Journal of Thermal Biology 58 (2016) 15–22

Contents lists available at ScienceDirect

Journal of Thermal Biology

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

Changes of brain monoamine levels and physiological indexes during heat acclimation in rats

Hikaru Nakagawa, Takeru Matsumura, Kota Suzuki, Chisa Ninomiya, Takayuki Ishiwata *

Graduate School of Community and Human Services, Rikkyo University, 1-2-26 Kitano, Niiza, Saitama 352-8558, Japan

ARTICLE INFO

Article history:
Received 14 December 2015
Received in revised form
20 March 2016
Accepted 21 March 2016
Available online 28 March 2016

Keywords:
Heat acclimation
Noradrenaline
Dopamine
Serotonin
Body temperature
Heart rate

ABSTRACT

Brain monoamines, such as noradrenaline (NA), dopamine (DA), and serotonin (5-HT), regulate many important physiological functions including thermoregulation. The purpose of this study was to clarify changes in NA, DA, and 5-HT levels in several brain regions in response to heat acclimation while also recording body temperature (Tb), heart rate (HR), and locomotor activity (Act). Rats were exposed to a heated environment (32 °C) for 3 h (3H), 1 day (1D), 7 days, 14 days (14D), 21 days, or 28 days (28D). After heat exposure, each of the following brain regions were immediately extracted and homogenized: the caudate putamen (CPu), preoptic area (PO), dorsomedial hypothalamus (DMH), frontal cortex (FC), and hippocampus (Hip). NA, DA, and 5-HT levels in the extract were measured by high performance liquid chromatography. Although Tb increased immediately after heat exposure, it decreased about 14D later. HR was maintained at a low level throughout heat exposure, and Act tended to increase near the end of heat exposure. After 3H, we observed a marked increase in NA level in the CPu. Although this response vanished after 1D, the level increased again after 28D. DA level in the CPu decreased significantly from 1D to 28D. 5-HT level in the PO and DMH decreased from 1D to 14D. It returned to control levels after 28D with increment of DA level. 5-HT level in the FC decreased at the start of heat exposure, but recovered after 28D; a time point at which DA level also increased. Monoamine levels in the Hip were unchanged after early heat exposure, but both 5-HT and DA levels increased after 28D. These results provide definitive evidence of changes in monoamines in individual brain regions involved in thermoregulation and behavioral, cognitive, and memory function during both acute and chronic heat exposure.

1. Introduction

Currently, global warming is one of the most serious problems in the world and many humans die of heat illnesses, such as heat cramps and heatstroke (Singh et al., 2013). A high temperature environment causes increases in body temperature (Tb) that can lead to hyperthermia, which impairs cognitive function and memory (Gaoua et al., 2012; Puumala and Sirviö, 1998; Racinais et al., 2008). Animals have several mechanisms for autonomous responses to heat. When mammals are exposed to heat over long periods, they adapt to the environment via thermoregulation. This response is called heat acclimation (Matthew, 1997; Wendt et al., 2007). Chronic heat exposure over several weeks induces adaptive changes in the thermoregulatory response such as sweating quickly (Buono et al., 2009), controlling salivation (Horowitz et al., 1983), and falling Tb (Buono et al., 1998). Heat-induced c-fos expression has been identified in many brain regions, including the hypothalamus, septum, cortex, and hippocampus (Hip) (Bratincsak and Palkovits, 2004; Kiyohara et al., 1995; Patronas et al., 1998). Previous studies suggest that many of the brain regions affected by heat exposure are concerned with physiological functions including regulation of Tb under hot conditions.

