Effect of chronic cold exposure on noradrenergic modulation in the ventromedial hypothalamus of core body temperature regulation in freely moving rats

慢性寒冷暴露が視床下部腹内側核ノルアドレナリン作動性神経の
自由行動下ラット体温調節に及ぼす影響

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Abstract

The present study intended to compare the involvement of noradrenalone (NA) in the ventoromedial hypothalamus (VMH) in core body temperature (Tcore) regulation between room temperature acclimated rat (RA group, kept at 23℃ for 2 wks) and cold acclimated rat (CA group, kept at 5℃ for 2wks). We quantified the release of NA in the VMH during 3 h cold exposure (5℃) in both groups. Cold exposure significantly increased the extracellular NA levels in the VMH only in the CA group: not in the RA group. To further evaluate relationships between these different results in VMH NA levels and T_{core} regulation in the cold, we measured T_{core} during perfusion of α-adrenoceptor antagonist phenoxybenzamine during cold exposure. This pharmacological procedure induced significant hypothermia only in the CA group: no changes were observed T_{core} in the RA group. These results suggested that NA in the VMH was an important neuromodulator system for thermoregulation under cold environment in cold acclimated animals.

Keywords: thermoregulation; ventromedial hypothalamus; noradrenaline; cold acclimation; microdialysis

概要

本研究では、定常飼育ラット（RA 群）と寒冷順化ラット（CA 群）における視床下部腹内側核（VMH）ノルアドレナリン（NA）の体温調節に対する役割についての比較を行った。3 時間の寒冷暴露（5℃）は CA 群において VMH の NA 量を増加させたが、RA 群においてはこの変化は観察されなかった。さらに寒冷暴露中のαアドレナリン受容体遮断薬である phenoxybenzamine の VMH への還流投与は、CA 群において深部体温（T_{core}）を低下させたが、RA 群の T_{core} には影響しなかった。本研究結果より、寒冷順化ラットにおいては、VMH の NA 作動性神経が寒冷環境での体温調節に重要な役割を果たしている可能性が示唆された。

Keywords：体温調節；視床下部腹内側核；ノルアドレナリン；寒冷順化；マイクロダイアリシス

Introduction

Chronic exposure to a cold environment for several weeks involves adaptive autonomic thermoregulatory changes in rodents. The threshold for shivering induction in muscles decreases, and the capacity for non-shivering thermogenesis in brown adipose tissue (BAT) increases: thus, the replacement of shivering thermogenesis by non-shivering thermogenesis occurs during continuous cold exposure [Cannon and Nedergaard, 2004; Griggio, 1982]. Chronic cold exposure also causes neuroadaptive changes in thermoregulatory sites of the brain [Pacak and Palcovits, 2001]. Miyata et al. (1995) have been reported that the extent of cold-induced Fos in the ventromedial hypothalamus (VMH, one of the locus of BAT controller [Thornhill and Halvorson, 1990; Woods and Stock, 1994]) was increased after 14 d cold exposure relative to those after 3 h and 24 h exposure [Miyata et al., 1995]. Li et al. (1997) reported that the thermoresponsiveness of cold-sensitive neurons within the VMH to scrotal cold stimulation is significantly higher in cold-acclimated rats than in normal rats [Li et al., 1997]. These results suggested that the neuronal adaptation of VMH neurons is a contributory step in increases the capacity for non-shivering thermogenesis during chronic cold exposure. However, it is still unclear what brain neuromodulater systems implicate in this VMH neuronal adaptation during long-term cold exposure.

One candidate system involves the noradrenergic neurons, because we previously reported that noradrenaline (NA) in tissue extracts of the VMH started to increase with some delay, becoming significantly elevated first on day 7, with a tendency for further increase over the course of 28 d cold exposure: however, concentrations of dopamine and serotonin in the VMH were not affected by acute and chronic cold exposure [Saito et al., 2005]. Furthermore, noradrenergic system in the brain is an important physiological modulator of cold and other types of stress [Pacak and Palkovits, 2001].

To further understanding of the effect of chronic cold exposure on thermoregulatory actions of NA within the VMH, we used microdialysis, high performance liquid chromatography (HPLC), and biotelemetry. The study was comprised of two parts. In the first part, NA release was monitored in the VMH in freely moving animals. In the second part, the VMH were stimulated pharmacologically with simultaneously measuring core body temperature (T_{core}). In both parts, room temperature and cold acclimated animals were evaluated during 3 h cold exposure (5°C).

