Life Sciences 83 (2008) 79–84

Contents lists available at ScienceDirect

Life Sciences

journal homepage: www.elsevier.com/locate/lifescie

Effect of chronic cold exposure on noradrenergic modulation in the preoptic area of thermoregulation in freely moving rats

Takehito Saito [a,b,*], Takayuki Ishiwata [c,e], Hiroshi Hasegawa [d], Shigeki Nomoto [a,e], Yasunori Kotani [f], Minoru Otokawa [g], Yasutsugu Aihara [a]

[a] Department of Kinesiology, Graduate School of Science, Tokyo Metropolitan University, 1-1 Minami-ohsawa, Hachioji, Tokyo 192-0397, Japan
[b] Daikin Industries Ltd., 1000-2 Ohtani, Okamoto, Kusatsu, Shiga 525-8526, Japan
[c] Department of Sport and Wellness, College of Community and Human Services, Rikkyo University, 1-2-26 Kitano, Niiza, Saitama 352-8558, Japan
[d] Graduate School of Integrated Arts and Sciences, Hiroshima University, 1-7-1 Kagamiyama, Higashi-hiroshima, Hiroshima 739-8521, Japan
[e] Department of Central Nervous System, Tokyo Metropolitan Institute of Gerontology, 35-2 Sakae-cho, Itabashi, Tokyo 173-0015, Japan
[f] Department of Human System Science, Tokyo Institute of Technology, 2-12-1 Ookayama, Meguro-ku, Tokyo, 152-8550, Japan
[g] Laboratory of Biochemistry, Faculty of Sociology, Hosei University, 4342 Aihara, Machida, Tokyo 194-0298, Japan

ARTICLE INFO

Article history:
Received 15 October 2007
Accepted 15 May 2008

Keywords:
Thermoregulation
Preoptic area
Noradrenaline
Cold acclimation
Microdialysis

ABSTRACT

For this study, we compared the thermoregulatory involvement of noradrenaline (NA) in the medial preoptic area (mPOA) of non-cold acclimated rats to that of cold-acclimated rats. We quantified the release of NA in the mPOA during 3 h cold (5 °C) exposure in room-temperature-acclimated rats (RA group, kept at 23 °C for 2 weeks) and cold-acclimated rats (CA group, kept at 5 °C for 2 weeks). We concurrently monitored the core body temperature (T_c), heart rate (HR), and tail skin temperature (T_t). Cold exposure significantly increased T_c and HR, and decreased T_t in both groups. However, the cold-induced increase of the extracellular NA levels in mPOA was observed only in the RA group: not in the CA group. To elucidate these different results in NA levels further, and to evaluate participation of the mPOA in thermoregulation in the cold, we measured T_c, HR, and T_t during perfusion of α-adrenoceptor antagonist phenoxybenzamine during cold exposure (5 °C). This pharmacological procedure induced marked hypothermia, with decreases in HR only in the RA group; no changes were observed in T_c or any thermoregulatory parameter in the CA group. These results suggest that NA in the mPOA modulates heat production in response to acute cold stress in the RA group. However, this thermoregulatory action of NA in the mPOA was attenuated in the CA group.

Introduction

Chronic exposure to a cold environment for several weeks improves cold tolerance in rodents and involves adaptive autonomic thermoregulatory changes in effector systems. The threshold for shivering induction in muscles decreases, and the capacity for non-shivering thermogenesis in brown adipose tissue (BAT) increases (Cannon and Nedergaard, 2004; Griggio, 1982; Zeisberger and Roth, 1996). Many aspects of the replacement of shivering thermogenesis by non-shivering thermogenesis in effector systems in response to cold stress during chronic continuous cold exposure are well understood: long-term cold exposure changes muscle characteristics (Bourhim et al., 1990; Soni and Katoch, 1997). Increases in all the following have been observed: sympathetic nerve activity, the expression of uncoupling protein-1 (Ashwell et al., 1983; Milner and Trayhurn, 1989; Jacobsson et al., 1994), total cellularity (Bukowiecki et al., 1982), and total mitochondria in BAT (Cannon and Nedergaard, 2004).

Central nervous system structures involved in coordinating both shivering and non-shivering thermogenesis include the medulla (Morrison, 2003; Nason and Mason, 2006; Taniguchi et al., 2003), the midbrain (Rathner and Morrison, 2006; Uno et al., 2003), and the hypothalamus (Amir, 1990a,b; Chen et al., 1998; Li and Thornhill, 1998; Nagashima et al., 2000; Thornhill and Halvorson, 1994; Zaretskaia et al., 2002). The hypothalamus is recognized as the primary regulator of many autonomic functions (Beverly et al., 2001; Saint-Mleux et al., 2004; Smith et al., 2007; Yanagita et al., 2007). Moreover, the preoptic area and the anterior hypothalamus are believed to be the center for thermoregulation that integrates thermal information from central and peripheral thermoreceptors; it also promotes appropriate heat loss and heat production responses (Bligh, 1979; Boulant and Dean, 1986; Dean and Boulant, 1989; Kanosue et al., 1991). Furthermore, previous studies have indicated that the medial portion of the rostral preoptic area (mPOA) is a more effective site for thermoregulation under a cold environment (Osaka, 2004; Chen et al., 1998; Zhang et al., 1995).

* Corresponding author. Daikin Industries Ltd., 1000-2 Ohtani, Okamoto, Kusatsu, Shiga 525-8526, Japan. Tel.: +81 77 565 3476; fax: +81 77 565 6652.
E-mail address: takehito.saitou@daikin.co.jp (T. Saito).

