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Distribution of Fos-immunoreactive cells in the ventral part of rat medulla following voluntary treadmill exercise

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ABSTRACT

The ventral part of the medulla, which contains important cardiovascular regions, is reportedly activated during exercise. Nevertheless, it was uncertain which region(s) in the ventral medulla are specifically activated by exercise. The present study aimed to demonstrate a general pattern of exercise-specific distribution of excited neuronal cells in the rat ventral medulla. Via immunohistochemical experiments, we mapped tyrosine hydroxylase- and Fos-immunoreactive cells (TH-IR and Fos-IR cells, respectively) on rat medullary coronal sections following a bout of voluntary treadmill exercise, a comparative control period, or after pharmacologically induced-hypotension under anesthesia. In the ventral medulla at the rostrocaudal level adjacent, but not rostral or caudal, to the caudal edge of the facial nucleus, voluntary treadmill exercise induced significant ($P < 0.05$) increases in Fos expression, similar to hypotension. The rostral ventrolateral medulla (RVLM), as compared with the rostral ventromedial medulla (RVMM), displayed a greater number of Fos-IR cells due to either exercise or hypotension. In the RVLM, either exercise or hypotension induced significant expression of Fos in both TH-IR and TH non-immunoreactive cells. In the caudal ventrolateral medulla (CVLM), hypotension, but not exercise, increased the ratio of Fos-IR cells in the TH-IR population. These findings demonstrate that RVLM adrenergic and non-adrenergic neurons are specifically excited by voluntary exercise in rats, while RVMM or CVLM neurons are not. We suggest that RVLM C1/non-C1 neurons are a major part of central circuitries underlying sympathetic adjustments to exercise.

1. Introduction

Voluntary exercise elicits sympathoexcitation and pressor and tachycardia responses (Mark et al., 1985; Miki et al., 2002). Although the central mechanisms underlying sympathetic adjustments to exercise are not fully understood, several brain regions have been suggested to be involved in exercise-related processes (Dampney, 2016). The ventral part of the medulla contains sympathoexcitatory regions, such as the rostral ventrolateral and ventromedial medulla (RVLM and RVMM, respectively), in which sympathetic premotor neurons are located (Strack et al., 1989). The ventral medulla is activated by exercise, as reportedly shown by an increase in the expression of Fos protein, a marker of neural activation (Sagar et al., 1988) after voluntary treadmill exercise in rats (Barna et al., 2012; Iwamoto et al., 1996; Ohiwa et al., 2006). In these studies, while the number of Fos-immunoreactive (Fos-IR) cells in each medullary region was compared between exercised rats and control animals, information was insufficient regarding the pattern of distribution in the medulla of excited neuronal cells due to exercise. Thus, it has been uncertain which region(s) in the ventral medulla are specifically activated during exercise. Identifying the region(s) in the ventral medulla that are primarily activated by voluntary exercise is an important step in understanding how central mechanisms underlie sympathetic adjustments to exercise.

The present study aimed to demonstrate a general pattern of voluntary exercise-specific distribution of excited neuronal cells in the rat ventral medulla. Via immunohistochemical experiments, we mapped Fos-IR cells as well as tyrosine hydroxylase (TH)-immunoreactive (TH-IR) neurons on coronal sections of the ventral medulla of 1) rats which had voluntarily run on the treadmill (Exercise rats), 2) non-exercised control rats (Control rats), and 3) rats that were exposed to

Abbreviations: CVLM, caudal ventrolateral medulla; Fos-IR, Fos-immunoreactive; Fos^+TH^+ cells, Fos-immunoreactive cells with TH immunoreactivity; Fos^+TH^- cells, Fos-immunoreactive cells without TH immunoreactivity; MLR, mesencephalic locomotor region; PVN, paraventricular hypothalamic nucleus; RVMM, rostral ventromedial medulla; RVLM, rostral ventrolateral medulla; TH, tyrosine hydroxylase; TH-IR, tyrosine hydroxylase-immunoreactive

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pharmacological hypotension under anesthesia (Hypotension rats). The hypotension protocol, that elicits sympathoexcitation (Scislo et al., 1998) as does exercise, was conducted to compare the pattern of distribution of Fos-IR cells in the ventral medulla with that resulting from voluntary treadmill exercise. TH immunoreactivity was examined to segment the medullary cardiovascular regions such as the RVLM, in which C1 adrenergic neurons are abundantly distributed, and differentiate between RVLM C1 and non-C1 neurons.

