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Oxidative damage and brain concentrations of free amino acid in chicks exposed to high ambient temperature

Vishwajit S. Chowdhury [a,*], Shozo Tomonaga [b,1], Taro Ikegami [b,2], Edi Erwan [b], Kentaro Ito [b], John F. Cockrem [c], Mitsuhiro Furuse [b]

[a] Division for Arts and Science, Faculty of Arts and Science, Kyushu University, Fukuoka 819–0395, Japan
[b] Laboratory of Regulation in Metabolism and Behavior, Faculty of Agriculture, Kyushu University, Fukuoka 812–8581, Japan
[c] Institute for Veterinary, Animal and Biomedical Sciences, Massey University, Palmerston North, New Zealand

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ABSTRACT

High ambient temperatures (HT) reduce food intake and body weight in young chickens, and HT can cause increased expression of hypothalamic neuropeptides. The mechanisms by which HT act, and the effects of HT on cellular homeostasis in the brain, are however not well understood. In the current study lipid peroxidation and amino acid metabolism were measured in the brains of 14 d old chicks exposed to HT (35 °C for 24- or 48-h) or to control thermoneutral temperature (CT; 30 °C). Malondialdehyde (MDA) was measured in the brain to determine the degree of oxidative damage. HT increased body temperature and reduced food intake and body weight gain. HT also increased diencephalic oxidative damage after 48 h, and altered some free amino acid concentrations in the diencephalon. Diencephalic MDA concentrations were increased by HT and time, with the effect of HT more prominent with increasing time. HT altered cystathionine, serine, tyrosine and isoleucine concentrations. Cystathionine was lower in HT birds compared with CT birds at 24 h, whilst serine, tyrosine and isoleucine were higher at 48 h in HT birds. An increase in oxidative damage and alterations in amino acid concentrations in the diencephalon may contribute to the physiological, behavioral and thermoregulatory responses of heat-exposed chicks.

1. Introduction

High ambient temperature (HT) can decrease food intake, live weight gain, and food efficiency in broilers (Howlider and Rose, 1987; Siegel, 1995; Niu et al., 2009; Azad et al., 2010a), and can affect egg production in laying hens (Marsden et al., 1987; Peguri and Coon, 1991; Yahav et al., 2000; Sterling et al., 2003; Lin et al., 2004; Franco-Jimenez and Beck, 2007; Ajakaiye et al., 2010). The exposure of birds to HT can lead to an increase in deep body temperature (rectal temperature; Yahav and Hurwitz, 1996; Chowdhury et al., 2012a,b) and may cause heat stress (Bartlett and Smith, 2003; Soleimani et al., 2010). It is thought that the negative effects of heat stress on growth rate and production are probably due to reduced food intake in birds (Hurwitz et al., 1980; Savory, 1986), and food consumption, growth rate, food efficiency and survivability all decline in poultry as environmental temperature increases (Mashaly et al., 2004; Yu et al., 2008).

Behavioral, physiological and molecular adjustments occur during heat stress (Etches et al., 1995; Chowdhury et al., 2012a), and heat stress is one of the major causes of oxidative stress. Oxidative stress is defined as an imbalance of pro-oxidants and antioxidants, or a disruption of redox signaling and control (Lin et al., 2000; Jones, 2006; Lin et al., 2006; Azad et al., 2010b). HT can also have adverse effects on the structure and physiology of cells, and can impair transcription, RNA processing, translation and oxidative metabolism, as well as cause disruption of membrane structure and function (Iwagami, 1996; Mujahid et al., 2009; Azad et al., 2010b). Oxidative deterioration or peroxidation of cellular membrane lipids has been suggested as forming part of the cellular ageing process and to contribute to several age-associated degenerative diseases and various pathological conditions (Bast and Goris, 1989). Lipid peroxidation is initiated following the abstraction of hydrogen from polyunsaturated fatty acids by hydroxyl radicals generated from hydrogen peroxide (Bhuyan et al., 1986), and lipid peroxidation plays a role in the disruption of the normal structure of membrane phospholipids. Malondialdehyde (MDA) is a major breakdown product of lipid peroxidases, and MDA concentrations have been used to assess lipid peroxidation. The thiobarbituric acid test for MDA is widely used as an indicator of lipid peroxidation (Satoh, 1978; Yagi, 1984; Likidlilid et al., 2010; Bhutia et al., 2011). Changes in cellular physiology during HT in poultry are not well known. Recently, it was reported that HT