0024-3205/$ – see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.lfs.2008.05.008

A number of neurotransmitter systems are implicated in the neural activity of the mPOA that occurs during thermoregulation in a cold environment. One candidate system involves the noradrenergic neurons. Noradrenergic activity in the brain is an important physiological modulator of cold and other types of stress, such as immobilization, hypoglycemia, hemorrhage, and pain (Beverly et al., 2001; Kumar et al., 2007; Daiguji et al., 1982; Madden et al., 2006; Pacák and Palkovits, 2001; Taylor et al., 2000). Cold exposure evoked Fos immunoreactivity in the locus coeruleus (LC) (Miyata et al., 1995; Pacák and Palkovits, 2001), and mPOA receives a dense noradrenergic input from the LC (Simerly and Swanson, 1986; Espana and Berridge, 2006). Indeed, involvement of mPOA noradrenaline (NA) in core body temperature (T_c) regulation has been well documented. Microinjection of NA into the mPOA induced an increase of T_c (Beckman, 1970; Veale and Whishaw, 1976). Feleder et al. (2004) demonstrated that perfusion of the α_1-adrenoceptor agonist cirazoline into the mPOA induced hyperthermia in freely moving guinea pigs. These results suggest that NA in the mPOA mediates thermogenic response. Furthermore, we recently observed that NA concentrations in tissue extracts of the mPOA increased only during the early phase (3 h) of cold exposure; concentrations subsequently returned to baseline levels during 4 weeks of cold exposure (Saito et al., 2005).

To further elucidate the effect of chronic, continuous cold exposure on thermoregulatory actions of NA within the mPOA, we used microdialysis, high-performance liquid chromatography (HPLC), and biotelemetry. The study comprises two parts. In the first part, neurotransmitter release was monitored in the mPOA with simultaneous measurement of T_c and thermoregulatory parameters in freely moving animals. In the second part, the mPOA was stimulated pharmacologically. In both parts, room temperature and cold acclimated animals were evaluated during cold exposure (5 °C).

Materials and methods

Animals

In all, 12 male Wistar rats (250–350 g body weight) were used in this study. Rats were housed separately in plastic cages under controlled conditions: 23 °C ambient temperature; 50% relative humidity; and a 12/12 h light/dark cycle, with lights on at 06:00 h. All rats had access to food and water ad libitum, 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.

Thermoregulatory parameter measurements

At least 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 of T_c and heart rate (HR), an indicator of heat production (Chambers et al., 2000; Hasegawa et al., 2005; Ishiwata et al., 2005; Saito et al., 2005). We measured the tail skin temperature (T_t), an index of heat loss response on dorsal surface of the skin ca. 10 mm from the tail base using an alumel–chromel thermocouple wire (Hasegawa et al., 2005; Ishiwata et al., 2002, 2005). The thermocouple wire was covered with a plastic tube and a metal spring; it was attached with tape at least 2 h before the beginning of the experiment. We also used the 'Nejiren' (RC-2000; Osaka Microsystems, Japan) to prevent tangling of the thermocouple and microdialysis tubes during experiments (Ishiwata et al., 2002, 2005).

Temperature acclimation

Rats were divided randomly into the RA group (N=6), kept at an ambient temperature (T_a) of 23 °C for 2 weeks, and a CA group (N=6), kept at a T_a of 5 °C for 2 weeks in a temperature-controlled chamber (Ishiwata et al., 2002, 2005; Saito et al., 2005).

Short-term cold exposure

For experiments 1 and 2, T_a was set at a room temperature (23 °C) for 60 min. Thereafter, it was set at a cold temperature (5 °C) for 180 min automatically in a temperature-controlled chamber.

Microdialysis

After acclimation trials, rats were anesthetized using 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 mPOA (AP −0.40 mm; L +0.2 mm; D −8.6 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 (147 mM NaCl, 4 mM KCl, 2.3 mM $CaCl_2$) at a 1 μl/min flow rate.

Experiment 1: cold exposure affects NA in the mPOA in freely moving rats

To investigate whether the release pattern of NA in the mPOA during cold exposure would be different between RA and CA groups, we collected microdialysate from mPOA during 180 min cold exposure (5 °C) in RA and CA groups while simultaneously measuring T_c, HR, and T_t. Microdialysates were analyzed for NA using HPLC.

Experiment 2: α-adrenoceptor blocking agent affects T_c and thermoregulatory parameters in freely moving rats

To investigate whether the effects of noradrenergic blockade in the mPOA on thermoregulation are different for the RA and CA groups, we perfused the mPOA with 10 mM of phenoxybenzamine (PB) (Alexis Platform, USA), an α-adrenoceptor blocking agent, during cold exposure (5 °C). The 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.

HPLC

Microdialysates were analyzed using HPLC. Identification of unknown peaks in samples was accomplished by matching the retention times of peaks with those of authentic standards (Chromograph Report Software; Bioanalytical Systems Inc., USA). A mixture of a sample and standard were sometimes analyzed for confirmation of identification of peaks. The HPLC system was equipped with an amperometric electrochemical detector (LC-4C; Bioanalytical Systems Inc., USA) and a pump (PM-70; BAS, Japan). We used a 5-μm C-18 polymeric column (1.0 mm internal diameter × 15 cm; BAS, Japan) for HPLC. Using an automated HPLC robot (CMA200; CMA/Microdialysis AB, Sweden), 10 μl of the sample was injected into HPLC. The mobile phases were modified from Tossman (Tossman, 1989) as follows: HPLC consisted of 0.1 M sodium acetate/0.1 M citrate, 0.1 mM EDTA-2Na, 3% acetonitrile, 1 mM sodium 1-decane sulfonate, pH was 5.8. The flow rate of the mobile phase was 60 μl min^{-1}. A glassy carbon electrode (BAS, Japan) was set at a potential of 550 mV relative to a Ag/AgCl reference electrode. It took about 30 min for analysis of one sample. The details of the HPLC method were described in a previous report (Hasegawa et al., 2000; Ishiwata et al., 2004; Saito et al., 2005; Yasumatsu et al., 1998).