Materials and methods

Male Wistar rats (250-350 g body weight) were housed separately in plastic cages under con-

trolled conditions of ambient temperature (23°C), relative humidity (50%), and a 12/12 h light/dark cycle (lights on at 06:00 h). Rats had free access to food and water except during the experiments. All experiments were carried out according to the Guiding Principles for the Care and Use of Animals in the Field of Physiological Science of the Physiological Society of Japan.

Six days before acclimation trials began, biotelemetry devices (TA10ETA-F20; Data Sciences International, USA) were implanted in the anesthetized rats' (pentobarbital, 50 mg/kg, i.p.) peritoneal cavities, which allowed continuous monitoring T_{core} [Hasegawa et al., 2005, Ishiwata et al., 2005, Saito et al., 2005]. Rats were divided randomly into the room temperature acclimated (RA) group (N=5), kept ambient temperature (T_{amb}) at 23°C for 2 weeks, and the cold temperature acclimated (CA) group (N=5), kept T_{amb} at 5°C for 2 weeks in a temperature-controlled chamber. After acclimation trials, rats were anesthetized with pentobarbital (50 mg/kg, i.p.) and a microdialysis probe (0.22 mm external diameter, 2.0-mm-long dialyzing membrane and molecular weight cutoff value of 50,000 cellulose membrane, A-I-8-02; Eicom Corp., Japan) was implanted stereotaxically in the left VMH (AP -3.30 mm; L +0.8 mm; D -10.2 mm from dura) [Paxinos and Watson, 2004]. The probe was secured to the skull using dental cement. Subsequently, rats recovered for at least 2 d. At least 2 h before the beginning of the experiment, a microdialysis probe was connected to a microinjection pump (CMA 100; CMA Microdialysis AB, Sweden) and perfused with Ringer's solution (in mM, 147 NaCl, 4 KCl, 2.3 $CaCl_2$) at a 1 μl / min flow rate.

Microdialysates were analyzed for NA using HPLC, as described previously [Hasegawa et al., 2000; Ishiwata et al., 2004; Saito et al., 2005; Yasumatsu et al., 1998]. Briefly, Identification of unknown peaks in a sample was accomplished by matching the reaction times of peaks with those of authentic standards (Chromograph Report software; Bioanalytical Systems Inc., USA). The HPLC system was equipped with an amperometric electrochemical detector (LC-4C; Bioanalytical Systems Inc., USA) and a pump (PM-70; BAS Inc., Japan). We used a 5 μm C-18 polymeric column (1.0 mm internal diameter × 15 cm; BAS Inc., Japan) for HPLC. A 10 μl of the sample was injected into HPLC by an automated HPLC robot (CMA200; CMA/Microdialysis AB, Sweden).

Rats were tested under two counterbalanced experimental protocols. 1) To investigate whether the release pattern of NA in the VMH during cold exposure would be different between RA and CA groups, we collected microdialysate from the VMH during 180 min cold exposure (5°C) in RA and CA groups. 2) To investigate the effects of noradrenergic blockade in the VMH on T_{core} are different for RA and CA groups, we perfused the VMH with 10 mM of phenoxybenzamine (PB) (Alexis Platform, USA), an α-adrenoceptor blocking agent, during cold exposure (5°C). PB was dissolved in Ringer's solution. This particular dosage of PB was chosen from previous rat microdialysis studies [Forray et al., 1999; Nakata et al., 1990] and our preliminary examination.

At the end of each experiment, rats were sacrificed using an overdose of pentobarbital (120 mg/kg, i.p.). The location of the microdialysis probe was verified in coronal sections stained with bromophenol blue using a photomicroscope (IX-70; Olympus Optical Co., Japan).

Microdialysates were collected every 10 min and the measured parameters were expressed as percentages of the baseline value. The value of the first sample was considered as 100%. We measured T_{core} every 1 min and averaged those values every 10 min. Differences among data were evaluated for statistical significance using repeated analysis of variance (ANOVA) followed by Bonferroni/Dunn's post hoc tests. Values are expressed as the mean ±SEM. A value of $P < 0.05$ was inferred as statistically significant.

