Abstract

The purpose of the present study was to investigate the relationship between thermoregulation and catecholamine release in the preoptic area and anterior hypothalamus (PO/AH) during incremental treadmill running in the rat. To this aim, we combined in vivo brain microdialysis, biotelemetry, and metabolic measurements for continuous monitoring of core body temperature (T_{core}), brain neurotransmitters, and thermoregulatory responses. The animals were exercised for 1 h at 23°C. Treadmill speed was increased every 20 min (10, 20, and 26 m min^{-1}). T_{core}, oxygen consumption ($\dot{V}O_2$, an index of heat production), and tail skin temperature (T_{tail}, an index of heat loss) were simultaneously measured. Brain microdialysis samples were collected every 10 min, and these samples were analyzed for noradrenaline (NA), dopamine (DA), and serotonin (5-HT). T_{core} (°C) and $\dot{V}O_2$ ($ml \cdot min^{-1} \cdot kg^{-1}$) gradually increased during treadmill exercise (T_{core}, 0 min: 37.3 ± 0.1, 60 min: 39.6 ± 0.1, $P < 0.05$; $\dot{V}O_2$, 0 min: 20.5 ± 0.5, 60 min: 61.8 ± 0.4; $P < 0.05$). After an initial drop in T_{tail} (°C), this value gradually increased during exercise (0 min: 27.1 ± 0.1, 60 min: 31.9 ± 0.2; $P < 0.05$). Both NA and DA levels in the PO/AH significantly increased during exercise (60 min, NA: 1838 ± 109%, DA: 1309 ± 97%). There was no effect on serotonin release. Our data suggest that thermoregulatory responses are dependent on the intensity of the exercise and that these responses are associated with changes in NA and DA release, but not in 5-HT release in the PO/AH.

***Key words**:* Microdialysis; Exercise; Thermoregulation; Neurotransmission

1. Introduction

Body temperature is regulated by a balance of heat production and heat loss responses during exercise. The preoptic area and anterior hypothalamus (PO/AH) are thought to be the primary regions for body temperature regulation (Boulant & Dean, 1986). This brain area integrates thermal information from central and peripheral thermoreceptors, and initiates appropriate heat loss and heat production responses (Romanovsky, 2007). We previously investigated the functional role of PO/AH in thermoregulation during exercise using brain microdialysis (Hasegawa *et al*. 2005). Perfusion of the sodium channel blocker tetrodotoxin (TTX) into the PO/AH induced an increase in body core temperature with a decrease in heat loss and an increase in heat production responses during exercise. These data strongly indicated that the PO/AH is the critical thermoregulatory site in the brain during exercise and neurotransmission in the PO/AH region is involved in the regulation of body temperature. However, these findings could not indicate which specific neurotransmitter system controls thermoregulation during exercise, since TTX acts non specifically to inhibit exocytosis of neurotransmitters.

Brain catecholamines are considered to be involved in thermoregulation especially in the PO/AH (Boulant & Dean, 1986). Catecholaminergic and serotonergic projections innervate areas of the hypothalamus (Dahlstrom & Fuxe, 1964; Descarries & Beaudet, 1981), and a change in the activity of these neurons may be expected to contribute to the control of body temperature during exercise. We have recently reported the importance of catecholamines on thermoregulation and exercise-induced fatigue both in human (Roelands *et al*. 2008a; 2008b; Watson *et al*. 2005) and animal studies (Hasegawa *et al*. 2008).

The purpose of the present study was to investigate the relationship between body

temperature regulation and brain catecholamines in the PO/AH during incremental treadmill running in the rat using a combination of biotelemetry, oxygen consumption measurements and in vivo microdialysis. Using this combination it is possible to demonstrate changes in physiological parameters in parallel with neurotransmitter changes in the brain which is an extremely powerful strategy when studying neurobiology of behavior and also exercise (Linthorst & Reul, 2008). Furthermore, oxygen consumption is not only a good index for the exercise intensity, but also the index of thermoregulatory heat production responses (Tanaka *et al*., 1988; 1993),

2. Materials and methods

2.1.Animals

Male Wistar rats (Shimizu jiken, Shizuoka, Japan, weighing 300-350g) were used in all experiments. Animals were housed in a room of normal ambient temperature, on a 12h light/dark cycle (lights on at 08:00 h). Animals had a standard diet with free access to food and water throughout the experiments. All experiments were approved by the Ethical Committee for Animal Experiments of Hiroshima University.