Histological examination

At the end of each experiment, the rats were killed 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. Ltd., Japan).

Data collection and statistical analysis

Microdialysates were collected every 10 min and the measured parameters were expressed as percentages of the baseline value. The value of the sample at 60 min was considered to be 100%. We measured T_c, HR, and T_t 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.

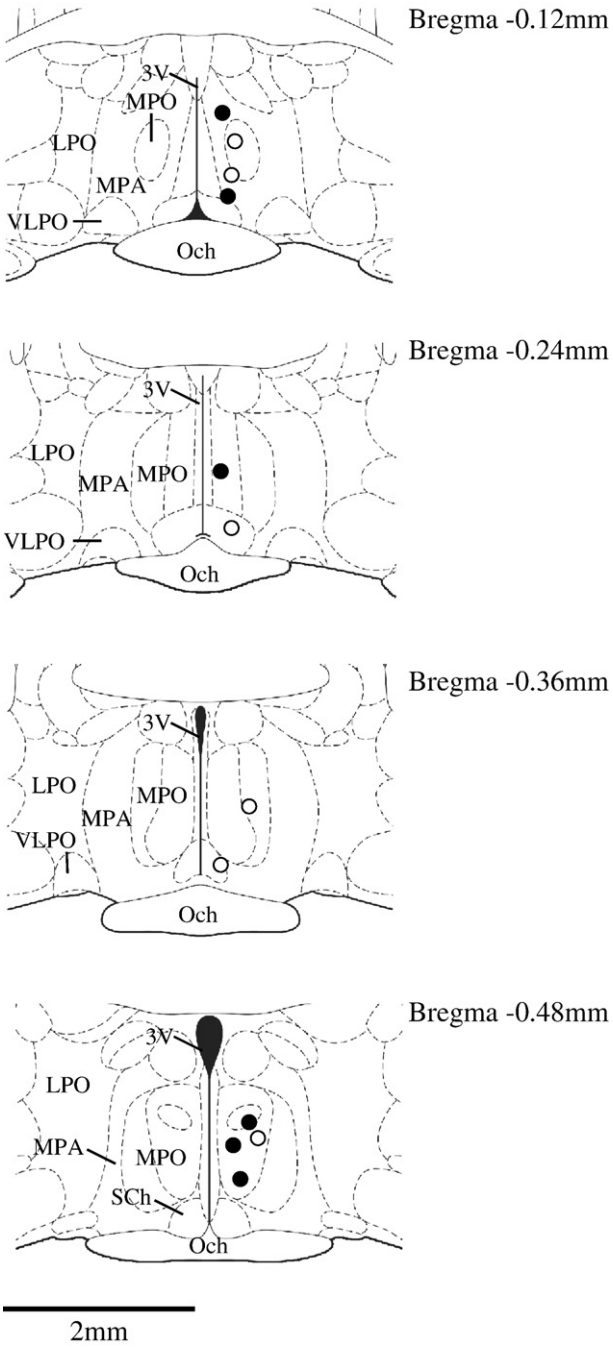

Fig. 1. Schematic presentation of the location of microdialysis probes tips in a coronal plane. Each symbol (open circle: RA group, close circle: CA group) indicates the location of the microdialysis probe. 3 V, third ventricle; LPO, lateral preoptic area; MPA, medial preoptic area; MPO, medial preoptic nucleus; Och, optic chiasm; Sch, suprachiasmatic nucleus; VLPO, ventrolateral preoptic area.

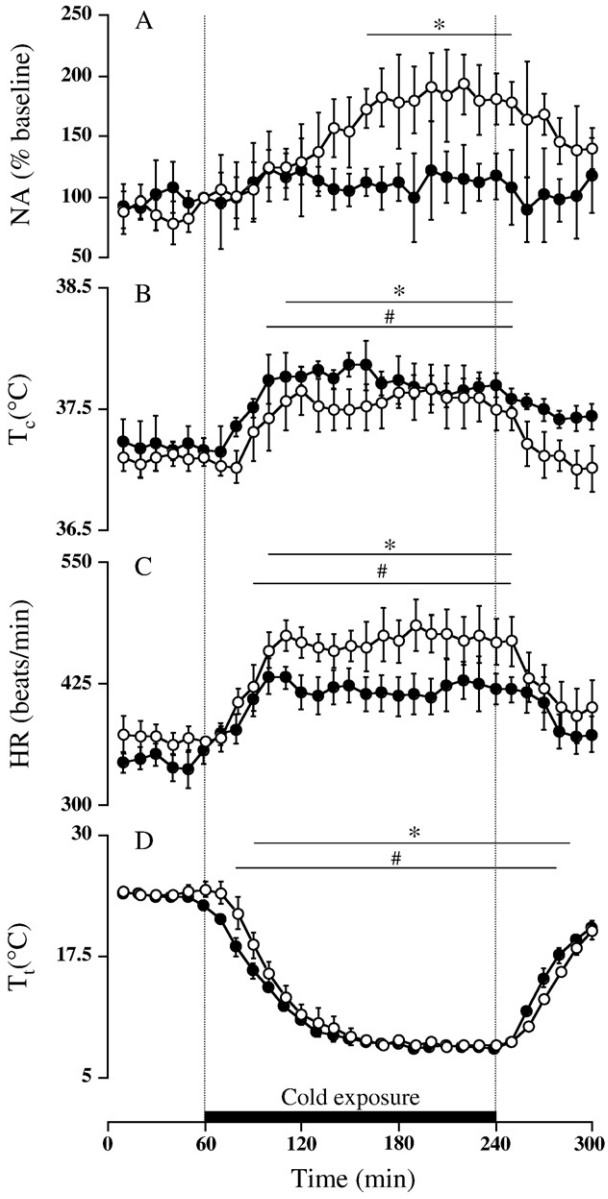

Fig. 2. Changes in extracellular NA level in the mPOA (A), T_c (B), HR (C) and T_t (D) during 3 h of cold exposure in the RA group (open circle, $n=6$) and the CA group (close circle, $n=6$). The value of NA in the sample at a point of 60 min was considered as 100%. Values are means ± SEM. *Significant difference from baseline value at a point of 60 min in the RA group, $P<0.05$. #Significant difference from baseline value at a point of 60 min in the CA group, $P<0.05$.