Results

We performed successful experiments on both RA (open circle) and CA (close circle) groups (RA: n = 5, CA: n = 5). As shown in Fig. 1, the tip of the microdialysis probe was correctly positioned into the VMH in all these animals.

Fig. 2 shows respective changes in extracellular NA levels in the VMH during cold exposure (from 60 min to 240 min) in the RA and CA groups. Samples were collected every 10 min. The value of the first sample in each rat was designated as 100%. Baseline concentrations (pg / 10 μl) of NA in the VMH were 0.79 ± 0.61 and 0.94 ± 0.32 in the RA and CA groups, respectively. In the RA group, no significant changes in extracellular levels of NA were observed during cold exposure. On the other hand, NA tended to increase from baseline values in the early stage of cold exposure (125 ± 7% at 110 min); significant increases were observed in the late stage (208 ± 40% and 205 ± 37% at 180 min and 240 min, respectively) and this significant high level of NA was maintained after cold exposure in the CA group.

Fig. 3 shows changes in T_{core} during perfusion of PB into the VMH (from 180 min to 240 min) during cold exposure in RA and CA groups. Cold exposure induced significant increases in T_{core} (RA: 37.8 ± 0.1°C, CA: 37.8 ± 0.1°C at 180 min) in both groups. Perfusion of PB into the VMH was not affected to T_{core} in the RA group. However, this pharmacological procedure induced significant hypothermia in the CA group. Significant decreases in T_{core} were observed from 200 min (37.4 ± 0.2°C) to 240 min (37.0 ± 0,2°C). T_{core} in both groups tended to return to baseline levels after cold exposure.

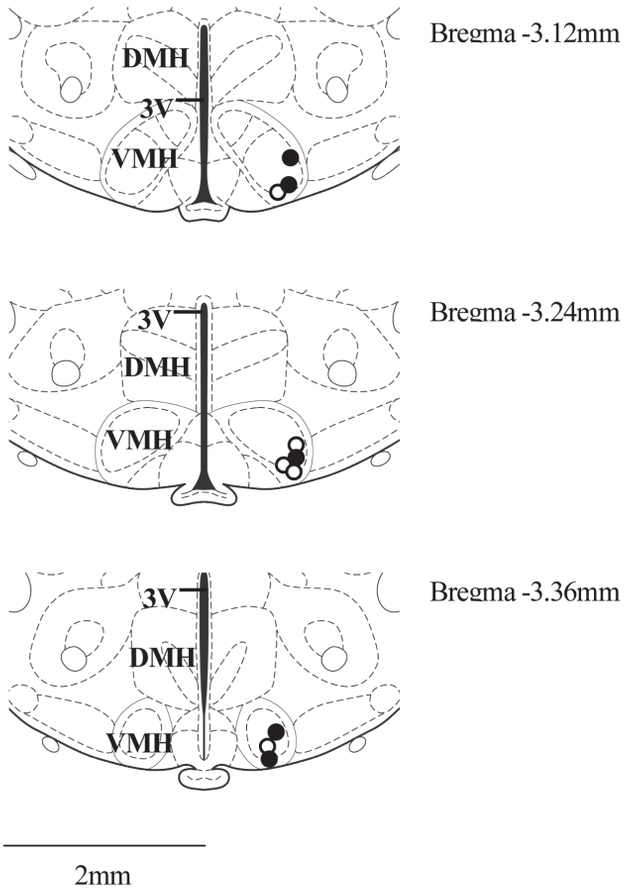

Fig.1. Schematic presentation of the location of microdialysis probes tips in a coronal plane. Each symbol (open circle: RA group, close circle: CA group) indicates the location of the microdialysis probe. 3 V, third ventricle; DMH, dorsomedial hypothalamus; VMH, ventromedial hypothalamus

