Effect of Several Durations of Cold Exposure on Brain Monoamines Concentrations in the Dorso Medial Hypothalamus in Rats

様々な期間の寒冷暴露がラット視床下部背内側核における脳内モノアミン濃度に及ぼす影響

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Abstract

The present study intended to clarify differences in concentrations of brain monoamines (norepinephrine, serotonin and dopamine) in the dorso medial hypothalamus (DMH) after cold exposure of several durations. Rats were exposed to a cold environment (5˚C) for 3 hours, 1 day, 7 days, 14 days or 28 days. After cold exposure, the DMH was immediately extracted and homogenized. Brain monoamines in the extract were measured by high performance liquid chromatography. Norepinephrine concentration in the DMH after 28 days cold exposure was significantly lower than control level. However, in other groups, no significant differences were observed. Furthermore, serotonin and dopamine concentrations in the DMH were not affected by all cold exposure sessions. These results suggested that long term exposure to cold decreases only noradorenergic activity in the DMH.

Keywords: Thermoregulation, monoamine, HPLC, hypothalamus

概要

本研究においては、様々な期間の寒冷暴露が視床下部背内側核（DMH）の脳内モノアミン類（ノルエピネフリン、セロトニン、ドーパミン）濃度にどのように影響するか検討することを目的とした。ラットを寒冷環境（5℃）に 3 時間、1、7、14、28 日間暴露した。暴露終了後、直ちに DMH を摘出し、組織をホモジナイズし、サンプルを抽出した。サンプル中のモノアミン濃度は高速液体クロマトグラフィーを用いて定量した結果、28 日間の寒冷暴露を行った群においてのみ、コントロール群と比較してノルエピネフリン濃度が有意に低い値を示した。さらにセロトニン及びドーパミンは寒冷暴露の影響を受けなかった。本研究より、長期間の寒冷暴露が DMH におけるノルエピネフリン作動性神経活動を低下させる可能性が示唆された。

キーワード：体温調節、モノアミン、HPLC、視床下部

1 Introduction

It is established that chronic exposure to cold improved cold tolerance by increasing non-shivering thermogenesis in the brown adipose tissue in rodents (Cannon and Nedergaard, 2004). Furthermore, previous Fos studies suggested that multiple hypothalamic regions involved body temperature regulation during chronic cold exposure (Miyata et al., 1995).

Several systems are implicated in the neural activity of the hypothalamus that occurs during thermoregulation in a cold environment. One candidate system involves monoaminergic neurons. Monoaminergic activity in the brain is an important physiological modulator in several situations (Beverly et al., 2001; Madden et al., 2006). We previously reported that chronic exposure to cold affected concentrations of monoamines in the preoptic area of hypothalamus, poseterior hypothalamus and vetro medial hypothalamus (Saito et al., 2005). Although these three hypothalamic regions have been generally recognized as critical sites for thermoregulation under a cold environment (Ishiwata et al., 2005; Li and Thornhill, 1998; Chen et al., 1998), recent studies suggested that the dorso medial hypothalamus (DMH) was also important for controlling body temperature (Dimicco and Zaretsky, 2006). However, there were no studies to investigate the monoamine change during cold exposure. The purpose of the present study was to determine the concentrations of monoamines (norepinephrine, serotonin and dopamine) in the DMH after different duration of cold exposure. We ultimately aimed to clarify the role of monoamines in the DMH of rat during cold acclimation.

2 Materials and methods

The study was conducted in 36 male Wistar rats (240-360 g body weight). The rats were housed separately in plastic cages under controlled conditions of ambient temperature (Ta) of 23 °C, relative humidity (RH) of 50%, and 12 h light/dark cycle (lights on at 06:00 h), and were allowed free access to food and water. All experiments were carried out according to the guiding principles for Care and Use of Animals in the Field of Physiological Science of the Physiological Society of Japan.