2. Materials and methods

2.1. Ethical approval

All procedures outlined in this study complied with the Guiding Principles for the Care and Use of Animals in the Fields of Physiological Sciences of the Physiological Society of Japan as well as the European Communities Council Directive of 1986 (86/609/EEC), and were approved by the Animal Care Committee of Tottori University (reference number: 15-Y-40). The experiments were performed on male Sprague–Dawley rats (N = 17, 8.4 ± 0.5 wks, 310 ± 12 g of body weight, means ± SEM). Rats were housed in standard rodent cages in a temperature-controlled room (24–25 °C) and were exposed to a 12:12 h light–dark schedule. Food and water were made available ad libitum.

2.2. Protocols

Thirteen rats were treadmill exercise-trained four-to-five times per week and for a total of nine-to-ten days according to a progressive exercise protocol described previously (Imaoka et al., 2013; Koba et al., 2014) (Table 1). On the first day of the protocol, the rats were acclimatized to a custom-built treadmill (MK-680-C; Muromachi, Tokyo, Japan) by running at 12–14 m/min, 0° incline for 10–15 min that followed a 5-min resting period and a 1-min buzzer sound (1 Hz) as a trigger of onset of the exercise. The speed, incline angle, and duration for running were then increased by 1–2 m/min, 0–2°, and 5–10 min per day over a week until rats ran at 20 m/min, 5° incline for 45 min. The exercise parameters were determined based on our previous observations that arterial pressure (AP) of conscious rats was increased and reached a steady value of about + 15 mm Hg within 10 min after the onset of voluntary treadmill exercise at 20 m/min, 5° incline (Imaoka et al., 2013). If the running pace became below the treadmill rate during each training session, a mild but aversive electrical stimulation of foot would be provided to rats with a shock grid installed at the rear of the treadmill. Nevertheless, the rats were administered very few shocks by receiving a gentle nudge with the cotton swab when they were about to touch the grid. Although we had planned to exclude rats that would exhibit refusal to run on the treadmill, all thirteen rats completed the training program. A period of one or two days was allowed between the final day for the training and the protocol day (Table 1).

On the protocol day, seven of the thirteen rats were randomly chosen as “Exercise” group. The Exercise rats were brought to the treadmill, on which any foot shocks had never been administered, and kept for 10 min in the resting period. After the 1-min buzzer period, they were subjected to 45-min voluntary treadmill exercise (20 m/min, 5°). Even the nudges were not necessary during actual data runs. The other six of the thirteen rats were brought to the treadmill but not subjected to voluntary exercise as a control (Control rats). Ninety minutes after the offset of the treadmill exercise or the control period, the rats were deeply anesthetized with 5% isoflurane in oxygen and perfused transcardially with heparinized saline followed by 4% paraformaldehyde in 0.1 mol/L PBS (pH 7.4). The brains were removed and postfixed for 4–12 h in 4% paraformaldehyde and then transferred to a 30% sucrose solution at 4 °C for 24–48 h. Then, the brains were embedded in Optimal Cutting Temperature Compound (Sakura Finetek,

Table 1
Timeline diagram demonstrating the protocol, from the training until brain extraction for immunohistochemical experiments.

Rat group	1st to 9–10th day		One or two days		Protocol day								
Control	Exercise training, 4–5/week	→	No exercise	→	10 min rest on the treadmill	→	1 min buzzer	→	45 min rest on the treadmill	→	90 min waiting	→	Perfusion under anesthesia, and brain extraction
Exercise	Exercise training, 4–5/week	→	No exercise	→	10 min rest on the treadmill	→	1 min buzzer	→	45 min treadmill exercise, 20 m/min, 5° incline	→	90 min waiting	→	Perfusion under anesthesia, and brain extraction
Hypotension	N/A		N/A		Catheter implantation under anesthesia			→	30–45 min hypotension under anesthesia	→	90 min waiting under anesthesia	→	Perfusion under anesthesia, and brain extraction