2.2.Exercise familiarization sessions and surgeries.

The rats exercised once a day, 5 days a week, for 1.5 weeks on a rodent treadmill. Exercise intensity and duration were gradually increased up to a speed of 26 m min^{-1} and duration for 80 min. We initially trained 30 rats in this study. After the training protocol, 24 rats that ran without the need of provocation with electric shocks were selected for telemetry implantation surgery. A telemetry device (TA10TA-F40, Data Science International, MN, USA) was implanted in the peritoneal cavity under pentobarbital anesthesia (50 mg kg^{-1}, $_{I.P.}$). The rats were then given at least 3 days to recover, upon which they were anaesthetized with pentobarbital (50 mg kg^{-1}, $_{I.P.}$) to implant the intracerebral

guide cannula (CXG-12, Eicom, Kyoto, Japan) in the left lateral PO/AH (anterior -0.3 mm; lateral +0.8 mm, ventral -6.7 mm, relative to bregma). The cannula was secured to the skull using dental cement (CARBO CEMENT, Shofu, Kyoto, Japan). Postoperative analgesia was provided to each rat by giving a single injection of ketoprofen (4 mg kg^{-1}, I.P.). This was followed by 4 days of recovery and 2 days of treadmill re-adaptation (Hasegawa *et al*. 2008). Some rats were not able to run smoothly during this period, and we did not use them for exercise experiments.

2.3.Experimental procedures.

On the day of the experiments, rats were anaesthetized with isoflurane 4% and oxygen insufflated into a transparent chamber. After induction, the rats were kept under anesthesia to change the probes, using 1.5% isoflurane delivered along with oxygen at 0.8 l/min via a face mask (Hasegawa *et al*. 2008). The dummy cannula was replaced by a microdialysis probe with a membrane length of 2 mm (CX-I-12-02, Eicom). The microdialysis probe was connected to a microinjection pump (CMA 100, CMA Microdialysis, Stockholm, Sweden) and was perfused with a modified Ringer's solution (147 mM NaCl, 4 mM KCl, and 2.3 mM $CaCl_2$) at a flow rate of 2µl min^{-1}. Microdialysis sampling was started 2 hours after probe implantation. The air-tight treadmill chamber (MK-680AT/02R, Muromachi Kikai, Tokyo, Japan) was adjusted by attaching the counterbalance arm of the microdialysis system. The thermocouple probe for tail skin temperature was attached using tape on the dorsal surface of the skin ~10 mm from the base of the tail. The rats were first monitored when they were resting on the treadmill for 120 min to verify stable basal conditions. The rats were then made to exercise for one hour at 0 grade incline. Treadmill speed was increased every 20 min (10, 20, and 26 m min^{-1}) corresponding to approximately 45, 60, and 80% of the $\dot{V}O_{2max}$, respectively (Mäkinen *et al*., 1996; Tanaka et al., 1988). The temperature in the treadmill chamber was set at 23°C. We continued to monitor for another hour during the recovery period after treadmill running.

2.4.Measurement of oxygen consumption.

The gas analysis system consisted of two air-tight treadmill chambers (as described above). 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 chambers at a rate of 3.0 l min^{-1}.

2.5.Sampling.

During the experiment, core body temperature (T_{core}) was measured and monitored by biotelemetry system (Dataquest A.R.T., Data Science International). Tail skin temperature (T_{tail}), an index of heat loss, and $\dot{V}O_2$, an index of heat production, were also simultaneously measured. Microdialysis samples (20 μl) were collected every 10 min. These parameters were recorded at rest, during incremental treadmill exercise, and during the 60 min of recovery.

2.6.Histological examination.

At the end of each experiment, the rats were killed with an overdose of pentobarbital and their brains were removed. The location of the microdialysis probe was verified in coronal sections (100 μm thick) stained with bromophenol blue and verified with the coordinates described by Paxinos & Watson (1986).

