Comparative Biochemistry and Physiology, Part A 204 (2017) 48–56

Contents lists available at ScienceDirect

Comparative Biochemistry and Physiology, Part A

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

L-Leucine acts as a potential agent in reducing body temperature at hatching and affords thermotolerance in broiler chicks

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ARTICLE INFO

Article history:
Received 19 June 2016
Received in revised form 23 September 2016
Accepted 28 October 2016
Available online 11 November 2016

Keywords:
L-Leucine
Thermal manipulation
In ovo injection
Body temperature
Thermotolerance
Chicks

ABSTRACT

Thermal manipulation (TM) of incubation temperature causes metabolic alterations and contributes to improving thermotolerance in chicks post hatching. However, there has been no report on amino acid metabolism during TM and the part it plays in thermotolerance. In this study, we therefore first analyzed free amino acid concentrations in the embryonic brain and liver during TM (38.6 °C, 6 h/d during embryonic day (ED) 10 to ED 18). It was found that leucine (Leu), phenylalanine and lysine were significantly decreased in the embryonic brain and liver. We then chose L-Leu and other branched-chain amino acids (L-isoleucine (L-Ile) and L-valine (L-Val)) for in ovo injection on ED 7 to reveal their roles in thermoregulation, growth, food intake and thermotolerance in chicks. It was found that in ovo injection of L-Leu, but not of L-Ileu or L-Val, caused a significant decline in body temperature at hatching and increased food intake and body weight gain in broiler chicks. Interestingly, in ovo injection of L-Leu resulted in the acquisition of thermotolerance under high ambient temperature (35 ± 1 °C for 180 min) in comparison with the control thermoneutral temperature (28 ± 1 °C for 180 min). These results indicate that the free amino acid concentrations during embryogenesis were altered by TM. L-Leu administration in eggs caused a reduction in body temperature at hatching, and afforded thermotolerance in heat-exposed young chicks, further suggesting that L-Leu may be one of the key metabolic factors involved in controlling body temperature in embryos, as well as in producing thermotolerance after hatching.

1. Introduction

Amino acids play important roles in growth and could also play critical roles in controlling body temperature (Erwan et al., 2014; Chowdhury et al., 2015), food intake (Furuse et al., 2006; Erwan et al., 2013; Tran et al., 2016) and behavior (Kabuki et al., 2011; Erwan et al., 2013; Ikeda et al., 2014; Tran et al., 2015). Several free amino acids were significantly increased in the blood, brain and muscle of chicks within 15 or 30 min of exposure to high ambient temperature (HT; 35 °C; Ito et al., 2014); however, most of those free amino acids declined in the brain and plasma when chicks were exposed to HT (35 °C) for a long time – that is, for either 24 or 48 h (Chowdhury et al., 2014). On the basis of these studies, we subsequently found that L-citrulline, which we administered orally, and which was increased and decreased in the chick plasma following short- and long-term heat exposure respectively, has a hypothermic function in chicks (Chowdhury et al., 2015). Therefore, the amino acids which are altered in chicks through exposure to HT may have important physiological functions.

Thermal manipulation (TM) during embryogenesis is carried out in order to increase the ambient temperature of incubation, which results in the acquisition of thermotolerance by neonatal chicks (Moraes et al., 2003; Yahav et al., 2004a,b; Collin et al., 2005; Piestun et al., 2008a) and chickens (Loyau et al., 2014) under HT. TM also has a long-lasting effect on energy balance that leads to an improved feed-conversion ratio (Piestun et al., 2013) and to decreased plasma glucose in neonatal broiler chicks (Yalçın et al., 2008a). A daily cyclical higher incubation temperature from embryonic day (ED) 10 to ED 18 decreased body temperature on the day of hatching (DOH) and improved thermotolerance in broiler (Yalçın et al., 2008b; Al-Zhgoul et al., 2013) and layer chicks (Walstra et al., 2010) after hatching. The effects on post-hatch performance of TM of incubation temperature during embryogenesis have been well studied (see review: Loyau et al., 2015), and it has been suggested that metabolic activities, especially energy metabolism, were significantly altered by TM (Piestun et al., 2009; Loyau et al., 2014; Piestun et al., 2015a). However, to the best of our knowledge, no report is available on amino acid metabolism during TM in any species. We hypothesized that if TM of incubation temperature could modulate amino acid metabolism in embryos, then the altered free amino acids in embryos would provide some clue as to later thermoregulatory functions in chicks. Therefore, the first aim of this study was to examine the

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http://dx.doi.org/10.1016/j.cbpa.2016.10.013
1095-6433/

changes in free amino acid concentrations in embryos resulting from TM of incubation temperature.