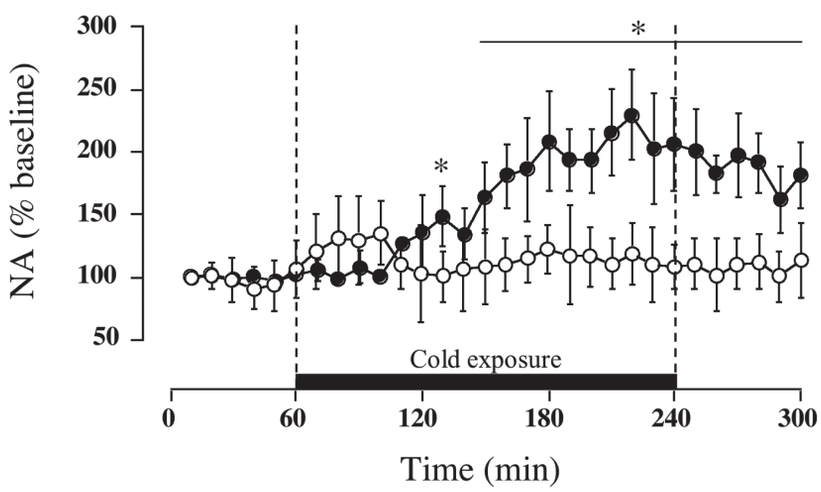

Fig.2. Changes in extracellular NA level in the VMH during 3 h of cold exposure in the RA group (open circle, n = 5) and the CA group (close circle, n = 5). The value of NA in the first sample was considered as 100%. Values are means ± SEM. *Significant differences compared with baseline value in the CA group, $P < 0.05$.

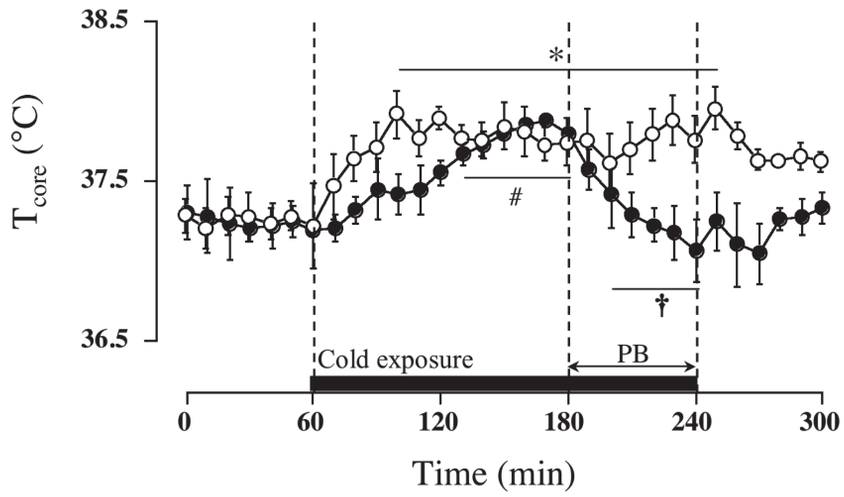

Fig.3. Changes in T_{core} elicited by perfusion of the VMH with 10 mM phenoxybenzamine (PB) during cold exposure (5℃) in the RA group (open circle, n = 5) and the CA group (close circle, n = 5). Values are means ± SEM. *Significant differences compared with baseline value at a point of 60 min in the RA group, $P < 0.05$. #Significant differences compared with baseline value at a point of 60 min in the CA group, $P < 0.05$. † Significant differences compared with value at a point of 180 min in the CA group, $P < 0.05$.

Discussion

In this study, we observed significant increases in NA efflux within the VMH during 3 h cold exposure only in the CA group not in the RA group. Also, blockade of α-adrenoceptor in the VMH during acute cold exposure by the microdialysis of PB caused a significant hypothermia only in the CA group.

Neurons in the VMH have been widely reported as playing an important role for controlling of non-shivering thermogenesis in BAT. For example, Thornhill and Halvorson (1990) demonstrated that electrical stimulation of the VMH increased BAT temperature [Thornhill and Halvorson, 1990]. Amir (1990) reported that microinjection of glutamate into the VMH activated BAT thermogenesis [Amir, 1990]. It has also been reported that colchicine lesions of the VMH suppress BAT activity [Preston et al., 1989]. Furthermore, cold-induced Fos expression in the VMH was significantly high only after 14 d cold exposure [Miyata] and thermosensitivity of individual VMH cold-sensitive neurons of cold-acclimated rats was significantly higher than those of room temperature acclimated rats [Li and Thornhill, 1997]. These previous studies suggested that neural adaptation within the VMH to cold stimuli occurred during chronic exposure to cold. However, little is known about what neuromodulator system is participated in this VMH neural adaptation. In the present study, we demonstrated that cold induced increase in NA in the VMH was observed only in the CA group.