* Corresponding author. Tel.: +81 92 642 2954; fax: +81 92 642 3098.
E-mail address: vc-sur@artsci.kyushu-u.ac.jp (V.S. Chowdhury).
[1] Shozo Tomonaga is now at the Laboratory of Nutritional Science for Animals, Graduate School of Agriculture, Kyoto University, Kyoto, 606–8502, Japan.
[2] Taro Ikegami is now at the Department of Chemistry, Biology and Marine Science, University of the Ryukyus, Okinawa 903–0213, Japan.

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increased the concentrations of some hypothalamic neuropeptides in young chicks (Chowdhury et al., 2012a). Lipid peroxidation can occur during HT, and thus the first goal of this study was to measure MDA levels to determine whether HT alters lipid peroxidation in the chick brain.

The administration of various amino acids may reduce the effects of psychological and physiological stress in chicks (Yamane et al., 2009; Hamasu et al., 2010; Erwan et al., 2012). Hamasu et al. (2009) reported that L-proline and L-arginine were decreased in the telencephalon and diencephalon of chicks under isolation or fasting stress, and L-proline attenuated the stress response in the central nervous system of chicks. However, to our knowledge, changes of free amino acids in the chicken brain have not been measured during HT. Therefore, the second aim of this study was to analyze the changes of free amino acids in the diencephalon and plasma of heat-exposed chicks.

2. Materials and methods

2.1. Animals

Day-old male layer chicks (Julia strain; *Gallus gallus domesticus*) were purchased from a local hatchery (Murata hatchery, Fukuoka, Japan) and housed in groups in metal cages at a constant temperature of 30 ± 1 °C under continuous light until 14 days of age. Food (Commercial starter diet (energy: 3050 kcal/kg and protein: 24%); Toyohashi Feed and Mills Co. Ltd., Aichi, Japan) and water were available *ad libitum*. On the basis of a preliminary experiment, 14- or 21-day chicks were more responsive to HT in terms of food intake than neonatal chicks (Chowdhury et al., 2012b). Thus, we used 14-day old chicks to examine the effect of HT (35 °C) compared with a control thermoneutral temperature (CT; 30 °C) in this study. Chicks were placed in individual cages 12 h before starting the experiment. Twenty-eight chicks ($n = 14$ in each group) were exposed to HT or CT for 24- or 48-h, with chicks allocated to groups according to body mass so the initial body masses (average 146 ± 1.5 g) were similar in the two groups. Chicks were given free access to water and feed during exposure to HT or CT. This study was performed according to the guidance for Animal Experiments in the Faculty of Agriculture of Kyushu University and the Law (No. 105) and Notification (No. 6) of the Japanese Government.

2.2. Experimental design

Body mass and rectal temperature were recorded immediately before the HT chicks were placed in their cages into a temperature-controlled chamber (Sanyo Electric Co. Ltd., Japan), whilst control chicks were placed in their cages on similar racks in CT as per the method described previously (Chowdhury et al., 2012a,b). Rectal temperature, food intake and body mass were measured after 24- or 48-h of HT or CT, after which birds were killed by exposure to anesthesia (isoflurane, Mylan Inc., Tokyo, Japan).