Rats were divided into six groups and exposed to a cold environment (Ta, 5 °C, RH, 50%) for 0 days (i.e., not exposed to cold, Control), 3 hours (3H), 1 day (1D), 7 days (7D), 14 days (14D) or 28 days (28D). After cold exposure, the rats were sacrificed and the DMH were immediately dissected out. The coordinate with respect to bregma was DMH: anterior – posterior -3.14 mm; lateral +0.5 mm; dorsal – ventral 8.3 mm (Paxinos and Watson, 1997; Figure 1). After washing the tissues with Ringer's solution (in mM, 147 NaCl, 4 KCl, and 2.3 $CaCl_2$), we ground the tissues gently with two frosted surface slide-glass slips (Matsunami, Japan) in 100 ml ice-cold 0.1 M perchloric acid (PCA). After homogenization was completed, the slide glasses were washed with another 100ml PCA. This procedure yielded samples of about 200ml from each tissue block. The homogenates were centrifuged at 12000g for 5min and the supernatants were then filtered (0.45mm, Millipore, Bedford, MA). Concentrations of norepinephrine, serotonin and dopamine in the sample were measured by high-performance liquid chromatography (HPLC) (Saito et al., 2005). Briefly, HPLC system was equipped with two amperometric electrochemical detectors (LC-4C, Bioanalytical Systems) and pumps (PM-70 and LC-100, BAS). We used a 5-mm C-18 polymetric column (1.0 mm id × 15 cm, BAS) for measurement of norepinephrine, and a 3-mm C-18 column (1.0 mm id × 10 cm, BAS) for measurement of serotonin and dopamine.

In analysis of monomaines, one-way ANOVA followed by Bonferroni/Dunn's post-hoc tests were performed. Data are expressed as mean ± SEM values. $P < 0.05$ was regarded as statistically significant.

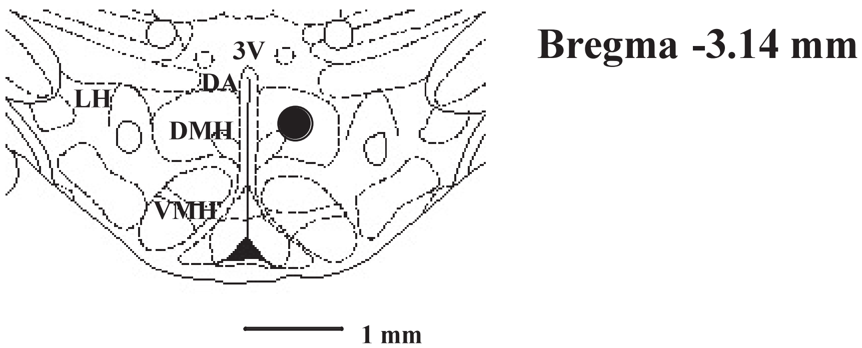

Figure 1. Schematic representation of a coronal section showing location of dissected site (●). 3V, 3rd ventricle; DA, dorsal hypothalamic area; DMD,dorsomedial hypothalamic nucleus, diffuse; LH, lateral hypothalamus; VMH, ventromedial hypothalamus.

3 Results

Figure 2 shows changes in monoamines in the DMH of control, 3H, 1D, 7D, 14D and 28D groups. The concentrations of both serotonin and dopamine (control values in the DMH were 0.80 ± 0.22 and 1.31 ± 0.55 pmol/mg w. w., respectively) were not affected by cold exposure. As for the concentration in norepinehprine, significant low level of norepinephrine in the 28D group compared to the control group was observed. Thus, norepinephrine in the control, 3H, 1D, 7D and 14D were: 6.96 ± 0.73 pmol/mg w. w., 9.03 ± 0.80 pmol/mg w. w., 6.27 ± 0.58 pmol/mg w. w., 5.00 ± 1.24 pmol/mg w. w., 6.54 ± 1.33 pmol/mg w. w., respectively, however, norerpinephrine in the 28D group (2.13 ± 0.48 pmol/mg w. w.) was significantly lower than control value.