Results

We performed experiments on both RA (open circle) and CA (close circle) groups (RA, $n=6$; CA, $n=6$). As presented in Fig. 1, the tip of the microdialysis probe was positioned correctly into the mPOA in all these animals.

Fig. 2 shows changes in extracellular NE level in the mPOA (A), T_c (B), HR (C) and T_t (D) during cold exposure (60–240 min) in RA (open circle) and CA (close circle) groups. Before the start of cold exposure

(0–60 min), NA, T_c, HR, and T_t were stable in both groups. In the RA group, baseline concentrations of NA in the mPOA were 0.71 ± 0.52 pg/10 μl at a point of 60 min. Actually, NA increased from baseline values in the early stage of cold exposure (129 ± 19% at 120 min) and significant increases were observed in the late stage (178 ± 39% and 181 ± 21%, respectively, at 180 min and 240 min). In the CA group, cold exposure did not affect NA efflux in the mPOA. Baseline concentrations of NA in the CA group were 0.84 ± 0.64 pg/10 μl at a point of 60 min. No significant changes were found in NA during cold exposure. On the other hand, each T_c and thermoregulatory parameters in the RA and CA groups showed a similar change during cold exposure. At least 15 min after the start of cold exposure, T_c was increased in both groups. Significant high levels of T_c in RA and CA groups were observed, respectively, from 115 min to 245 min and 110 min to 245 min. Each maximum value was 37.7 ± 0.1 °C (RA group) and 37.9 ± 0.1 °C (CA group). The elevation of HR occurred more rapidly than T_c elevation in both groups. In the RA group, HR was significantly increased from 110 min to 245 min, while in the CA group, significant increases in HR were observed from 105 min to 245 min. The respective maximum values of HR in RA and CA groups were 487.5 ± 26.8 bpm and 433.1 ± 18.8 bpm. Although a significant high level of HR was observed during cold exposure in both groups, the elevation level tended to be higher in the RA group. After cold exposure, T_c and HR were gradually decreased in both groups. In contrast to changes in T_c and HR, T_t in both groups was decreased during cold exposure. Significant low levels of T_t in RA and CA group were respectively observed from 75 min to 240 min and 70 min to 240 min. Minimum values of T_t in RA and CA groups were, respectively, 8.1 ± 0.5 °C and 7.9 ± 0.4 °C. These significant low levels were maintained after cold exposure (RA, up to 290 min; CA, up to 285 min).

Fig. 3 shows changes in T_c (A), HR (B), and T_t (C) during perfusion of PB into the mPOA (180–240 min) during cold exposure in RA (open circle) and CA (close circle) groups. In both groups, T_c, HR and T_t were stable before the start of cold exposure (0–60 min). In the RA group, cold exposure induced significant increases in T_c and HR (37.8 ± 0.1 °C and 500.1 ± 14.4 bpm, respectively, at 180 min). At the start of cold exposure, T_t decreased rapidly. This decrease was statistically significant from 80 min (18.9 ± 1.4 °C) to 240 min (8.3 ± 0.2 °C). Perfusion of PB into the mPOA induced significant decreases in T_c and HR. Significant decreases in T_c were observed at 210, 220, and 240 min (37.2 ± 0.2, 37.2 ± 0.2 and 37.3 ± 0.1 °C, respectively). Moreover, HR was decreased significantly from 190 min (457.1 ± 14.7 bpm) to 240 min (454.3 ± 13.9 bpm). On the other hand, perfusion of PB did not influence T_t. After cold exposure, T_c, HR and T_t all tended to return to baseline levels. In the CA group, cold exposure induced significant increases in T_c and HR (37.7 ± 0.1 °C and 412.4 ± 6.7 bpm at 180 min, respectively). However, the rise of HR tended to be lower than that of the RA group. During cold exposure, T_t was significantly decreased (8.6 ± 0.4 °C). This result was identical to that for the RA group. Perfusion of PB had no effect on T_c (37.7 ± 0.1 °C at 240 min), HR (420.5 ± 15.7 bpm at 240 min), or T_t (7.8 ± 0.2 °C). These results were in marked contrast to that of RA group, except for the T_t result. After cold exposure, T_c, HR, and T_t all tended to return to baseline levels.