Brains were dissected (as shown in Fig. 1) and the diencephalon (thalamus and hypothalamus), telencephalon, mesencephalon and cerebellum collected and snap frozen for analyzing oxidative damage. The food intake of each bird during the treatment period was recorded by subtracting the weight of feed remaining in the feeder from the initial or initial plus added food during the 24- or 48-h experimental period. Adequate precautions were taken against food spillage, namely, food was provided up to the half of the feeders and the opening of the feeders was adequate for the chicks to feed without spillage of food. Rectal temperatures of chicks were measured using a digital thermometer with an accuracy of ±0.1 °C (Thermalert TH-5, Physitemp Instruments Inc., USA), by inserting the thermistor probe in the cloaca to a depth of 1–2 cm. Diencephalons were also collected from chicks maintained under identical HT conditions to the first experiment for 24- or 48-h of HT, and at the same time diencephalons were collected from control chicks. Randomly selected diencephalons from 24- and 48- h experimental and control groups ($n = 7$ in each group) were used for the analysis of free amino acid concentrations.

2.3. Brain tissue MDA

Brain tissues (diencephalon, telencephalon, mesencephalon and cerebellum) were used for MDA measurements. Tissues were homogenized in buffer (100 mM KCl, 50 mM Tris–HCl and 2 mM ethyleneglycol tetraacetic acid, pH 7.4) for 1 min and then sonication for 15 s was performed. The homogenates were centrifuged at 700 *g* for 10 min and supernatants were collected. A Thiobarbituric Acid Reactive Substance (TBARS) assay kit (Cayman Chemical Company, Ann Arbor, MI, USA) was used for assaying lipid peroxidation in the brain tissue. Briefly, tissue homogenates were mixed with the supplied sodium dodecyl sulfate solution then a color reagent (thiobarbituric acid (TBA), diluted TBA acidic acid solution, diluted TBA sodium hydroxide solution) was prepared as per the manufacturer's protocol and added to the homogenates. After vortexing, samples were placed on vigorously boiled water for an hour. After one hour vials were removed and placed in an ice bath for 10 min to stop the reaction. After 10 min, the vials were centrifuged at 1600 *g* at 4 °C for 10 min. Vials were stable with pink color solution at room temperature for around 30 min. Absorbance of the supernatant was measured at 562 nm. The content of TBARS is expressed as the MDA equivalent. The protein content of tissue homogenates was

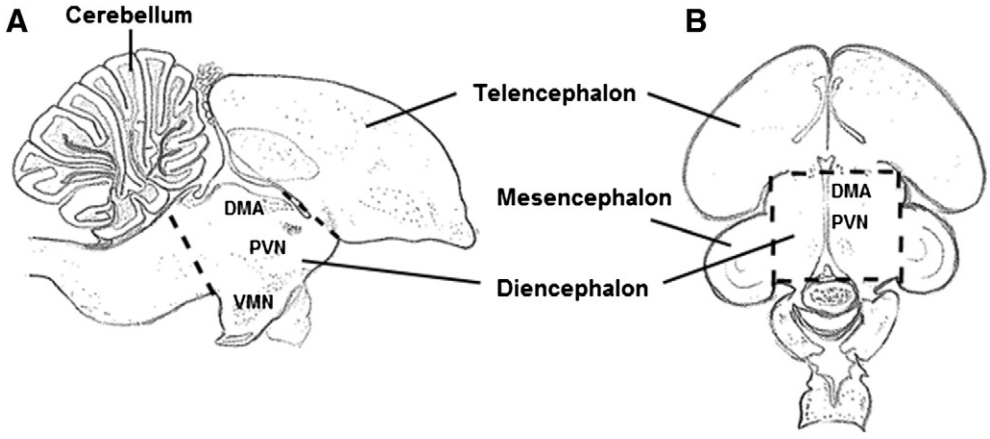

Fig. 1. Schematic drawing of sagittal (A) and coronal (B) sections depicting different dissected parts of the chick brain (See Kuenzel and Masson, 1988). Broken line shows the diencephalic area to get an image for whole diencephalon, which was used for the analysis of MDA and free amino acid concentrations. Some nuclei of the diencephalon are shown, namely DMA, dorsomedial anterior nucleus; PVN, paraventricular nucleus; VMN, entromedial nicleus.