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

For the analysis of NA, DA, and 5-HT in the dialysate, an off-line high performance

liquid chromatography (HPLC) assay was used, as described previously in detail (Hasegawa *et al*. 2008). In summary, the assay was based on ion-pair reversed-phase chromatography (5 μm particle size; 2.0 × 200 mm ID, EICOMPAK CAX column, Eicom), coupled to single-channel electrochemical detection (ECD-300, Eicom) with automatic injection (10 μl) of the samples (M-500, Eicom). 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.

2.8.Data collection and statistical analysis.

All values are presented as means ± SEM. The average concentration of 3 microdialysis samples for 30 min before exercise was considered as the baseline and was defined as 100 %. The average thermoregulatory parameters at the start of exercise were also considered as the baseline. Differences between data were evaluated for statistical significance by using one-way analysis of variance (ANOVA) for repeated measures followed by Dunnet's post hoc tests. $P < 0.05$ was regarded as statistically significant.

3. Results

We performed successful experiments in 11 exercising rats. These rats were able to run for 1hour during which the running speed was incrementally changed. We excluded experimental data, where the tips of the microdialysis probe were not correctly positioned

into the PO/AH. The success rate of experiment was about 40-50% because some rats were not able to complete the exercise protocol and in some cases technical problems occurred.

Figure 1 shows the mean changes in T_{core} (A), $\dot{V}O_2$ (B), and T_{tail} (C) of the eleven exercising rats during incremental treadmill running. Before the start of exercise these parameters were stable for at least 60 min. Treadmill running produced a progressive increase in T_{core}, and these levels were significantly higher than basal resting T_{core}. T_{core} levels were dependent on the exercise intensity, and T_{core} increased in a linear fashion during the final stage of high-intensity exercise. During recovery T_{core} gradually decreased, but remained significantly higher than basal levels at the end of the experiment. Treadmill running immediately increased $\dot{V}O_2$, and levels were stable at the end of each exercise stage. The $\dot{V}O_2$ levels during exercise were significantly higher than basal levels. The increase in $\dot{V}O_2$ indicates that thermoregulatory heat production was enhanced during incremental treadmill running. After treadmill exercise, $\dot{V}O_2$ immediately decreased within 20 min. T_{tail} dropped at the beginning of exercise, followed by a gradual increase until the end of the moderate-intensity exercise. During high-intensity exercise, T_{tail} reached a plateau. Although T_{tail} at the end of low-intensity exercise did not differ from baseline, T_{tail} with both moderate- and high-intensity exercise was significantly greater. During recovery, T_{tail} gradually decreased to baseline.

Figure 2 shows the mean changes in extracellular NA (A), DA (B), and 5-HT (C) in the PO/AH during incremental treadmill running. Before the start of exercise, the neurotransmitter levels were stable. Extracellular concentrations in the PO/AH of NA and DA increased during incremental running. The increase in both NA and DA release was dependent on the increase in exercise intensity. During recovery, both NA and DA levels

gradually decreased to the basal resting level. Extracellular 5-HT levels in the PO/AH were not altered by treadmill running.

4. Discussion

To the best of our knowledge, this is the first study to employ continuous monitoring to investigate the actual changes in brain neurotransmitters and the detailed thermoregulatory responses in the same exercising rats. This combined approach allowed a significant reduction in the number of animals in order to obtain reliable results. Using this methodology, we show that the increase in core body temperature, oxygen consumption, and tail heat loss during incremental treadmill running is accompanied by an increase in hypothalamic NA and DA release, but without an effect on serotonergic neurotransmission.

To maintain a thermal balance, heat produced by exercising muscles must be offset by heat loss; otherwise, physical activity could result in hyperthermia (Shellock & Rubin, 1984; Sores *et al*. 2004). Because rats do not sweat or pant and cannot employ heat loss behaviors such as saliva spreading and body extension while running on a treadmill, tail skin vasodilatation is the main route for heat loss during exercise (Tanaka *et al*. 1988; 1993). In this study, T_{tail} dropped at the start of exercise as a result of vasoconstriction and blood redistribution to the exercising muscles (Wilson *et al.* 1978), T_{tail} increased after 10 min of exercise and remained elevated. Our results indicate that vasodilatation occurred with the activation of heat loss responses during incremental running. Although the heat dissipation mechanism was engaged, it was insufficient to overcome the intense heat production by exercise thermogenesis; core body temperatures did not plateau during high-intensity treadmill running (Fig. 1A). This suggests that the rat's tail has sufficient heat-dissipating capacity to counterbalance the heat load of mild exercise, but the animals could not maintain thermal balance during high-intensity exercise.

