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ORIGINAL ARTICLE

Central L-alanine reduces energy expenditure with a hypnotic effect under an acute stressful condition in neonatal chicks

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Abstract Recently, we reported that intracerebroventricular (i.c.v.) injection of L-alanine attenuated the stress response under an acute stressful condition in chicks. However, no information of L-alanine was available for the influence on energy expenditure and changes in the posture under stressful conditions. The purpose of the present study was to clarify whether central L-alanine affects heat production (HP) of neonatal chicks, and whether HP is correlated with the behavior after isolation-induced stress. The i.c.v. injection of L-alanine (0.8 μmol) decreased oxygen consumption, carbon dioxide production and HP shortly after injection. Central L-alanine reduced the posture for active wakefulness, but increased the posture for sitting motionless with head drooped (sleeping posture). The present study demonstrates that central L-alanine decreases energy expenditure and causes a hypnotic effect in chicks exposed to an acute stressful condition.

Keywords L-alanine · Intracerebroventricular injection · Heat production · Neonatal chick · Posture · Social separation stress

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Introduction

L-Alanine is an α-amino acid and is one of the 20 proteinogenic amino acids, i.e. the building blocks of proteins. On the other hand, β-alanine is the only naturally occurring β-amino acid and is not used in the biosynthesis of any proteins. Accordingly, as least with regards to protein synthesis, the roles of L-alanine and β-alanine are greatly different. However, both L-alanine and β-alanine can activate the glycine receptor (Olsen and DeLorey 1999). Under an isolation-induced stress, intracerebroventricular (i.c.v.) injection of glycine significantly decreased spontaneous activity induced by stress in chicks (Asechi et al. 2006). Social separation stress increased spontaneous activity and vocalization of chicks, but these responses were attenuated by the i.c.v. injection L-alanine (Kurauchi et al. 2006). β-Alanine induced hypoactivity with lower spontaneous activity and less vocalization manifested as sleep-like behavior (Tomonaga et al. 2004). However, the effects of L-alanine on sleeping behavior and energy metabolism have not been investigated, possible because it is nutritionally dispensable.

The objectives of the present study were to clarify (1) whether central L-alanine modifies behavior, including sleep, and (2) whether central L-alanine alters energy expenditure in chicks under a social separation stress. Finally, we discussed the relationship between behavioral changes and energy expenditure.

Materials and methods

Animals and food

One-day-old male layer type chicks (Julia) were purchased from a local hatchery (Murata Hatchery, Fukuoka, Japan)

and housed in a windowless room at a constant temperature of 30 ± 1°C. Continuous lighting was provided. The birds were given free access to a commercial starter diet (AX, Toyohashi Feed and Mills Co. Ltd, Aichi, Japan) and water. Chicks were reared in a group (25/cage) till the experiment. On the day of the experiment, chicks (3 or 4-day-old) were assigned to treatment groups based on their body weight in order to produce uniform treatment groups. Experimental procedures followed the guidance for animal experiments in Faculty of Agriculture and in the Graduate Course of Kyushu University and the law (no. 105) and notification (no. 6) of the government.

Preparation of drugs

L-Alanine was purchased from Wako Pure Chemical Industries, Ltd (Osaka, Japan) and was dissolved in 0.85% saline containing 0.1% Evans blue solution.

Experimental procedure

The i.c.v. injections were made using a microsyringe according to the method of Davis et al. (1979); Koutoku et al. (2005). The stress and pain induced by this method is minimal (Koutoku et al. 2005). Chicks were given i.c.v. injections of 0 and 0.8 μmol of L-alanine based on the dose by Asechi et al. (2006). The injected volume was 10 μl. The birds were placed in an acrylic chamber mentioned below just after i.c.v. injection to induce social isolation stress.

