Journal of Thermal Biology xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Journal of Thermal Biology

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

Moderate intensity, exercise-induced catecholamine release in the preoptic area and anterior hypothalamus in rats is enhanced in a warm environment

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ARTICLE INFO

Keywords:
Thermoregulation
Monoamine
Thermoregulatory center
Exercise
Warm ambient temperature

ABSTRACT

Thermoeffector responses and core body temperature (T_{core}) homeostasis during exercise are affected by both ambient temperature and exercise intensity. We have previously reported that T_{core}, heat loss responses, and catecholamine release in the preoptic area and anterior hypothalamus (PO/AH) increased during incremental treadmill running. However, no previous study has examined whether changes in the thermoregulatory responses at warm ambient temperature are related to catecholamine responses during moderate intensity exercise in rats. Therefore, the aim of the present study was to investigate the responsiveness of neurotransmission in the PO/AH to moderate intensity exercise at different ambient temperatures, and to relate this to changes in thermoregulation. We measured the monoamine levels in the PO/AH and the thermoregulatory responses in exercising rats simultaneously using a combination of methods, including in vivo microdialysis, biotelemetry, and animal O^2/CO^2 metabolism measuring system. On the day of experiments, rats ran for 60 min at a speed of 18 m min^{-1} on a treadmill at a 5% gradient, in an ambient temperature of 23 °C or 30 °C. T_{core}, tail skin temperature (T_{tail}; an index of heat loss), and oxygen consumption ($\dot{V}O_2$: an index of heat production) were monitored. Dopamine (DA), noradrenaline (NA), and serotonin (5-HT) levels were measured by high performance liquid chromatography (HPLC) with electrochemical detection. Exercise significantly increased the T_{core}, T_{tail}, and $\dot{V}O_2$ values, as well as DA and NA release in the PO/AH at both temperatures, and the increases were more pronounced at the warm ambient temperature. The results suggest that the increase in the T_{core}, heat production, and heat loss responses even during moderate intensity running in a warm environment are likely associated with an increase in DA and NA release in the PO/AH region.

1. Introduction

Body temperature regulation is controlled by the central nervous system, which can modulate the balance between metabolic heat production and heat loss, depending on environmental conditions (Nagashima, 2006; Romanovsky, 2007). During exercise, autonomic heat-loss mechanisms are activated to prevent an excessive rise in core body temperature. More specifically, the heat produced by exercising muscles increases primarily in proportion to work intensity during exercise, which is then counteracted by an increase in heat loss to maintain thermal balance.

A hot and humid environment can significantly add to the challenge that physical exercise imposes on the thermoregulatory system, as heat exchange between the body and the environment is substantially impaired under such conditions (Fuller et al., 1998; Walters et al., 2000). Disturbances in the thermal balance in such situations can induce hyperthermia. Thus, the extent of the increase in body temperature during exercise can differ according to environmental conditions (Tanaka et al., 1988).

The preoptic area and anterior hypothalamus (PO/AH) are generally considered to be the major brain regions involved in thermoregulation, which integrates thermoafferent signals from the skin and other parts of the body and exerts control over the efferent pathways to thermoeffectors (Nagashima, 2006; Romanovsky, 2007). There is evidence to suggest a role for a variety of neurotransmitters in this brain region in thermoregulation (Boulant and Dean, 1986; Yasumatsu et al., 1998). In our previous work, we showed that the PO/AH is a critical thermoregulatory site in the brain during exercise, and that neurotransmission in the PO/AH region is involved in the regulation of body temperature (Hasegawa et al., 2005). This brain area contains a high concentration of noradrenergic, dopaminergic, and serotonergic terminals (Dahlström and Fuxe, 1964), and these three monoamines may play an important role in thermoregulation. However, their precise functions in heat production and heat loss responses remain unknown.

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https://doi.org/10.1016/j.jtherbio.2017.11.003
Received 6 April 2017; Received in revised form 5 November 2017; Accepted 8 November 2017

Physical exercise has been shown to influence central neurotransmitter systems (Lin and Kuo, 2013). Several studies have examined the effect of exercise on brain noradrenaline (NA), dopamine (DA), and serotonin (5-hydroxytryptamine: 5-HT) levels (Meeusen and De Meirleir, 1995; Hasegawa et al., 2011). Using brain microdialysis, we observed that the core body temperature and thermoregulatory responses depended on the intensity of the exercise, and that thermoregulatory responses were associated with changes in the release of NA and DA, but not 5-HT, in the PO/AH region. Thus, changes in NA and DA release in the PO/AH may be involved in thermoregulation during exercise (Hasegawa et al., 2011).

However, (Wanner et al., 2014) suggested that the core body temperature of running mice was influenced greatly by the ambient temperature, but not by the exercise protocols or intensities used in that study. They did not measure the neurotransmission in the PO/AH, and the precise mechanisms have not been expounded. According to these studies, we can infer that different species have different responses to ambient temperature and exercise intensity. Although a previous study reported that body temperature, heat loss responses, and catecholamine release in the PO/AH increased during high intensity exercise (Hasegawa et al., 2008), no previous study has examined whether changes in the thermoregulatory response at warm ambient temperature are related to catecholamine responses during moderate intensity exercise in rats.

Therefore, the purpose of this study was to investigate the influence of a warm ambient temperature (30 °C) on thermoregulation and monoamine release in the PO/AH during moderate intensity exercise in rats.