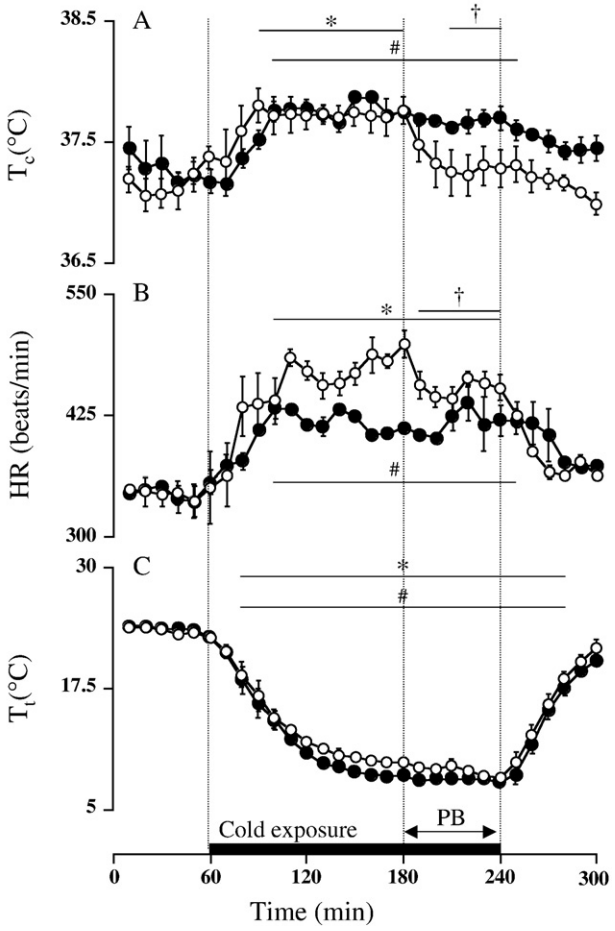

Fig. 3. Changes in T_c (A), HR (B) and T_t (C) elicited by perfusion of the mPOA with 10 mM phenoxybenzamine (PB) during cold exposure (5 °C) in the RA group (open circle, n=6) and the CA group (close circle, n=6). Values are means ± SEM. *Significant difference from baseline value at a point of 60 min in the RA group, $P<0.05$. #Significant difference from baseline value at a point of 60 min in the CA group, $P<0.05$. †Significant difference from value at a point of 180 min in the RA group, $P<0.05$.

Discussion

In this study, although 3 h exposure to cold (5 °C) significantly increased T_c and HR and decreased T_t in both the RA and CA groups, cold-induced elevation of NA efflux within the mPOA was observed only in the RA group. Perfusion of PB, an α-adrenoceptor blocking agent, into the mPOA significantly decreased T_c and HR in the RA group under acute cold exposure. However, T_c and thermoregulatory effectors in the CA group did not change during perfusion of the α-adrenoceptor antagonist during cold exposure.

Although many studies have been explored the role of NA in the mPOA in thermoregulation of normal ambient temperature housed rats, different experimental results and differences of opinion persist. For example, local application of NA into the mPOA induced a decrease of T_c (Poole and Stephenson, 1979). Feldberg and Myers (1963) also suggested that NA might regulate heat loss response in a hot environment. On the other hand, microinjections of NA into the mPOA have led to increases in T_c and the authors of those reports have asserted that NA release in the POA mediates a heat production response (Beckman, 1970; Veale and Whishaw, 1976). Therefore, in their summary of the literature on neurotransmitters and thermoregulation, Clark and Lipton (1986) noted that the role of NA in the mPOA remained unclear. In the RA group of this study, acute cold exposure significantly increased the extracellular level of NA in the mPOA, and blocking NA receptors in the mPOA in a cold environment by microdialysis of an α-adrenoceptor antagonist causing marked hypothermia. Furthermore, perfusion of PB into the mPOA decreased heat production (HR decrease), and had no effect on a heat loss effector (T_t). These results suggested that NA within the mPOA is an important neurotransmitter modulating heat production in response to cold stress in animals housed at normal temperatures.

The present study did not clarify which adrenoceptor subtype (α_1, α_2, or β) was exactly responsible for NA hyperthermic effect during acute cold exposure because PB is non-selective α-adrenergic antagonist. However, specific physiological functions of each adrenoceptor subtype in the mPOA were clarified by the work of Mallick and colleagues. They reported that sleep and wakefulness were mediated by α_2- and β-adrenoceptors, respectively, whereas α_1-adrenoceptors

involved thermoregulation (Mallick and Alam, 1992; Ramesh et al., 1995). They further reported that α_1-adrenoceptors were present on thermosensitive neurons of the mPOA (Mallick et al., 2002). Moreover, it was reported that warm-sensitive neurons in the mPOA are more abundant than cold-sensitive neurons (Dean and Boulant, 1989); our recent studies as well as those of others have demonstrated that the functional role of mPOA warm-sensitive neurons in heat production involves the inhibitory control of thermogenesis (Chen et al., 1998; Ishiwata et al., 2002, 2005; Zhang et al., 1995). Interestingly, it has been reported that NA (Watanabe et al., 1986) or an α_1-adrenoceptor agonist cirazoline inhibits the activity of warm-sensitive neurons in the mPOA in the rat slice preparation (Imbery et al., 2008), and that perfusion of cirazoline into the mPOA induced prompt hyperthermia in an in vivo microdialysis study (Feleder et al., 2004). Taking these observations into account the α_1-adrenoceptor mediated inhibition of warm-sensitive neurons in the mPOA probably activates a heat production response against acute cold environments in normal-temperature housed animals.

Extracellular NA levels in the mPOA did not change during 3 h of cold exposure in the CA group. This observation concurs with those reported from our previous study: the NA content in the POA after 2 weeks' cold exposure was nearly equal to that of the control level (Saito et al., 2005). Moreover, the perfusion of an α-adrenoceptor antagonist had no effect on T_c or thermoregulatory effectors under a cold environment for the CA group. These results suggest that the thermoregulatory action of NA within the mPOA in response to a cold stimulus is attenuated during chronic cold exposure. Although the exact mechanism remains unclear, the noradrenergic cells of the LC might participate in this attenuation. Results of previous neuroanatomical studies indicate that the LC sends dense noradrenergic fibers to the mPOA. Furthermore, the detailed origin and organization of noradrenergic efferents from the LC to the mPOA have been identified (Simerly and Swanson, 1986; Espana and Berridge, 2006). Referring to these previous studies, it was considered that regions in which microdialysis probes located in this study receive noradrenergic innervation from the LC. Miyata et al. (1995) reported that cold-induced Fos expression in the LC was increased significantly after 3 h cold exposure (10 °C). However, the number of these cells in cold-exposed rats reverted to baseline levels after 2 weeks. Moreover, chronic cold exposure at 5 °C (3 weeks) significantly reduces tyrosine hydroxylase (a rate-limiting enzyme for catechole amine biosynthesis) mRNA expression in the LC (Featherby and Lawrence, 2004). The findings described above are consistent with our results and the fact that activity of noradrenergic neurons in the LC decreases during chronic cold exposure. Therefore, the results suggest that because chronic cold exposure might decrease the responsibility of the LC noradrenergic neurons for cold stress, cold-induced NA releases in the mPOA of the CA group were not observed in this study.