Respiratory measurement

Oxygen (O_2) consumption, carbon dioxide (CO_2) production and respiratory quotient (RQ) were measured using an open circuit calorimeter system to determine heat production (HP) (MK-5000RQ, Muromachi Kikai Co. Ltd, Tokyo, Japan). For these measurements, an acrylic chamber (150 × 150 × 150 mm) with a stainless steel grid floor was used. Fresh atmospheric air was drawn at a rate of 500 ml/min and then passed through O_2 and CO_2 detectors (MM202R, Muromachi Kikai Co. Ltd, Tokyo, Japan). The concentrations of these gases were recorded every 3 min. The analyzer was calibrated using primary gas standards of high purity (Sumitomo Seika Chemicals Co. Ltd., Osaka, Japan) every 1 h. HP was calculated by the method of Romijn and Lokhorst (1961) as follows: HP (kcal/min) = the volume of O_2 consumed (ml/min) × 3.871 + the volume of CO_2 produced (ml/min) × 1.194. The units for HP were converted to joules from calories by multiplying by 4.184, and the values were normalized with the body weight.

Observation of posture

A video camera was positioned at the front of the chamber to record the behaviors of chicks. Based on the method by van Luijtelaar et al. (1987), the chick's behaviors were classified into four categories: (1) active wakefulness; (2) standing/sitting motionless with eyes open; (3) standing motionless with eyes closed; and (4) sitting motionless with head drooped (sleeping posture) by watching the DVD. They demonstrated the correlation between sleeping posture and electrophysiological sleep with electroencephalography measurement (van Luijtelaar et al. 1987). The monitoring systems were set in a separate room to avoid disturbing the animals. Measurements were done during 30 min.

Finally, the birds were decapitated after an overdose of sodium pentobarbital. The brains were removed and the location of the Evans blue dye was confirmed. Data of chicks without dye in the lateral ventricle were deleted.

Statistical analysis

Data were statistically analyzed by repeated measure two-way analysis of variance (ANOVA) using a commercially available package, StatView (Version 5, SAS Institute, Cary, USA, 1998). Significant differences implied $P < 0.05$. Values are presented as mean ± SEM.

Results

Figure 1 shows the effect of i.c.v. injection of L-alanine on O_2 consumption, CO_2 production, HP and RQ during the 30 min isolation. The effects for time post-injection in overall O_2 consumption ($F(8, 72) = 20.174$, $P < 0.0001$), CO_2 production ($F(8, 72) = 11.201$, $P < 0.0001$), HP ($F(8, 72) = 17.914$, $P < 0.0001$) and RQ ($F(8, 72) = 13.264$, $P < 0.0001$) were significant. The values for O_2 consumption, CO_2 production and HP increased with time and reached a plateau 15 min post-injection. Conversely, the RQ decreased with time and had a constant value after 15 min. Significant interactions between L-alanine and time were detected in O_2 consumption ($F(8, 72) = 2.080$, $P < 0.05$), CO_2 production ($F(8, 72) = 2.207$, $P < 0.05$) and HP ($F(8, 72) = 2.122$, $P < 0.05$). These three parameters were lowered by L-alanine soon after i.c.v. injection, but the effect of L-alanine disappeared with time.

Changes in the posture after i.c.v. administration of L-alanine are shown in Fig. 2. Significant effects of L-alanine were observed in active wakefulness ($F(1, 12) = 5.215$, $P < 0.05$) and sleeping posture ($F(1, 12) = 6.668$, $P < 0.05$). The effects of time post-injection in active

