◀Research Note▶

Intracerebroventricular Injection of L-Alanine Induces a Sedative Effect under an Acute Stressful Condition in Neonatal Chicks

Isao Kurauchi¹, Mari Asechi^a, Tetsuya Tachibana^{1,4}, Li Han², Kohsuke Hayamizu²,
D. Michael Denbow³ and Mitsuhiro Furuse¹

¹ Laboratory of Advanced Animal and Marine Bioresources, Graduate School of Bioresources
and Bioenvironmental Sciences, Kyushu University, Fukuoka 812-8581, Japan

² FANCL Research Institute, 12-13 Kamishinano, Totsuka-ku, Yokohama, Kanagawa, 244-0806, Japan

³ Department of Animal and Poultry Sciences, Virginia Polytechnic Institute and State University,
Blacksburg, VA 24061-0306, USA

⁴ Present address : Laboratory of Animal Science, Department of Agrobiological Science,
Faculty of Agriculture, Ehime University, Matsuyama 790-8566, Japan

Pyruvate can be synthesized from amino acids such as L-alanine, L-serine and L-cysteine. Recently, we reported that intracerebroventricular (i.c.v.) injection of L-serine and L-cysteine attenuated acute stress in chicks. This fact implies that amino acid substrates for pyruvate play a sedative role in the brain. However, no information was available for L-alanine. To elucidate the central effect of L-alanine on stress responses, L-alanine (0.8 μ mol) or saline was administered i.c.v. just before exposure to social separation stress. The social separation stress increased spontaneous activity and vocalization of chicks, but these responses were attenuated by the i.c.v. injection of L-alanine. In conclusion, in addition to L-serine and L-cysteine, centrally administered L-alanine may be effective in attenuating anxiety induced by a psychological stressor.

Key words : L-alanine, intracerebroventricular injection, neonatal chick ; pyruvate, social separation stress

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Introduction

With regards to nutrition, amino acids are divided into two categories, i.e., essential and nonessential amino acids. Pyruvate is the entry point of the three-carbon nonessential amino acids — L-alanine, L-serine, and L-cysteine — into the metabolic mainstream as shown in Fig. 1. The transamination of L-alanine, involved in the metabolism of L-tryptophan and the vitamin pyridoxine, directly yields pyruvate. Another simple reaction in the degrada-

tion of amino acids is the deamination of L-serine to pyruvate by serine dehydratase. L-Cysteine can be converted into pyruvate by several pathways, with its sulfur atom emerging in H₂S, SCN⁻, or SO₃²⁻ (Berg *et al.*, 2002). Among these amino acids, intracerebroventricular (i.c.v.) injection of L-serine or L-cysteine induced sedative and hypnotic effects in chicks under an acute stress (Asechi *et al.*, 2006). Beta-alanine, the only naturally occurring beta amino acid, is not used in the biosynthesis of any major proteins or enzymes. Centrally administered

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Correspondence to : Dr. M. Furuse, Laboratory of Advanced Animal and marine Bioresources, Faculty of Agriculture, Kyushu University, Fukuoka 812-8581, Japan

Tel : (81) (92) 642-2953 Fax : (81) (92) 642-2953 E-mail : furuse@brs.kyushu-u.ac.jp

Fig. 1. Pyruvate formation from amino acids.

beta-alanine also induced hypoactivity in chicks (Tomo-naga *et al.*, 2004), but beta-alanine does not produce pyruvate.

So date, no information is available for central functions of L-alanine on the stress response. In the present study, we investigated whether the i.c.v. administration of the three-carbon nonessential amino acid alanine, which serves as a precursor to pyruvate, can attenuate behavioral changes caused by social separation stress in neonatal chicks.

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 $30 \pm 1^\circ\text{C}$. 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 (20–25/cage) till the experiment. On the day of the experiment, chicks (3-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

I.c.v. injections were made using a microsyringe according to the method of Davis *et al.* (1979) and Koutoku *et al.* (2005). The stress and pain suffered by this method is minimal as described elsewhere (Koutoku *et al.*, 2005). Chicks were given i.c.v. injections of 0 and $0.8 \mu\text{mol}$ of L-alanine based on the dose by Asechi *et al.* (2006). The injected volume was $10 \mu\text{l}$. After the injection, chicks were immediately placed in an acrylic monitoring cage ($40 \text{ cm} \times 30 \text{ cm} \times 20 \text{ cm}$) individually, and behavioral observations were made for 10 min. During this period, chicks were deprived of feed and water. Spontaneous activity was automatically determined by utilizing infrared beam sensors (Neuroscience Inc., Tokyo, Japan) placed above the center of the monitoring cage and analyzed by the software DAS-008 (Neuroscience Inc.). Chick vocalizations were simultaneously recorded using a computer with the software Windows Media Player (Microsoft Corporation, USA) and the numbers of distress vocalizations, which are shrill and intense calls, were counted using Gretchen software (Excla Inc., Japan).

At the conclusion of the experiment, 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 commercially available package, StatView (Version 5, SAS Institute, Cary, U.S.A., 1998). Significant differences implied $P < 0.05$. Values are presented as mean $\pm$ SEM.

Results

Figure 2 shows the effect of i.c.v. injection of L-alanine on spontaneous activity (upper panel) and distress vocalizations (lower panel) during the 10

Fig. 2. Effect of i.c.v. injection of saline (control) and L-alanine on cumulative spontaneous activity (upper panel) and distress vocalizations (lower panel) during 10 min isolation in 3-day-old layer chicks. Results are expressed as means $\pm$ S.E.M. The number of chicks used in each group was 6.

min isolation. The effect of i.c.v. L-alanine administration on spontaneous activity, although not significant, tended to be significant ($F(1, 10) = 3.883$, $P = 0.0771$). The time post-injection ($F(9, 90) = 23.691$, $P < 0.0001$) and an interaction between treatment and time ($F(9, 90) = 5.130$, $P < 0.0001$) was significant. Compared with the control, L-alanine gradually induced hypoactivity in neonatal chicks. The effect of L-alanine on vocalizations was significant ($F(1, 10) = 7.219$, $P < 0.05$). The time post-injection ($F(9, 90) = 129.930$, $P < 0.0001$), but not an interaction between treatment and time ($F(9, 90) = 1.773$, $P = 0.0844$), was significant. The increased number of vocalizations due to an acute stress was attenuated by central L-alanine.

Discussion

Recently, Asechi *et al.* (2006) revealed that the nonessential amino acid L-serine, a constituent of proteins and a precursor for the synthesis of glycine and L-cysteine, is effective in improving anxiety or

sleep disorders induced by psychological stressor. L-Cysteine was similarly effective. L-Serine and L-cysteine are sources for pyruvate synthesis, as is L-alanine (Fig. 1). The present results clearly showed that i.c.v. injection of L-alanine had a similar effect to that of L-serine and L-cysteine in its ability to reduced social stress-induced vocalizations. As a result, it is clear that all of the three-carbon nonessential amino acids have a sedative function. These facts suggest that pyruvate may have a central function in the stress response, since these three-carbon amino acids produce pyruvate. The concern of pyruvate remains to be studied in the future.

Glycine is widely recognized as a major inhibitory neurotransmitter. The amino acids that can activate the glycine receptor include β -alanine, taurine, L-alanine, L-serine and proline (Olsen and DeLorey, 1999). Thus, the sedative effect of L-alanine observed here may be through the glycine receptor.

In conclusion, the nonessential amino acid L-alanine administered i.c.v. has an important function in the central nervous system with special reference to attenuation of the stress response.

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