^[8]. Only rats with a total or subtotal loss of TH immunoreactivity in the SNc were used to analyze electrophysiological recordings (Fig. 1).

Fig. 1 Immunocytochemistry of TH showing the degeneration of dopaminergic neurons in the SNc on the 6-OHDA injected side (right) compared to normal side (left). Scale bar, 1 mm.

2.5 Statistics The neurons which met the following criteria were included for off-line analysis in this study: (1) a positive-negative waveform with a notch on the ascending limb (Fig. 2A) and biphasic excitation-inhibition responses to the pressure applied to the contralateral hind paw ('paw pinch') (Fig. 2B)^[9]; (2) the histology verified the neurons which were recorded within the LC (Fig. 2C). The spontaneous activity of each neuron was recorded for 5 min. The firing patterns were determined by interspike interval histograms (ISIHS) and the mean interspike interval (ISI) coefficient of variation^[10]. Comparisons between different groups of the firing rates were performed using an ANOVA test. The coefficient of variation was analyzed using the Mann-Whitney *U* test, as these data

were not normally distributed. The proportions of firing patterns of noradrenergic neurons of the LC in the different groups were compared using the χ^2 test. All data were expressed as mean $\pm$ SD. Statistical analyses were performed using SPSS 10.0 for Windows. $P < 0.05$ was considered statistically significant.

3 Results

All the recording sites used in normal and 6-OHDA-

lesioned rats were verified to be within the LC (Fig. 2C). 6-OHDA-lesioned rats in the study turned consistently towards the side contralateral to the side of the lesion with more than 20 turns per 5 min after apomorphine stimulation and displayed a total loss of TH immunoreactivity in the SNc. All neurons recorded had electrophysiological characteristics of noradrenergic neurons.

A total of 19 LC noradrenergic neurons were recorded in 10 normal rats. Firing rates of these neurons ranged from 0.31

Fig. 2 Determination of noradrenergic neuron of LC. A: The positive-negative waveform firing of a LC noradrenergic neuron. The arrow indicates the notch on the ascending limb. Bar = 0.5 ms. B: The typical response pattern of a LC neuron to brief paw compression (PC) of the contralateral hind paw. The arrow shows that an increase in depolarization spikes occurs when PC is applied, followed by a period of drop of firing rate. Bar = 0.5 s. C: Histological photomicrographs showing the recording site (arrow) in the LC marked with iontophoretically injected Pontamine Sky Blue. Scale bar, 200 μ m. 4V, the fourth ventricle; Me5, mesencephalic trigeminal nucleus; scp, superior cerebellar peduncle.

to 4.82 spikes/s with a mean of 2.69 ± 1.23 spikes/s ($n = 19$; Fig. 3). The mean ISI coefficient of variation had a mean value of 0.69 ± 0.35 (Fig. 4). In this group, noradrenergic neurons exhibited three different firing patterns, 52% of the neurons displaying a regular firing pattern, 32% discharging an irregular and 16% having a bursting firing pattern (Fig. 5). With 6-OHDA lesion of the SNc, a total of 34 LC noradrenergic neurons were recorded in 21 rats after 2 weeks and 46 neurons in 23 rats after 4 weeks, respectively. During the second week, firing rates of the neurons varied from 1.19 to 8.61 spikes/s. The mean firing rate increased significantly to 4.16 ± 1.79 spikes/s compared to the normal rats ($n = 34$,

Fig. 3 Distribution of mean firing rate of LC noradrenergic neurons in normal rats and rats with 6-OHDA lesions of the SNc (PD-2 week and PD-4 week). * $P < 0.05$ vs normal rats.

Fig. 4 Distribution of mean ISI coefficient of variation of LC noradrenergic neurons in normal rats and rats with 6-OHDA lesions of the SNc (PD-2 week and PD-4 week). * $P < 0.05$ vs normal rats.

Fig. 5 Distribution of firing patterns of LC noradrenergic neurons in normal rats and rats with 6-OHDA lesions of the SNc (PD-2 week and PD-4 week).

$P < 0.05$; Fig. 3). The mean ISI coefficient of variation, 0.48 ± 0.2 , was significantly lower than that of the normal rats ($P < 0.05$; Fig. 4). No significant changes were observed in the firing pattern with 38% regular, 53% irregular and 9% bursting neurons (Fig. 5). However, moving on to the fourth week, the firing rates of noradrenergic neurons ranged from 1.02 to 13.69 spikes/s. The mean firing rate was 4.41 ± 2.27 spikes/s, which was significantly higher than the value in normal rats ($n = 46$, $P < 0.05$; Fig. 3). The mean ISI coefficient of variation (0.52 ± 0.18) was significantly lower than the value in normal rats ($P < 0.05$; Fig. 4). The percentage of neurons with regular firing pattern increased to 37% and those firing irregularly increased significantly to 63% ($P < 0.05$; Fig. 5). The burst-firing neurons were not observed in this group.