It has been demonstrated that in ovo injection of amino acids improves the growth performance of chick embryos without affecting their hatchability (Ohta et al., 1999, 2001). Furthermore, in ovo injection of branched-chain amino acids (BCAAs) which include L-leucine (L-Leu), L-isoleucine (L-Ile) and L-valine (L-Val) promoted the growth of broiler chicks (Bhanja and Mandal, 2005). It has also been reported that supplementation of L-Leu and/or BCAAs has improved embryonic growth as well as food intake and muscle development in chicks (Anthony et al., 2000; Izumi et al., 2004; Bai et al., 2015; Kita et al., 2015). Recently, we reported that free BCAAs were significantly increased in the chick brain (diencephalon) and plasma under short-term exposure to a comparatively strong HT (40 °C for 5 h; Ito et al., 2015), and suggested that L-Leu or other BCAAs may have some critical functions in chicks under heat stress. Although central injection of L-Leu stimulated food intake in chicks (Izumi et al., 2004; Wang et al., 2012), it was unknown whether L-Leu and/or BCAAs have any functions related to thermoregulation in chickens. With this background in mind, and in connection with TM, the second aim of this study was to examine whether the in ovo injection of BCAAs could regulate body temperature and contribute to food intake, growth and also thermotolerance in chicks.

2. Materials and methods

2.1. Incubation of fertilized broiler eggs and rearing of chicks

Four experiments were conducted with a total of 266 Chunky broiler eggs. Fertilized eggs were purchased from a local hatchery (Murata Hatchery, Fukuoka, Japan), individually weighed, and then placed in the incubator (Showa P008 type incubator, Showa Furanki Company, Saitama, Japan). Eggs were grouped on the basis of their weight to produce uniformity among the groups, and they were marked with a soft lead pencil for identification. Incubators had been set up with a temperature of 37.6 °C and about 60% relative humidity. All eggs were candled on ED 7 and auto turned every h until ED 18. Eggs were transferred onto hatching trays from ED 19 after the eggs with undeveloped and dead embryos had been removed. After hatching, day-old broiler chicks were housed in groups in metal cages at a constant temperature of 30 ± 1 °C under continuous light until they were 4 days old. The room temperature was decreased to 28 ± 1 °C once the chicks were 5 days old. Food (Adjust diets (metabolizable energy: >12.55 MJ/kg, protein: >23%)); Toyohashi Feed and Mills Co. Ltd., Aichi, Japan) and water were provided ad libitum.

2.2. Experimental design

In Experiment 1, sixty eggs were distributed into two incubators and set as two groups – namely, control treatment (CT) and TM. The CT group was incubated as described above, while the incubation temperature of the TM group was increased from 37.6 °C to 38.6 °C daily for 6 h (10:00–16:00) from ED 10 to 18 as reported previously by Yalçın et al. (2009). We conducted a preliminary study on the daily changes in temperature and relative humidity of the two incubators and found that both incubators showed similar changes regarding these parameters (data not shown). Furthermore, no differences were found in daily changes in temperature and relative humidity between the incubators used in the current study (data not shown); however, we cannot rule out incubator effects if there is any. Future study with cross-over design of the incubators will clarify the issue. At ED 14 and 19, developing embryos (n = 8–10) were randomly selected from each group and euthanized by cervical dislocation. The euthanization of chicks at DOH was carried out using proper anesthesia with isoflurane (Mylan Inc., Tokyo, Japan). The brain and liver were collected and stored at −80 °C in the deep freezer until they were analyzed for free amino acid concentrations. As the brain is the center of thermoregulation (Yahav, 2015) and the liver plays a critical role in amino acid metabolism (Gan and Jeffay, 1967), we collected these tissues for amino acid analysis. However, it could be relevant to measure the free amino acids also in muscle, a main tissue involved in thermoregulation, protein synthesis and glycogen storage in birds. Therefore, further research will be conducted to analyze the changes in free amino acids in the muscles of developing embryos.