We investigated only the thermoregulatory involvement of NA in the mPOA of RA and CA rats in this study, but previous reports have described that other neurotransmitter systems such as GABAergic system or cholinergic system in the mPOA also participate in thermoregulation. For example, it was reported that GABA-A receptor antagonist picrotoxin affects thermosensitive neurons in the mPOA (Jha et al., 2001); our previous study and others have demonstrated that GABAergic pharmacological manipulation in the mPOA changes body temperature (Ishiwata et al., 2005; Osaka, 2004). We have identified that acute cold exposure increases GABA efflux in the mPOA of RA rats (Ishiwata et al., 2005). These previous studies indicated that the GABAergic system in the mPOA was an important neurotransmitter system for thermoregulation under a cold environment. Furthermore, regulation of body temperature by interaction of noradrenergic and cholinergic system in the mPOA has also been reported (Mallick and Joseph, 1998). Therefore, further studies must clarify the respective involvements of these systems in the mPOA in thermoregulation during cold acclimation.

In the present study, a cold-induced increase of extracellular NA in the mPOA was observed only in the RA group. Perfusion of phenoxybenzamine, an α-adrenoceptor antagonist, into the mPOA induced significant hypothermia and decreased heat production only in the RA group. This treatment had no effect on the CA group in a cold environment. These results show that, although NA release within the POA facilitates heat production in response to cold stimuli in the early stages of cold acclimation, following chronic cold exposure, NA is not released in the mPOA. Furthermore, in cold-acclimated animals, this area is not responsive to noradrenergic pharmaceutical manipulation.

Acknowledgements

We are grateful to Dr. Kita, Department of Kinesiology, Graduate School of Science, Tokyo Metropolitan University, for the valuable discussion.

References

Amir, S., 1990a. Activation of brown adipose tissue thermogenesis by chemical stimulation of the posterior hypothalamus. Brain Research 534, 303–308.
Amir, S., 1990b. Intra-ventromedial hypothalamic injection of glutamate stimulates brown adipose tissue thermogenesis in the rat. Brain Research 511, 341–344.
Ashwell, M., Jennings, G., Richard, D., Stirling, D.M., Trayhurn, P., 1983. Effect of acclimation temperature on the concentration of the mitochondrial "uncoupling" protein measured by radioimmunoassay in mouse brown adipose tissue. FEBS Letters 161, 108–112.
Beverly, J.L., de-Vries, M.G., Beverly, M.F., Bouman, S.D., Arseneau, L.M., 2001. Noradrenergic and GABAergic systems in the medial hypothalamus are activated during hypoglycemia. American Journal of Physiology. Regulatory, Integrative and Comparative Physiology 280, R563–R569.
Beckman, A.L., 1970. Effect of intrahypothalamic norepinephrine on thermoregulatory responses in the rat. American Journal of Physiology 218, 1596–1604.
Bligh, J., 1979. The central neurology of mammalian thermoregulation. Neuroscience 4, 1213–1216.
Boulant, J.A., Dean, J.B., 1986. Temperature receptors in the central nervous system. Annual Review of Physiology 48, 639–654.
Bourhim, M., Barre, H., Oufara, S., Minaire, Y., Chatonnet, J., Cohen-Adad, F., Rouanet, J.L., 1990. Increase in cytochrome oxidase capacity of BAT and other tissues in cold-acclimated gerbils. American Journal of Physiology. Regulatory, Integrative and Comparative Physiology 258, R1291–R1298.
Bukowiecki, L.J., Collet, A.J., Follea, N., Guay, G., Jahjah, L., 1982. Brown adipose tissue hyperplasia: a fundamental mechanism of adaptation to cold and hyperphagia. American Journal of Physiology. Endocrinology and Metabolism 242, E353–E359.
Cannon, B., Nedergaard, J., 2004. Brown adipose tissue: function and physiological significance. Physiological Revews 84, 277–359.
Chambers, J.B., Williams, T.D., Nakamura, A., Henderson, R.P., Overton, J.M., Rashotte, M.E., 2000. Cardiovascular and metabolic responses of hypertensive and normotensive rats to one week of cold exposure. American Journal of Physiology. Regulatory, Integrative and Comparative Physiology 279, R1486–R1494.
Chen, X.M., Hosono, T., Yoda, T., Fukuda, Y., Kanosue, K., 1998. Efferent projection from the preoptic area for the control of non-shivering thermogenesis in rats. Journal of Physiology 512, 883–892.
Clark, W.G., Lipton, J.M., 1986. Changes in body temperature after administration of adrenergic and serotonergic agents and related drugs including antidepressants: II. Neuroscience and Biobehavioral Reviews 10, 153–220.
Daiguji, M., Okada, F., Yamashita, I., 1982. Dopamine-beta-hydroxylase activity in the locus coeruleus and hypothalamus in cold-stressed rats. Psychoneuroendocrinology 7, 223–227.
Dean, J.B., Boulant, J.A., 1989. In vitro localization of thermosensitive neurons in the rat diencephalon. American Journal of Physiology. Regulatory, Integrative and Comparative Physiology 257, R57–R64.
Espana, R.A., Berridge, C.W., 2006. Organization of noradrenergic efferents to arousal-related basal forebrain structures. The Journal of Comparative Neurology 496, 668–683.
Feldberg, W., Myers, R.D., 1963. A new concept of temperature regulation by amines in the hypothalamus. Nature 28, 1325.
Feleder, C., Perlik, V., Blatteis, C.M., 2004. Preoptic alpha 1- and alpha 2-noradrenergic agonists induce, respectively, PGE2-independent and PGE2-dependent hyperthermic responses in guinea pigs. American Journal of Physiology. Regulatory, Integrative and Comparative Physiology 286, R1156–R1166.
Featherby, T., Lawrence, A.J., 2004. Chronic cold stress regulates ascending noradrenergic pathways. Neuroscience 127, 949–960.
Forray, M.I., Bustos, G., Gysling, K., 1999. Noradrenaline inhibits glutamate release in the rat bed nucleus of the stria terminalis: in vivo microdialysis studies. Journal of Neuroscience Research 55, 311–320.
Griggio, M.A., 1982. The participation of shivering and nonshivering thermogenesis in warm and cold-acclimated rats. Comparative Biochemistry and Physiology 73, 481–484.