4 Discussion

All the recorded neurons located in the LC and displayed electrophysiological characteristics of noradrenergic neurons in this study, corresponding to previously reported^[11]. The results of the present study showed that the unilateral lesion of the nigrostriatal pathway induced an obvious increase in the firing rate with change in the firing pattern of noradrenergic neurons recorded in the LC of rats. α_2 adrenoceptors are located on the soma and dendrites of noradrenergic neurons where they act as autoreceptors^[12], and α_2 subtype is most dense in the LC. Activation of α_2 adrenoceptors by NA causes suppression of LC noradrenergic neuron firing^[13]. Intracellular recording studies have demonstrated that the α_2 adrenoceptor-mediated inhibition of LC neurons results from a hyperpolarization of the somadendritic membranes, due to an increase in K^+ conductance^[14]. Furthermore, α_2 adrenergic autoreceptors of LC neurons are activated by NA released from the terminals of recurrent axon collaterals and/or from presynaptic dendrites of LC neurons. Therefore, the α_2 adrenoceptor-mediated collateral inhibition of LC neurons operates as a negative feedback mechanism to regulate the electrical activity of LC neurons. Results from neurochemical studies have shown that a marked reduction of NA in post-mortem samples of various brain regions of PD patients and 6-OHDA-lesioned rats^[15]. The reduction of NA is attributable to loss of noradrenergic neurons in the LC. In fact, the neuronal loss was found to be greater in the LC than in the substantia nigra in Parkinsonian patients^[4]. These studies demonstrate LC noradrenergic system is severely affected in PD. On the basis of these studies and our results, we can postulate that the 6-OHDA lesions of the SNc caused loss of noradrenergic neurons and decrease in concentration of NA in the LC of rats, leading to the overactivity of residual noradrenergic neurons.

In addition to α_2 adrenergic autoreceptors in the LC, the firing of these noradrenergic neurons is also affected by inputs from the dorsal raphe nucleus (DRN) and paragigantocellularis nucleus. The LC receives a dense 5-hydroxytryptamine (5-HT) innervation and electrophysiological and biochemical studies have suggested that the 5-HT system exerts a tonic inhibition of LC noradrenergic neurons^[16]. In

addition, the lesion of 5-HT neurons with 5,7-dihydroxytryptamine increased the spontaneous firing activity of LC noradrenergic neurons^[17]. Neurochemical and neuropathological studies have shown that 5-HT concentration is significantly reduced in the some areas of the brain and cerebrospinal fluid, and loss of 5-HT neurons in the raphe nuclei of Parkinsonian patients^[18]. Several studies have also observed a reduction of 5-HT_{1A} binding sites in the raphe nuclei of patients with PD and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-treated monkeys^[19]. Thus, the reduction of inhibitory inputs from the raphe nuclei to the LC after lesion of the SNc causes an increase of activity of LC noradrenergic neurons. The LC also receives excitatory glutamatergic afferents from the paragigantocellularis nucleus^[20]. Stimulation of the paragigantocellularis nucleus activates LC noradrenergic neurons and this response is blocked by excitatory amino acid receptor antagonists^[21]. However, the activity of paragigantocellularis nucleus is still unclear. From all the mentioned above, the activity of LC noradrenergic neurons is influenced by the complex interaction of various afferent projections to this nucleus, and the balance between these inhibitory and excitatory drives could be responsible for the overactivity of LC noradrenergic neurons. These results also indicate the overactivity of LC noradrenergic neurons in the LC may represent a compensatory mechanism for the dopaminergic dysfunction in PD.

Acknowledgements: This work was supported by the Foundation of Science and Technological Program of Shaanxi Province, China (No. 2007K15-G1).

References:

- [1] Gesi M, Soldani P, Giorgi FS, Santinami A, Bonaccorsi I, Fornai F. The role of the locus coeruleus in the development of Parkinson's disease. *Neurosci Biobehav Rev* 2000, 24: 655-668.
- [2] Halliday GM, Blumbergs PC, Cotton RG, Blessing WW, Geffen LB. Loss of brainstem serotonin- and substance P-containing neurons in Parkinson's disease. *Brain Res* 1990, 510: 104-107.
- [3] Perry RH, Irving D, Tomlinson BE. Lewy body prevalence in the aging brain: relationship to neuropsychiatric disorders, Alzheimer-type pathology and catecholaminergic nuclei. *J Neurol Sci* 1990, 100: 223-233.
- [4] Zarow C, Lyness SA, Mortimer JA, Chui HC. Neuronal loss is greater in the locus coeruleus than nucleus basalis and substantia nigra in Alzheimer and Parkinson diseases. *Arch Neurol* 2003, 60: 337-341.
- [5] Cash R, Ruberg M, Raisman R, Agid Y. Adrenergic receptors in Parkinson's disease. *Brain Res* 1984, 322: 269-275.
- [6] Paxinos G, Watson C. The rat brain in stereotaxic coordinates. 4th ed. In: Academic Press. Sydney, 1998.
- [7] Cao J, Liu J, Zhang QJ, Wang T, Wang S, Han LN. The selective 5-HT_{1A} receptor antagonist WAY-100635 inhibits neuronal activity of the ventromedial prefrontal cortex in a rodent model of Parkinson's disease. *Neurosci Bull* 2007, 23: 315-22.
- [8] Vlamings R, Visser-Vandewalle V, Koopmans G, Joosten EA, Kozan R, Kaplan S, *et al.* High frequency stimulation of the subthalamic nucleus improves speed of locomotion but impairs forelimb movement in Parkinsonian rats. *Neuroscience* 2007, 148: 815-823.
- [9] Pineda J, Torrecilla M, Martin-Ruiz R, Ugedo L. Attenuation of withdrawal-induced hyperactivity of locus coeruleus neurones by inhibitors of nitric oxide synthase in morphine-dependent rats. *Neuropharmacology* 1998, 37: 759-767.
- [10] Wang Y, Zhang QJ, Liu J, Feng J, Chu YX, Gao R, *et al.* Increase of firing rate with changes in firing pattern of neurons of the pedunculopontine nucleus in 6-hydrodopamine lesioned rats. *Neurosci Bull* 2005, 21: 146-152.
- [11] Borsody MK, Weiss JM. Influence of corticotropin-releasing hormone on electrophysiological activity of locus coeruleus neurons. *Brain Res* 1996, 724: 149-168.
- [12] Starke K. Presynaptic alpha-autoreceptors. *Rev Physiol Biochem Pharmacol* 1987, 107: 73-146.
- [13] Pineda J, Ruiz-Ortega JA, Ugedo L. Receptor reserve and turnover of alpha-2 adrenoceptors that mediate the clonidine-induced inhibition of rat locus coeruleus neurons *in vivo*. *J Pharmacol Exp Ther* 1997, 281: 690-698.
- [14] Arima J, Kubo C, Ishibashi H, Akaike N. alpha2-Adrenoceptor-mediated potassium currents in acutely dissociated rat locus coeruleus neurones. *J Physiol* 1998, 508 (Pt 1): 57-66.
- [15] Kish SJ, Shannak KS, Rajput AH, Gilbert JJ, Hornykiewicz O. Cerebellar norepinephrine in patients with Parkinson's disease and control subjects. *Arch Neurol* 1984, 41: 612-614.
- [16] Maeda T, Kojima Y, Arai R, Fujimiya M, Kimura H, Kitahama K, *et al.* Monoaminergic interaction in the central nervous system: a morphological analysis in the locus coeruleus of the rat. *Comp Biochem Physiol C* 1991, 98: 193-202.
- [17] Luppi PH, Aston-Jones G, Akaoka H, Chouvet G, Jouvet M. Afferent projections to the rat locus coeruleus demonstrated by retrograde and anterograde tracing with cholera-toxin B subunit and Phaseolus vulgaris leucoagglutinin. *Neuroscience* 1995, 65: 119-160.

- [18] Kerenyi L, Ricaurte GA, Schretlen DJ, McCann U, Varga J, Mathews WB, *et al*. Positron emission tomography of striatal serotonin transporters in Parkinson disease. *Arch Neurol* 2003, 60: 1223-1229.
- [19] Kumar JS, Mann JJ. PET tracers for 5-HT(1A) receptors and uses thereof. *Drug Discov Today* 2007, 12: 748-756.
- [20] Aston-Jones G, Ennis M, Pieribone VA, Nickell WT, Shipley MT. The brain nucleus locus coeruleus: restricted afferent control of a broad efferent network. *Science* 1986, 234: 734-737.
- [21] Ennis M, Aston-Jones G. Activation of locus coeruleus from nucleus paragigantocellularis: a new excitatory amino acid pathway in brain. *J Neurosci* 1988, 8: 3644-3657.

帕金森病模型大鼠蓝斑核肾上腺素能神经元电活动增强

王涛¹, 张巧俊², 刘健^{1,3}, 吴仲恒², 王爽¹

¹ 西安交通大学医学院生理学与病理生理学系, 西安 710061

² 西安交通大学第二附属医院神经内科, 西安 710004

³ 环境与疾病相关基因教育部重点实验室, 西安 710061

摘要: **目的** 观察帕金森病模型大鼠蓝斑核肾上腺素能神经元电活动的改变。**方法** 在黑质致密部局部注射 6-羟多巴胺损毁右侧黑质纹状体通路后, 采用细胞外记录方法分别于损毁术后第2周和第4周观察大鼠蓝斑核肾上腺素能神经元的电活动变化。**结果** 6-羟多巴胺损毁术后第2周和第4周, 蓝斑核肾上腺素能神经元放电频率比对照组显著增加($P < 0.05$)。损毁术后第4周, 蓝斑核呈不规则放电的神经元比例显著高于对照组($P < 0.05$)。**结论** 6-羟多巴胺损毁术后, 大鼠蓝斑核肾上腺素能神经元活动增强, 且更不规则, 提示蓝斑核参与帕金森病的发病。

关键词: 蓝斑核; 6-羟多巴胺; 帕金森病; 电生理学