It is well known that the preoptic area (PO) in the hypothalamus plays an important role in thermoregulation (Ishiwata et al., 2005; Nagashima et al., 2000; Nakayama, 1985). In addition, recent studies have reported that the dorsomedial hypothalamus (DMH) also plays a key role (Dimicco and Zaretsky, 2007; Nakamura, 2011), especially implicating neurons in this region in the control of non-shivering thermogenesis in brown adipose tissue (BAT) (de Menezes et al., 2006; Zaretskaia et al., 2002). Before physiological reactions to short-term heat exposure arise, animals often retreat to shade or a waterfront. This behavior is called behavioral thermoregulation. The caudate putamen (CPu) is an area involved in

Abbreviations: 5-HT, serotonin; NA, noradrenaline; DA, dopamine; CPu, caudate putamen; PO, preoptic area; DMH, dorsomedial hypothalamus; Hip, hippocampus; FC, frontal cortex; HR, heart rate; Tb, body temperature; Act, locomotor activity; BAT, brown adipose tissue; Ta, ambient temperature; RH, relative humidity; 3H, 3 h; 1D, 1 day; 7D, 7 days; 14D, 14 days; 21D, 21 days; 28D, 28 days

* Corresponding author.
E-mail address: ishiwata@rikkyo.ac.jp (T. Ishiwata).

http://dx.doi.org/10.1016/j.jtherbio.2016.03.010
0306-4565/

controlling behavior and locomotor activity (Act), and its function is strongly influenced by dopamine (DA) receptors (Charntikov et al., 2011; Der-Ghazarian et al., 2012). It is also reported that cognitive function and memory are impaired by hyperthermia (Gaoua et al., 2012; Puumala and Sirviö, 1998; Racinais et al., 2008). The frontal cortex (FC) and Hip have been shown to control these functions (Kesner and Churchwell, 2011; Warburton and Brown, 2015).

These functions are regulated by neurotransmitters called monoamines, such as NA, DA, and serotonin (5-HT). Monoamines play essential roles in several functions with homeostatic roles; including thermoregulation, behavior, cognitive function, and memory (Clark and Lipton, 1985, 1986). Monoamines have an important role for thermoregulation in the PO. Previous studies have shown that an increase in DA and NA in the PO is involved in thermoregulation in exercising rats (Hasegawa et al., 2008). It was reported that 5-HT in the PO also has an important role in mediating Tb (Ishiwata 2014; Jacobs and Azmitia, 1992), but an acute change of Tb was not observed after cold and heat exposure, or after perfusing 5-HT reuptake inhibitors into the PO and anterior hypothlamus (Ishiwata et al., 2004). Thermogenesis in the BAT is mainly mediated by γ-aminobutyric acid (Nakamura, 2011). However, no studies have investigated the monoamine changes in the DMH during heat exposure or heat acclimation. Puumala and Sirviö (1998) showed that DA and 5-HT play important roles in the FC by using the 5-choice serial reaction time task. Monoamine signaling, especially by 5-HT, in the Hip is related to learning and memory (Adams et al., 2008). Moreover, many stressors can induce impairments of spatial memory (Pattij et al., 2001; Zethof et al., 1994). Therefore, during heat exposure, changes in monoamine levels in any of these brain regions can influence neural and physiological functions related to thermoregulation.

However, it is currently unknown which monoamines signal in these brain regions to mediate the adaptive changes of the thermogenic response during heat acclimation. The purpose of this study was to measure changes in the concentrations of NA, 5-HT, and DA in the PO, DMH, CPu, FC, and Hip during chronic heat exposure while also measuring the Tb, heart rate (HR), and Act of rats.

2. Materials and methods

2.1. Animals

The study was conducted using 49 male Wistar rats (CLEA Japan, Inc., Japan; 330–360 g body weight). The rats were housed in plastic cages under controlled conditions with an ambient temperature (Ta) of 23 °C, a relative humidity (RH) of 50%, a 12 h light/dark cycle (lights on at 07:00), and were allowed free access to food and water. 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 and approved by a committee for safety and ethics in research and experiments related to Rikkyo University Life Sciences (No. LS13027A).

2.2. Measurement of thermoregulatory parameters

To determine the timing for measurement of monoamines, the core Tb, HR, and Act of 7 rats was measured for 4 weeks. They were anesthetized using somnopentyl anesthesia (50 mg/kg, i.p.) and a telemetry device (TA11CTA-F40; Data Sciences International, Saint Paul, MN) was implanted in the abdominal cavity. After surgery, rats were housed separately in a normal environment (Ta: 23 °C, RH: 50%) for 1 week to recover from the operation. Then, rats were exposed to a heated environment (Ta: 32 °C, RH: 50%) while Tb, HR, and Act were continuously monitored for 28 days. It should however be noted that an environmental temperature of 23 °C is lower than the thermoneutrality temperature (28 °C) in the laboratory rat (Gordon, 1990; Maloney et al., 2014).