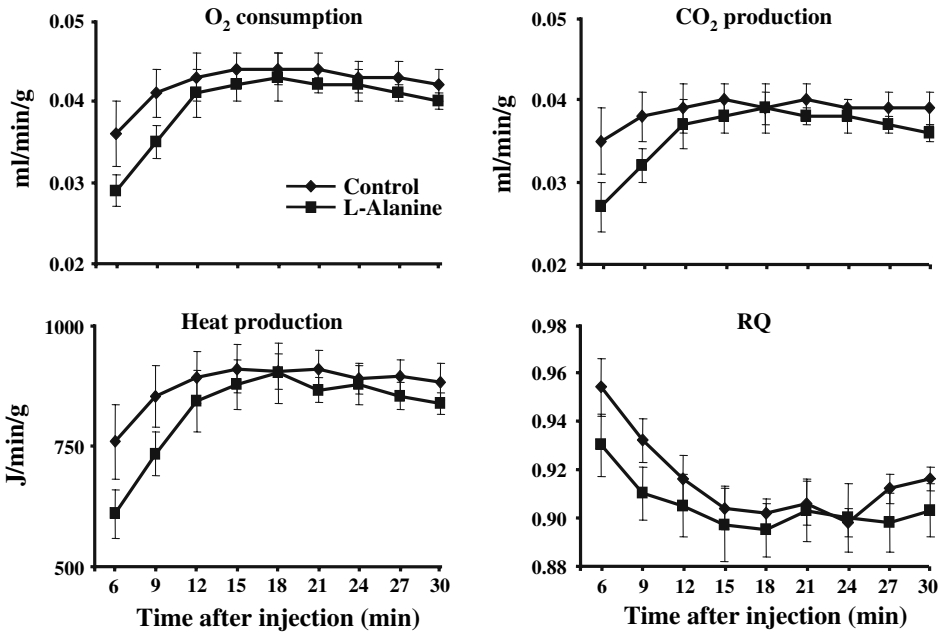

Fig. 1 Changes in O_2 consumption, CO_2 production, HP and RQ after i.c.v. injection of L-alanine. $N = 7$. Data are expressed as means ± SEM

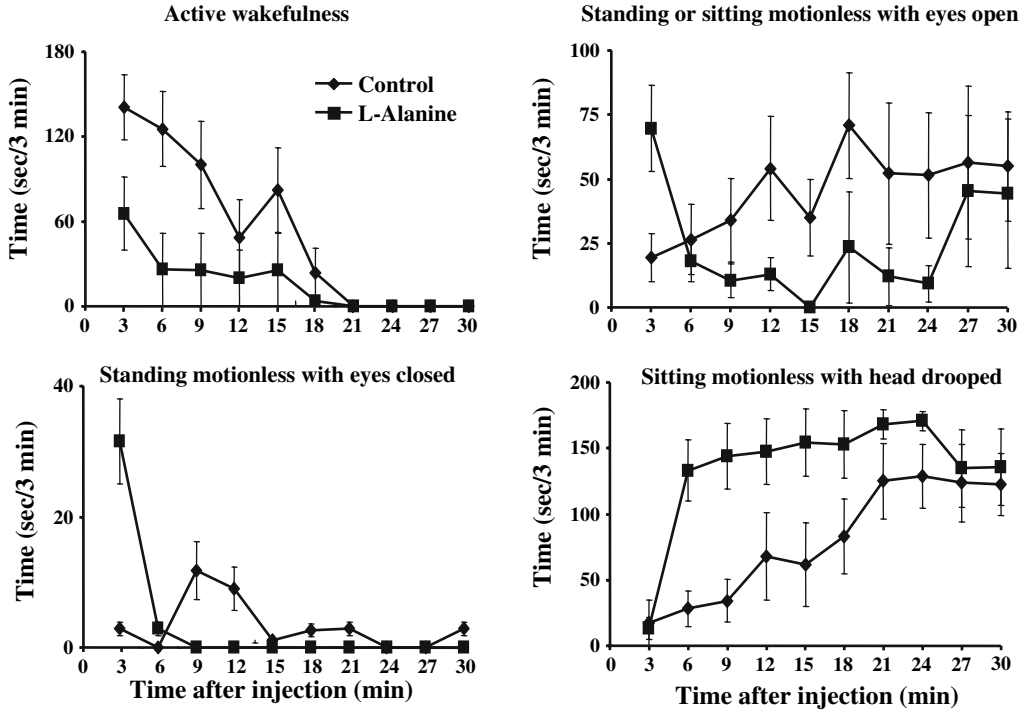

Fig. 2 Effect of i.c.v. injection of L-alanine on various behaviors of chicks exposed to social separation stress for 30 min. The behaviors observed were: (1) active wakefulness; (2) standing/sitting motionless with eyes open; (3) standing motionless with eyes closed; and (4) sitting motionless with head drooped (sleeping posture). $N = 7$. Data are expressed as means ± SEM