We were able to confirm that the use of oxygen consumption is a practical index for heat production responses during exercise. Tanaka et al. (1988) examined the relationship

between body temperature, tail vasomotor response, and work intensity in rats, using a treadmill and continuous $\dot{V}O_2$ monitoring. They reported that rectal temperature during treadmill running was proportional to work intensity based on $\dot{V}O_2$ at 24°C. We observed a gradual increase in $\dot{V}O_2$ during incremental treadmill running at 23°C (Fig. 1B). These results are consistent with their results that $\dot{V}O_2$ during exercise is dependent on the exercise intensity. These authors measured core temperature using a rectal thermocouple probes during exercise. This approach may stress the rats and could cause emotional hyperthermia (Gordon, 1990). The core temperature of an unstressed rat varies between 37.0-37.4°C during the day (Gordon, 1990). The peritoneal core temperature before exercise in our rats was 37.3°C. This agrees well with previous reports and confirms that the use of biotelemetry in this study resulted in undisturbed rats before exercise.

Previous findings show that a high core body temperature enforces the development of fatigue during prolonged exercise, particularly in a hot environment (Nybo & Secher, 2004). Additionally, hyperthermia has been demonstrated to exert a profound effect on the central nervous system (Nybo, 2008). However, previous studies have not combined brain catecholamine measurements with exercise intensity and core temperature. Therefore, animal experiments are necessary to compare continuous physiological parameters and brain neurotransmitters during exercise (Meeusen *et al*. 2001; 2006). During incremental treadmill running, we observed increases in core body temperature and hypothalamic catecholamines, while serotonergic neurotransmission remained unchanged. In addition, results from our previous microdialysis studies clearly indicate that dopaminergic neurotransmission is important to the control of core temperature by modulating metabolic levels during exercise (Hasegawa *et al*. 2000). PO/AH neurotransmission is critical for thermoregulation during exercise, as shown by our previous experiments (Hasegawa *et al*. 2005). Furthermore, acute injection of a dual DA/NA re-uptake inhibitor in rats improves exercise performance and increases core and brain temperature during treadmill exercise in a warm environment by increasing extracellular concentrations of DA and NA (but not 5-HT) in the PO/AH (Hasegawa *et al*. 2008). Recently, we observed no effects of a

low-intensity exercise in a warm environment on extracellular 5-HT in the PO/AH, and treatment with a selective 5-HT re-uptake inhibitor did not produce acute changes in thermoregulation (Takatsu *et al*., 2010). Based on our previous studies and the current literature, it is clear that the physiological mechanisms involved in exercise thermoregulation are influenced by catecholaminergic NA and DA neurotransmitter activity in the PO/AH, and do not involve serotonergic neurotransmission. However, more research is necessary to elucidate the exact role of NA and DA in thermoregulation during exercise.

5. Conclusion

We employed in vivo brain microdialysis, biotelemetry, and metabolic measurements to perform continuous monitoring of brain neurotransmitters, core body temperature, and thermoregulatory responses, during incremental running in a rat model. The results provide new evidence that the increase in core body temperature during incremental treadmill running is accompanied by an increased release of NA and DA in the thermoregulatory center of the brain. Furthermore, the activity of heat loss mechanisms and heat production parallel exercise intensity. The physiological mechanisms of thermoregulation during exercise appear to be influenced by catecholamine neurotransmitter activity in the PO/AH, rather than serotonergic neurotransmission. These new findings also suggest that the running animal model may be useful for examining the neurotransmitter-governed mechanisms of thermoregulation and central fatigue during exercise.