2.3. Measurement of neurotransmitters levels

Based on the results of monitoring Tb, HR, and Act during 28 days of exposure to a heated environment (Fig. 1); 42 rats were divided into 7 groups and exposed to the heated environment (Ta: 32 °C, RH: 50%) for 3 h (3H), 1 day (1D), 7 days (7D), 14 days (14D), 21 days (21D), or 28 days (28D). The control group (Con, Ta: 23 °C, RH: 50%) was not exposed to heat. After heat exposure, the rats were killed, and their brains were immediately sliced at 300-μm thickness using a LinearSlicer (PRO7, Dosaka EM CO., Ltd., Kyoto, Japan). The PO, DMH, CPu, FC, and Hip were dissected out using disposable biopsy punches (BP-10F, Kai Medical, Gifu, Japan) with a 1-mm radius (0.2 mg tissue). The coordinates with respect to bregma were: FC-AP +3.2 mm, L+0.8 mm, D 3.5 mm; CPu-AP +0.2 mm, L+4.0 mm, D 6.0 mm; PO-AP −0.4 mm, L+0.5 mm, D 8.3 mm; DMH-AP −3.6 mm, L+0.4 mm, D 9.2 mm; pH-AP −3.6 mm, L+0.4 mm, D 8.2 mm; Hip-AP −2.4 mm, L+1.8 mm, D 2.8 mm (Paxinos and Watson, 1986). After washing the tissue with Ringer's solution (147 mM NaCl, 4 mM KCl, 2.3 mM $CaCl_2$), the tissue was gently ground in a pestle, and then placed in a microtube (As One, Osaka, Japan) in 100 μl of ice-cold 0.1 M perchloric acid (PCA). The completed homogenate samples were iced for 30 min in a 0 °C freezer. The homogenates were centrifuged at 12,000 × *g* for 5 min at 0 °C (CF15RX II, Hitachi Koki, Co. Ltd., Tokyo, Japan) and the supernatants were then filtered (0.45 μm, Millipore, Billerica, MA) prior to high performance liquid chromatography (HPLC) analysis.

2.4. HPLC

The NA, DA, and 5-HT concentrations in the sample were measured by high performance liquid chromatography (HPLC-ECD 700 system, Eicom, Kyoto, Japan). Peaks in a sample were identified by matching the retention times of peaks with those of authentic standards (PowerChrom, eDAQ Pty Ltd, Denistone East, Australia). A mixture of a sample and a standard were sometimes analyzed for confirmation of identification of peaks.

We measured the concentration of NA, DA, and 5-HT in the same run. The HPLC system was equipped with an amperometric electrochemical detector (ECD-700), a pump (EP-700), a column temperature controller (ATC-700), and a data processor (EPC-300). We used a EICOMPAK column (SC-5ODS, 3.0 mm i.d. × 150 mm, Eicom) with a precolumn (PC-04-AC, 4.0 mm i.d. × 5.0 mm, Eicom). A 20-μl aliquot of the sample was injected directly into the HPLC by an automated HPLC sample injector (M-504, Eicom). The mobile phase solution contained 85% 0.1 M acetic acid–citric acid buffer (pH 3.5), 15% methanol, sodium 1-octane sulfonate (170 mg/mL), and EDTA-2Na (5 mg/mL). The flow rate of the mobile phase was 0.5 mL/min. The graphite electrode (WE-3G, Eicom) was set at a potential of 750 mV, relative to an Ag/AgCl reference electrode. Analysis of one sample took about 25 min (Matsumura et al., 2015).

2.5. Statistical analysis

Tb, HR, and Act were measured every 1 min and averaged for 12 h (7:00–19:00, 19:00–7:00). The samples were grouped into periods of 7 days to enable weekly analysis of neurotransmitter levels. Weekly changes in physiological parameters were evaluated for statistical significance with repeated ANOVAs, followed by