This result consisted with our recent study, reported that remarkable increases in concentrations of NA in the VMH of 14 d cold exposed rats [Saito et al., 2005]. Moreover, perfusion of α-adrenoceptor antagonist into the VMH during cold exposure induced significant hypothermia only in the CA group. These results suggested that NA release in the VMH in response to cold stimuli was increased during chronic cold exposure, and the improvement of VMH NA activity was important neuromodulator system involves thermoregulation under cold environment in cold acclimated animals.

Although we observed significant hypothermia after perfusion of α-adrenoceptor blocking agent into the VMH during acute cold exposure in the CA group, what thermoregulatory responses types (i.e. shivering thermogenesis, non-shivering thermogenesis and/or peripheral vasoconstriction) inhibition caused this hypothermia was remain unclear. However, previous studies reported that shivering thermogenesis was decreased in cold acclimated rodents under cold environment [Gautier et al., 1991; Golozoubova et al., 2001]. Furthermore, we verified that microdialysing PB into the VMH of CA rats had no effect on tail temperature changing (index of vosoconstriction) during acute cold exposure in preliminary examination. Many previous studies indicated that non-shivering thermogenesis was a major mechanism for heat production in cold acclimated animals [Himms-Hagen, 1984; Cannon et al., 2004; Li and Thornhill, 1997; Golozoubova et al., 2001; Thornhill and Halvorson, 1990]. Taking these observations into account, it would be reasonable to assume that perfusion of α-adrenoceptor antagonist into the VMH of CA group decreased non-shivering thermogenesis and then induced significant hypothermia in this study. However, further studies, such as simultaneous measurement of BAT temperature of cold acclimated animals during current pharmacological procedure, will be needed.

In the RA group we tested here, 3h cold exposure did not affect on extracellar levels of NA in the VMH and blockade of α-adrenoceptor did not induce hypothermia. These results were expected because we previously observed concentrations of NA in the VMH tissue extract of 3h and 1d cold exposed rats were equal to that of control rat [Saito et al., 2005]. With respect to the well-known transition from shivering to non-shivering thermogenesis in cold acclimation [Himms-Hagen, 1984], shivering thermogenesis play a dominant role during 3h cold exposure in the RA group. It has been reported that shivering thermogenesis was controlled by posterior hypothalamus (PH), because cold sensitive neurons were located within the PH [Li and Thornhill, 1998] and PH stimulation evoked shivering thermogenesis in non-cold acclimated rat [Halvorson and Thornhill, 1993]. Thus, it was suggested that the action of neuron in the PH but not in the VMH was important for thermoregulation of the RA group during cold exposure. However, relationship between the neuromodulator system in the PH and thermoregulation under cold environment has been still unclear.

Although we observed NA but not serotonin and dopamine in the PH was decreased in an early phase of cold acclimation [Saito et al., 2005], further pharmaco-physiological examination should be required.

Previous studies have been reported that other brain regions such as the preoptic area and anterior hypothalamus [McAllen 2007], the hypothalamic paraventricular nucleus [Amir, 1990], the dorsomedial hypothalamus [Zaretskaia et al., 2002], the periaqueductal gray [Rathner and Morisson, 2006] the medullary raphe [Nason and Morisson, 2006] also have an important role for controlling BAT thermogenesis. Fos studies have been indicated that acute and chronic cold exposure affected on neuronal activities in these area [Miyata et al., 1995; Bratinesak and Palcovits, 2004; Yoshida et al., 2005; Mckitrick, 2000]. Thus, it was strongly suggested that not only the VMH but also another brain sites involve the development of non-shivering thermogenesis in course of cold acclimation. Furthermore, it was expected to clarify the involvement of other brain neuromodulator/transmitter system such as histaminergic, GABAergic and gulatamatergic neurons in the VMH in cold acclimation.

In summary, our results suggested that the improvement of noradrenergic activity in the VMH might be important neuromodulator system for maintaining T_{core} under cold environment in cold acclimated animals.

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