In Experiment 2, fifty eggs were randomly divided into five groups (n = 10 eggs) as follows: control (sterile water injected); L-Leu; L-Ile; L-Val; and combined BCAAs (L-Leu + L-Ile + L-Val). Amino acids were dissolved into sterile water with doses of 35, 21 and 29 μmol/500 μl/egg for L-Leu, L-Ile and L-Val, respectively, as suggested by Ohta et al. (1999), and the combined BCAAs group was the combination of the above doses. In our preliminary study, we confirmed that there was no effect of injection of 500 μl of sterile water compared to no manipulation of eggs (no whole, no injection) on body temperature at hatching (data not shown). The in ovo injection was performed on ED 7 following the method described previously (Ohta et al., 2001). In brief, the large end of the egg (the injection site) was sterilized with 70% ethanol prior to a small hole being drilled in it. Sterile water or a solution (or solutions) of amino acids were injected through the yolk sac to a depth of 2.5 cm with a 1-ml disposable syringe that had a 25-gauge needle. The small holes at the large end were sealed immediately with Scotch tape upon completion of the injection. After injection, the eggs were returned to the incubator for incubation under CT. On DOH, time until hatching, hatchability, body weight and rectal temperature were recorded at 2 h after hatching. Rectal temperature was recorded by a digital thermometer with an accuracy of ±0.1 °C (Thermalert TH-5, Physitemp Instruments Inc., USA) by inserting the thermistor probe into the colon (rectum) through the cloaca to a depth of 2 cm.

In Experiment 3, fifty-six eggs were randomly divided into 2 groups (n = 28) as follows: control group (sterile water injection); and L-Leu-injected group. On ED 7, in ovo injection of sterile water (500 μl/egg) or L-Leu (35 μmol/500 μl/egg) was performed as described in Experiment 2. On DOH, hatching performance (i.e. time until hatching, hatchability, body weight and rectal temperature) was recorded. The chicks were reared until 5 or 6 days of age. On DOH, forty chicks (n = 20 per group (control and L-Leu-injected)) were further divided into five chicks per cage (floor space: 18 cm × 50 cm; height: 30 cm). On day 2, birds from each group were gradually isolated with two chicks per cage. On day 3, each chick was placed in an individual cage (floor space: 20 cm × 28 cm; height: 30 cm). Body weight and rectal temperature were measured every day; however, it was only possible to measure individual food intake after the chicks had been placed in individual cages. On day 5 or 6, chicks (n = 10) were subjected to a heat challenge by being placed randomly into one of two chambers (Sanyo Electric Co. Ltd., Japan; Catalog number: Sanyo MIR-253) to expose them to either HT (35 ± 1 °C for 180 min) or CT (28 ± 1 °C for 180 min). All birds were provided with ad libitum access to food and water when exposed to either HT or CT. Rectal temperature was recorded at 0, 60, 120 and 180 min after they were transferred into the chambers.

In Experiment 4, one hundred eggs were randomly divided into two groups (n = 50) as follows: control group (sterile water injection); and L-Leu-injected group. The dose of L-Leu, the in ovo injection, and the recording of the hatching performance were as described in Experiment 2. The rearing conditions and the process by which the chicks were gradually isolated were the same as described in Experiment 3. However, sex identification was carried out in this experiment on day 2 after hatching. Males were selected for the experiment, because male chicks (Julia strain) had been used in our previous experiments concerning heat-stress administration (Chowdhury et al., 2012, 2014; Ito et al., 2014, 2015). On day 9, chicks were exposed to HT or CT as described above in Experiment 3. All birds were provided with ad libitum access to the food and water during the 180 min of the heat challenge. Rectal temperature was recorded at 0, 60, 120 and 180 min from the start of the heat challenge.