wakefulness ($F(9, 108) = 10.786$, $P < 0.0001$) and sleeping posture ($F(9, 108) = 8.138$, $P < 0.0001$) were significant. Significant interactions between central L-alanine and time were detected in active wakefulness ($F(9, 108) = 2.735$, $P < 0.01$) and sleeping posture ($F(9, 108) = 2.213$, $P < 0.05$). These results suggest that just after isolation the i.c.v. injection of L-alanine decreased active wakefulness while sleeping posture increased. L-Alanine had no effect on postures for standing or sitting motionless with eyes open or standing motionless with eyes closed.

Discussion

Corticosterone is released from the adrenal glands in response to activation of the hypothalamic–pituitary–adrenal (HPA) axis and is one of the biological markers of stress in birds. Corticotropin-releasing factor (CRF), a key hypothalamic activator of the HPA axis, is a stress-related peptide in the brain of birds. The central injection of CRF drastically increases corticosterone concentrations in the plasma of chicks (Furuse et al. 1997; Zhang et al. 2001a, b). We have previously demonstrated that neonatal chicks injected i.c.v. with CRF were excited, had increased locomotion, vocalized loudly, and demonstrated increased rectal temperature and plasma corticosterone concentrations (Tachibana et al. 2004; Zhang et al. 2001a, 2003, 2004). Plasma corticosterone concentrations and vocalizations were also highly increased in isolated chicks (Feltenstein et al. 2003). In the present study, the time spent for the posture of active wakefulness was higher in the saline injected control. This behavior was due to isolation stress. However, central L-alanine decreased the time spent for active wakefulness and increased the posture for sleeping behavior. L-Alanine may be one of the regulators of the stress response.

I.c.v. injection of CRF induced hyperthermia (Tachibana et al. 2004). In addition, i.c.v. injection of CRF dose-dependently increased O_2 consumption, CO_2 production and HP (Tachibana et al. 2006). The hypnotic effect of L-alanine clearly resulted in decreased O_2 consumption and CO_2 production compared with the control. As a result, HP was significantly reduced by the i.c.v. injection of L-alanine. It can be concluded that the reduced HP obtained in the present study was due mainly to changes in physical activity, not internal changes in metabolism. On the other hand, RQ values quickly decreased in both groups suggesting that carbohydrate reserves in the body of neonatal chicks are very limited and rapidly depleted.

The actions of central L-alanine may be mediated through the glycine receptor. Glycine is widely recognized as a major inhibitory neurotransmitter, and L-alanine can activate the glycine receptor (Olsen and DeLorey 1999). In fact, Mandelbrod et al. (1983) reported that the pharmacological sensitivity of neurons in the region containing the highest concentration of CRF was inhibited by glycine. Furthermore, L-alanine is metabolized to pyruvate, and finally glucose (Berg et al. 2002). Recently, Asechi et al. (2008) revealed that i.c.v. administration of glucose triggered sedative and hypnotic effects. These facts suggested, that the metabolic pathway from L-alanine to glucose via pyruvate may have involvement in the sedative and hypnotic effects of L-alanine. However, the intermediate product pyruvate has no influence on the stress-related behaviors and plasma corticosterone level (Asechi et al. 2008). Further, the sedative effects of glucose seem independent of that of L-alanine. This can be explained by the fact that pyruvate itself is the precursor of glucose as well as L-alanine. The effect of i.c.v. injection of 0.8 μmol of L-alanine disappeared within 30 min. The precise mechanism by which L-alanine induced hypnotic effects remains to be investigated.

In conclusion, L-alanine administered i.c.v. can attenuate the stress response through the reduction in activity induced by the hypnotic effect. In additon, L-alanine reduces energy expenditure in chicks.

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