2. Materials and methods

2.1. Animal

Male Wistar rats were used in all experiments. The rats were housed separately in plastic cages under controlled conditions (lights on between 6:00 and 18:00 h; ambient temperature 23–24 °C) and had free access to food and water. All experiments were approved by the Ethics Committee for Animal Experiments of the Hiroshima University.

2.2. General procedures

A telemetry device (TA10TA-F40, Data Science International, MN, USA) was implanted into the peritoneal cavity under pentobarbital anesthesia (50 mg kg^{-1}, i.p.); then, a guide cannula (CXG-12, Eicom, Kyoto, Japan) was implanted in the left side PO/AH (anterior −0.3 mm, lateral + 0.8 mm, ventral −6.7 mm, relative to bregma) on the same day. The rats were allowed to recover for 1 week before exercise familiarization. The animals were initially familiarized with exercising on a treadmill for 5 days. Each daily session consisted of running on the treadmill for 5 min at a 5% gradient, at a speed of 18 m min^{-1} in a temperate environment (Sonne and Galbo, 1980).

2.3. Experimental procedures

On the day of the experiments, the rats were anesthetized with isoflurane 4% and oxygen insufflated into a transparent chamber. After induction, the rats were kept under anesthesia using 1.5% isoflurane, delivered along with oxygen at 0.8 L min^{-1} via a face mask (Hasegawa et al., 2008), and the dummy cannula was replaced by a microdialysis probe, with a membrane length of 2 mm (CX-I-12-02, EICOM, Kyoto, Japan). The microdialysis probe was connected to a microinjection pump (CMA 100, CMA Microdialysis, Stockholm, Sweden) and was perfused with a modified Ringer solution (147 mM NaCl, 4 mM KCl, and 2.3 mM $CaCl_2$) at a flow rate of 2 μL min^{-1}. The air-tight treadmill chamber (MK-680AT/02 R, Muromachi Kikai, Tokyo, Japan) was adjusted by attaching the counterbalance arm of the microdialysis system. Oxygen consumption ($\dot{V}O_2$) was continuously measured with an O_2/CO_2 metabolism measuring system (MK-5000RQ/02; Muromachi Kikai). Room air was pumped through the chamber at a rate of 3.0 L min^{-1}. Microdialysis sampling collection was started 3 h after probe implantation. The rats were first monitored, under both temperate and warm conditions, when they were resting on the treadmill for 60 min at 23 °C, to verify stable basal conditions. The thermocouple for tail skin temperature (T_{tail}; an index of heat loss responses) was attached on the dorsal surface of the skin at ~ 10 mm, using tape. Under the temperate environmental condition (temperate), the rats were made to exercise at a moderate intensity (18 m min^{-1} and 5% gradient) on a treadmill, which corresponds to an oxygen uptake of approximately 67% $\dot{V}O_{2max}$ (Sonne and Galbo, 1980), for 60 min at 23 °C. Recovery from exercise was monitored on the treadmill for 60 min. Under warm conditions (warm), the treadmill chamber was warmed for 10 min before exercise, and the temperature was maintained at 30 °C during exercise. The exercise protocol was the same as that at 23 °C. All rats completed both experimental conditions, in random order, separated by 5 days to recover exercise capacity. All experiments were performed at the same time of day.

2.4. Sampling

During the experiments, the core body temperature (T_{core}) and T_{tail} values were measured and monitored using a biotelemetry system (Dataquest A.R.T., Data Sciences, MN, USA). $\dot{V}O_2$, was also simultaneously measured as an index of heat production. Microdialysates (20 μL) were collected every 10 min using a fraction collector (EFC-82, EICOM, Kyoto, Japan).

2.5. Chromatographic assay for the determination of DA, NA and 5-HT in dialysates from PO/AH

The levels of 5-HT, NA and DA in the dialysates were determined using off-line high-performance liquid chromatography (HPLC) with automatic injection (10 μL) of samples (M-500, EICOM, Kyoto, Japan). In summary, the assay was based on ion-pair reversed-phase chromatography (5 μm particle size; 2.0 × 200 mm ID, EICOMPAK CAX, EICOM, Kyoto, Japan) coupled to single-channel electrochemical detection (ECD-300, EICOM, Kyoto, Japan). The mobile phase consisted of 30% methanol in the following solution: 0.1 M ammonium acetate buffer (pH 6.0), 0.05 M sodium octanesulphonate and 50 mg L^{-1} Na-EDTA. The flow rate through the column was 250 μL min^{-1}. Because of the high pH (6.0) of the mobile phase, a low oxidation potential was set (450 mV vs. Ag/AgCl). The retention times for NA, DA and 5-HT were 5, 7 and 13 min, respectively.

2.6. Histology

At the end of each experiment, the rats were deeply anesthetized pentobarbital (100 mg kg^{-1}, i.p.) and their brains were removed. Correct placement of the microdialysis probe was verified according to the coordinates described by Paxinos and Watson (1998) in coronal sections (100-μm thick). Coronal sections (100 μm) were cut on a vibrotome (MA752, Campden Instruments, Loughborough, England) and stained with bromophenol blue for histological confirmation of the microdialysis probe in the PO/AH.

2.7. Data collection and statistical analysis

All values are presented as means ± SD. The average concentration of 3 microdialysis samples for 30 min was considered as the baseline and was defined as 100%. The values obtained subsequently were expressed as a percentage of this basal value. The effects of environmental conditions on the T_{core}, T_{tail}, $\dot{V}O_2$, 5-HT, NA and DA levels were assessed by two-way (environmental condition × time) repeated measures