Hasegawa, H., Yazawa, T., Yasumatsu, M., Otokawa, M., Aihara, Y., 2000. Alteration in dopamine metabolism in the thermoregulatory center of exercising rats. Neuroscience Letters 289, 161–164.
Hasegawa, H., Ishiwata, T., Saito, T., Yazawa, T., Aihara, Y., Meeusen, R., 2005. Inhibition of the preoptic area nucleus by tetrodotoxin alters thermoregulatory functions in exercising rats. Journal of Applied Physiology 98, 1458–1462.
Imbery, T.E., Irdmusa, M.S., Speidell, A.P., Streer, M.S., Griffin, J.D., 2008. The effects of Cirazoline, an alpha-1 adrenoreceptor agonist, on the firing rates of thermally classified anterior hypothalamic neurons in rat brain slices. Brain Research 1193, 93–101.
Ishiwata, T., Hasegawa, H., Yazawa, T., Otokawa, M., Aihara, Y., 2002. Functional role of the preoptic area and anterior hypothalamus in thermoregulation in freely moving rats. Neuroscience Letters 325, 167–170.
Ishiwata, T., Saito, T., Hasegawa, H., Yazawa, T., Otokawa, M., Aihara, Y., 2004. Changes of body temperature and extracellular serotonin level in the preoptic area and anterior hypothalamus after thermal or serotonergic pharmacological stimulation of freely moving rats. Life Sciences 75, 2665–2675.
Ishiwata, T., Saito, T., Hasegawa, H., Yazawa, T., Kotani, Y., Otokawa, M., Aihara, Y., 2005. Changes of body temperature and thermoregulatory responses of freely moving rats during GABAergic pharmacological stimulation to the preoptic area and anterior hypothalamus in several ambient temperatures. Brain Research 1048, 32–40.
Jacobsson, A., Mühleisen, M., Cannon, B., Nedergaard, J., 1994. The uncoupling protein thermogenin during acclimation: indications for pretranslational control. American Journal of Physiology. Regulatory, Integrative and Comparative Physiology 267, R999–R1007.
Jha, S.K., Yadav, V., Mallick, B.N., 2001. GABA-A receptors in mPOAH simultaneously regulate sleep and body temperature in freely moving rats. Pharmacology Biochemistry and Behavior 70, 115–121.
Kanosue, K., Niwa, K., Andrew, P.D., Yasuda, H., Yanase, M., Tanaka, H., Matsumura, K., 1991. Lateral distribution of hypothalamic signals controlling thermoregulatory vasomotor activity and shivering in rats. American Journal of Physiology. Regulatory, Integrative and Comparative Physiology 260, R486–R493.
Kumar, V.M., Vetrivelan, R., Mallick, H.N., 2007. Noradrenergic afferents and receptors in the medial preoptic area: neuroanatomical and neurochemical links between the regulation of sleep and body temperature. Neurochemistry International 50 (6), 783–790.
Li, Q., Thornhill, J., 1998. Thermoresponsiveness of posterior hypothalamic (PH) neurons of rats to scrotal and abdominal thermal stimulation. Brain Research 794, 80–87.
Madden, C.J., Stocker, S.D., Sved, A.F., 2006. Attenuation of homeostatic responses to hypotension and glucoprivation after destruction of catecholaminergic rostral ventrolateral medulla neurons. American Journal of Physiology. Regulatory, Integrative and Comparative Physiology 291, R751–R759.
Mallick, B.N., Alam, M.N., 1992. Different types of norepinephrinergic receptors are involved in preoptic area mediated independent modulation of sleep-wakefulness and body temperature. Brain Research 591, 8–19.
Mallick, B.N., Joseph, M.M., 1998. Adrenergic and cholinergic inputs in preoptic area of rats interact for sleep–wake thermoregulation. Pharmacology Biochemistry and Behavior 61, 193–199.
Mallick, B.N., Jha, S.K., Islam, F., 2002. Presence of alpha-1 adrenoreceptors on thermosensitive neurons in the medial preoptico-anterior hypothalamic area in rats. Neuropharmacology 42, 697–705.
Morrison, S.F., 2003. Raphe pallidus neurons mediate prostaglandin E2-evoked increases in brown adipose tissue thermogenesis. Neuroscience 121, 17–24.
Milner, R.E., Trayhurn, P., 1989. Cold-induced changes in uncoupling protein and GDP binding sites in brown fat of *ob/ob* mice. American Journal of Physiology. Regulatory, Integrative and Comparative Physiology 257, R292–R299.
Miyata, S., Ishiyama, M., Shido, O., Nakashima, T., Shibata, M., Kiyohara, T., 1995. Central mechanism of neural activation with cold acclimation of rats using Fos immunohistochemistry. Neuroscience Research 22, 209–218.
Nakata, T., Berard, W., Kogosov, E., Alexander, N., 1990. Microdialysis in the posterior hypothalamus: sodium chloride affects norepinephrine release, mean arterial pressure, heart rate and behavior in awake rats. Brain Research Bulletin 25, 593–598.
Nason Jr., M.W., Mason, P., 2006. Medullary raphe neurons facilitate brown adipose tissue activation. The Journal of Neuroscience 26, 1190–1198.