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Figure Legends

Figure 1. Effect of incremental treadmill running on core body temperature (A), oxygen consumption (an index of heat production) (B), and tail skin temperature (an index of heat loss) (C) in rats. The dashed lines indicate the incremental treadmill running. Treadmill speed was increased every 20 min (10, 20, and 26 m min^{-1}). *Significant difference compared with baseline ($P < 0.05$). Values are means ± SEM (n=11).

Figure 2. Effect of incremental treadmill running on extracellular noradrenaline (NA) (A), dopamine (DA) (B), and serotonin (5-HT) (C) in the PO/AH in rats. The average concentration of three microdialysis samples before treadmill exercise was set as the baseline, defined as 100%. Microdialysis sample results were expressed relative to the baseline value (means ± SEM, n=11). The dashed lines indicate the incremental treadmill running. Treadmill speed was increased every 20 min (10, 20, and 26 m min^{-1}). *Significant difference compared with baseline ($P < 0.05$).

Figure 1

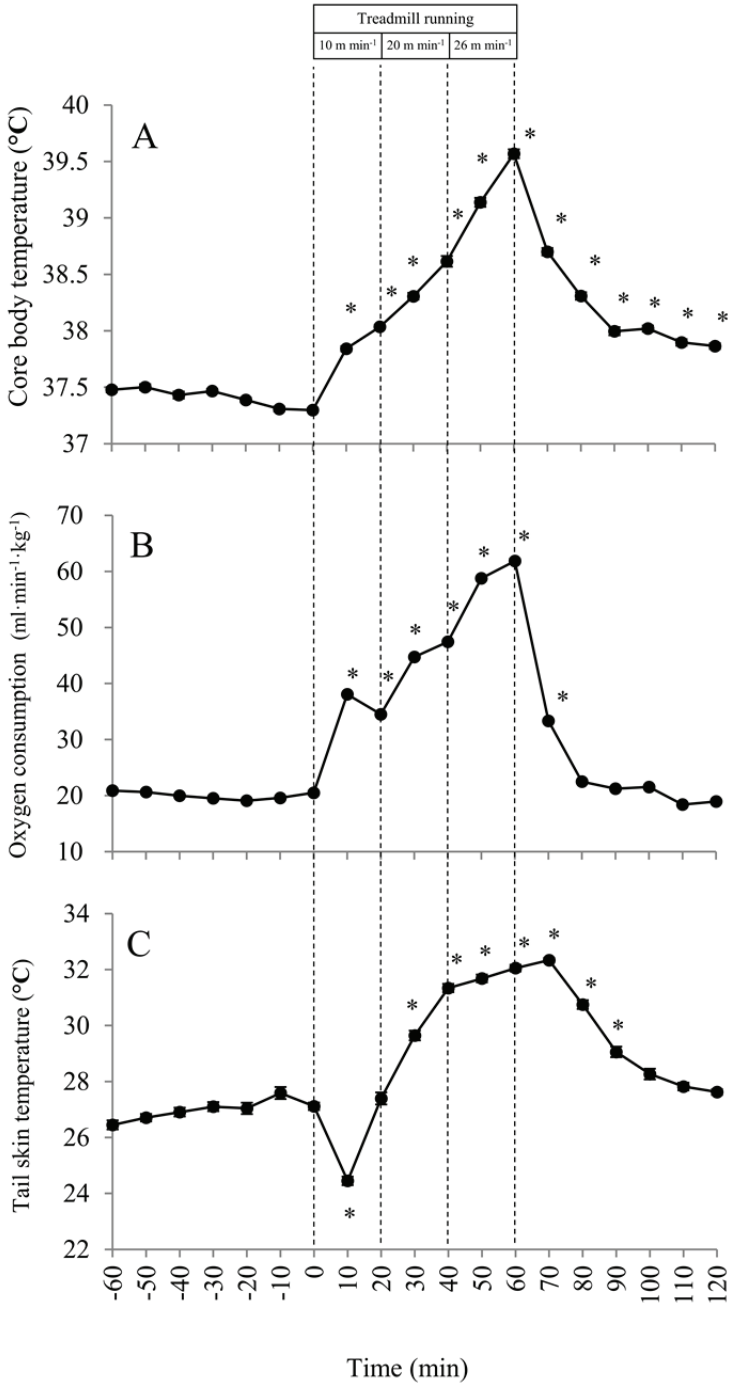
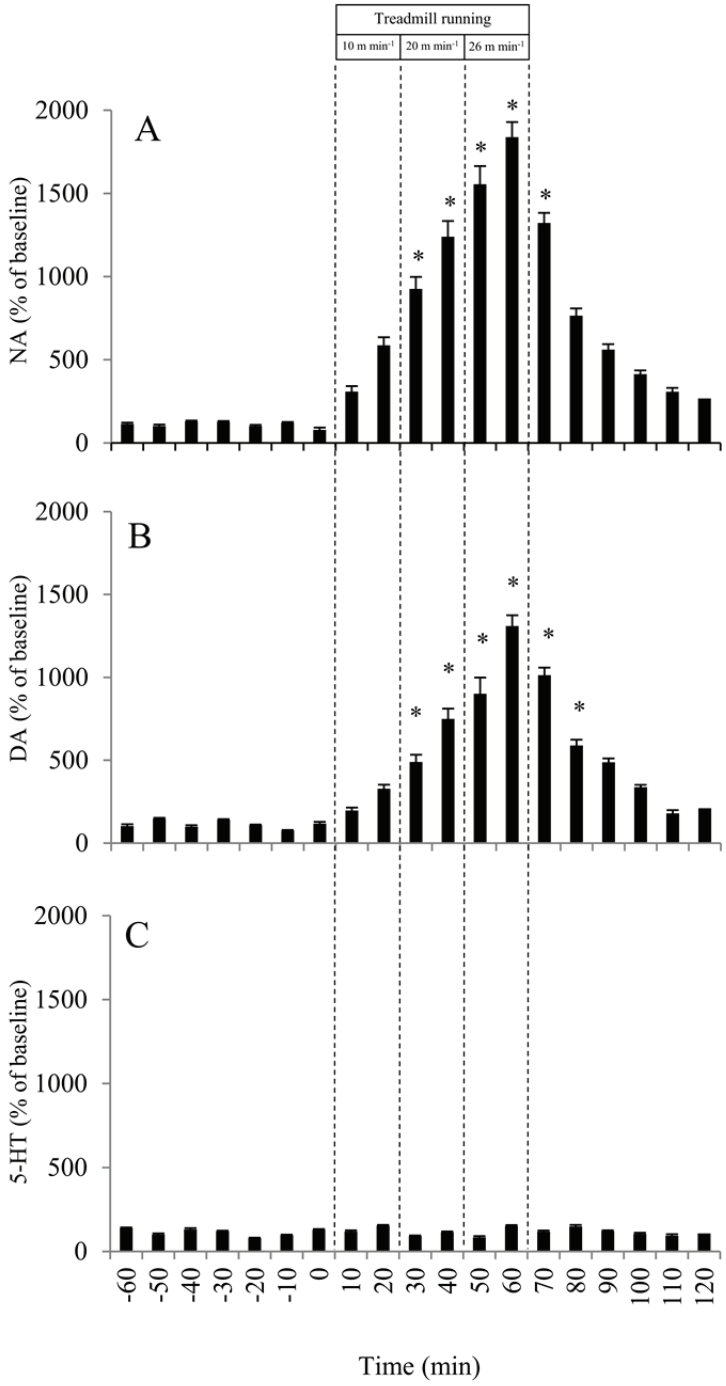

Treadmill running
10 m min-1
20 m min-1
26 m min-1
A
Core body temperature (°C)
40
39.5
39
38.5
38
37.5
37
B
Oxygen consumption (ml·min-1·kg-1)
70
60
50
40
30
20
10
C
Tail skin temperature (°C)
34
32
30
28
26
24
22
-60
-50
-40
-30
-20
-10
0
10
20
30
40
50
60
70
80
90
100
110
120
Time (min)

Figure 2


Treadmill running
10 m min-1
20 m min-1
26 m min-1
A
B
C
NA (% of baseline)
DA (% of baseline)
5-HT (% of baseline)
2000
1500
1000
500
0
-60
-50
-40
-30
-20
-10
0
10
20
30
40
50
60
70
80
90
100
110
120
Time (min)