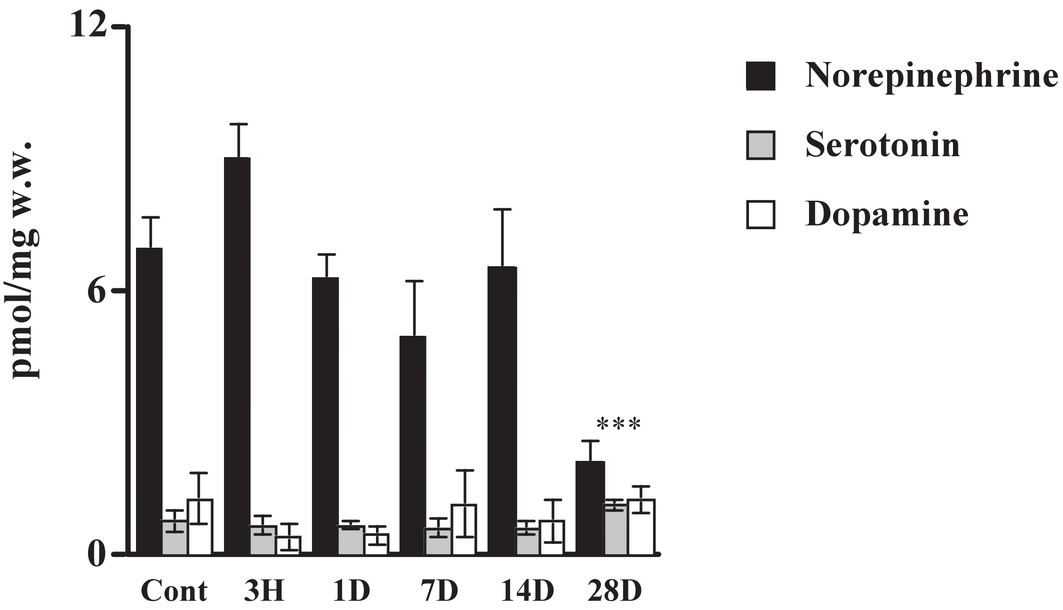

Figure 2. Concentrations of norepinephrine (solid bars), serotonin (tippled bars) and dopamine (open bars) in the dorso medial hypothalamus in control, 3H, 1D, 7D, 14D and 28D groups.
Data are means ± SEM of 6 rats.
Asterisk indicates a significant difference between the control and each conditions.
*** $P < 0.001$, compared with the control.

4 Discussion

In this study, although short and long terms exposure to cold did not affect on serotonin and dopamine, we observed time related differences in the concentration of norepinephrine in the DMH. Norepinephrine concentrations of 3H, 1D, 7D and 14D groups were similar to control value, however, it was observed significantly low level of norepinephrine only in the 28D group. Thus, with respect to the well-known transition from shivering to non-shivering thermogenesis in cold acclimation (Bruck et al., 1970), the data of the present study suggest the involvement of low level norepinephrine in the DMH in the adjustment of non shivering thermogenesis.

Dimicco and colleagues reported that chemical stimulation of the DMH evoked non-shivering thermogenesis (Zarestskaia et al., 2002), and other studies also indicated that the DMH was one of the critical region for thermoregulation especially by controlling non shivering thermogenesis (Nakamura and Morrison, 2007). Although it has been recognized that norepinephrine in the DMH plays essential role for several stress responses, such as panic and anxiety responses, there were no studies investigated that the involvement of this neurotransmitter systems in the DMH in thermoregulation. Since cold stress produce similar aspects to panic or anxiety in rats (R), it is suggested that norepinephrine in the DMH have an important role for thermoregulation in cold acclimated rats. However, further pharmacological and physiological studies are required to investigate the effects of norepinephrine in the DMH on thermoregulation in cold acclimated and non-cold acclimated rats. Such studies should help in clarifying the role of the DMH noradrenergic system in rat cold acclimation.

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