Nagashima, K., Nakai, S., Tanaka, M., Kanosue, K., 2000. Neuronal circuitries involved in thermoregulation. Autonomic Neuroscience 85, 18–25.
Osaka, T., 2004. Cold-induced thermogenesis mediated by GABA in the preoptic area of anesthetized rats. American Journal of Physiology. Regulatory, Integrative and Comparative Physiology 287, R306–R313.
Pacák, K., Palkovits, M., 2001. Stressor specificity of central neuroendocrine responses: implications for stress-related disorders. Endocrine Reviews 22, 502–548.
Paxinos, G., Watson, C., 2004. The Rat Brain in Stereotaxic Coordinates, 5th Edition. Academic Press Inc.
Poole, S., Stephenson, J.D., 1979. Effects of noradrenaline and carbachol on temperature regulation of rats. British Journal of Pharmacology 65, 43–51.
Ramesh, V., Kumar, V.M., John, J., Mallick, H., 1995. Medial preoptic alpha-2 adrenoceptors in the regulation of sleep-wakefulness. Physiology and Behavior 57, 171–175.
Rathner, J.A., Morrison, S.F., 2006. Rostral ventromedial periaqueductal gray: a source of inhibition of the sympathetic outflow to brown adipose tissue. Brain Research 1077, 99–107.
Saint-Mleux, B., Eggermann, E., Bisetti, A., Bayer, L., Machard, D., Jones, B.E., Muhlethaler, M., Serafin, M., 2004. Nicotinic enhancement of the noradrenergic inhibition of sleep-promoting neurons in the ventrolateral preoptic area. The Journal of Neuroscience 24, 63–67.
Saito, T., Ishiwata, T., Hasegawa, H., Nomoto, S., Otokawa, M., Aihara, Y., 2005. Changes in monoamines in rat hypothalamus during cold acclimation. Journal of Thermal Biology 30, 229–235.
Simerly, R.B., Swanson, L.W., 1986. The organization of neural inputs to the medial preoptic nucleus of the rat. The Journal of Comparative Neurology 246, 312–342.
Smith, P.M., Samson, W.K., Ferguson, A.V., 2007. Cardiovascular actions of orexin-a in the rat subfornical organ. Journal of Neuroendocrinology 19, 7–13.
Soni, A., Katoch, S.S., 1997. Structural and metabolic changes in skeletal muscle of cold acclimated rats. Journal of Thermal Biology 22, 95–107.
Taniguchi, A., Chen, X.M., Nagashima, K., Tanaka, M., Kanosue, K., 2003. Involvement of the raphe pallidus in the suppressive effect of preoptic warming on non-shivering thermogenesis in rats. Brain Research 966, 103–109.
Taylor, B.K., Roderick, R.E., Basbaum, A.I., 2000. Brainstem noradrenergic control of nociception is abnormal in the spontaneously hypertensive rat. Neuroscience Letters 291, 139–142.
Thornhill, J., Halvorson, I., 1994. Activation of shivering and non-shivering thermogenesis by electrical stimulation of the lateral and medial preoptic areas. Brain Research 656, 367–374.
Tossman, U., 1989. Sample Analysis. User's Guide. Library of Microdialysis. Stockholm Carnegie Medicine, vol. 1.
Uno, T., Roth, J., Shibata, M., 2003. Influence of the hypothalamus on the midbrain tonic inhibitory mechanism on metabolic heat production in rats. Brain Research Bulletin 61, 129–138.
Veale, W.L., Whishaw, I.Q., 1976. Body temperature responses at different ambient temperatures following injections of prostaglandin E1 and noradrenaline into the brain. Pharmacology Biochemistry and Behavior 4, 143–150.
Watanabe, T., Morimoto, A., Murakami, N., 1986. Effect of amine on temperature-responsive neuron in slice preparation of rat brain stem. American Journal of Physiology. Regulatory, Integrative and Comparative Physiology 250, R553–R559.
Yanagita, S., Amemiya, S., Suzuki, S., Kita, I., 2007. Effects of spontaneous and forced running on activation of hypothalamic corticotropin-releasing hormone neurons in rats. Life Sciences 80, 356–363.
Yasumatsu, M., Yazawa, T., Otokawa, M., Kuwasawa, K., Hasegawa, H., Aihara, Y., 1998. Monoamines, amino acids and acetylcholine in the preoptic area and anterior hypothalamus of rats: measurements of tissue extracts and in vivo microdialysates. Comparative Biochemistry and Physiology 121, 13–23.
Zeisberger, E., Roth, J., 1996. Central regulation of adaptive responses to heat and cold. Handbook of Physiology, Section 4, Environmental Physiology. American Physiological Society, vol. 1. Oxford University Press, pp. 579–595.
Zaretskaia, M.V., Zaretsky, D.V., Shekhar, A., DiMicco, J.A., 2002. Chemical stimulation of the dorsomedial hypothalamus evokes non-shivering thermogenesis in anesthetized rats. Brain Research 928, 113–125.
Zhang, Y.H., Yanase-Fujiwara, M., Hosono, T., Kanosue, K., 1995. Warm and cold signals from the preoptic area: which contribute more to the control of shivering in rats? The Journal of Physiology 485, 195–202.