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Plasma Amino Acid Concentration in Neonatal Chicks Modified by Acute Stress

[1]I. Kurauchi, [1]K. Hamasu, [2]D.M. Denbow and [1]M. Furuse
[1]Laboratory of Advanced Animal and Marine Bioresources,
Graduate School of Bioresource and Bioenvironmental Sciences,
Kyushu University, Fukuoka 812-8581, Japan
[2]Department of Animal and Poultry Sciences,
Virginia Polytechnic Institute and State University, Blacksburg, VA 24061-0306, USA

Abstract: To clarify the influence of acute stress on plasma amino acid concentrations, chicks were exposed to either restraint with isolation stress or fasting stress. In restraint with isolation-induced stress, plasma L-hydroxyproline, L-serine, L-asparagine, β-alanine, L-alanine, L-histidine, L-arginine, L-proline, L-methionine, L-leucine, L-phenylalanine and L-ornithine decreased compared with the control. During fasting stress, L-asparagine, β-alanine, L-histidine, GABA, L-threonine, L-arginine, L-proline, L-methionine, L-leucine, L-phenylalanine and L-ornithine linearly decreased while, tryptophan increased. Most of the amino acids modified in both acute stresses have been recognized to have a role in sedation and/or hypnosis. Amino acids quickly metabolized during acute stress should be supplemented before and/or after stressful conditions to support chicken health.

Key words: Amino acid, chick, stress, plasma, fasting stress, L-serine

INTRODUCTION

The amino acid compositions of poultry diets have been formulated to match the requirements for maintenance, growth and production of meat and eggs (NRC, 1994). Controlling the environment is an important management tool to attenuation the stress response and to improve poultry production. However, the relationship between dietary amino acid requirements and stress conditions has not been clarified. Recently, it has been demonstrated that several amino acids function in the central nervous system in the attenuation of the stress response. For instance, among basic amino acids, L-lysine and its metabolites (Takagi *et al*., 2001) and L-arginine and its metabolites (Suenaga *et al*., 2008a, b) attenuate the acute stress responses in neonatal chicks. However, L-histidine had no effect on acute stress (Tomonaga *et al*., 2004). Non-essential three-carbon amino acids such as L-alanine (Kurauchi *et al*., 2006), L-serine (Asechi *et al*., 2006) and L-cysteine (Asechi *et al*., 2006) also attenuated stress responses. While, the nutritional significance of amino acids on stressful conditions was clarified, changes in plasma amino acid concentration have not been determined during stressful conditions. Since, plasma amino acid concentration is one of the parameters to determine amino acid requirement (Ishibashi *et al*., 1998), the present study determined the changes in plasma amino acids during stressful conditions. These findings suggest new amino acid requirements for stressful conditions.

MATERIALS AND METHODS

Animals and food: One-day-old male layer chicks (Julia) purchased from a local hatchery (Murata Hatchery, Fukuoka, Japan) were maintained in a windowless room at a constant temperature of 30±1°C. Lighting was provided continuously. Chicks were given free access to a commercial starter diet (Toyohashi Feed and Mills Co. Ltd., Aichi, Japan) and water. On the experimental day, chicks were distributed into experimental groups based on their body weight, so that the average body weight was as uniform as possible within the same experiment. Experimental procedures followed the guidance for Animal Experiments in the Faculty of Agriculture and in the Graduate Course of Kyushu University and the Law (No. 105) and Notification (No. 6) of the Japanese Government.

Stress: In the present study, neonatal chicks (5 and 6 days old) were stressed by different processes. The first was a combination of restraint with isolation-induced stress and the second was a fasting stress. In the restraint

Corresponding Author: M. Furuse, Graduate School of Bioresource and Bioenvironmental Sciences, Kyushu University, Fukuoka 812-8581, Japan

with isolation-induced stress, the body and legs of each chick were bound with rubber bands and placed in an acrylic cage separated from the group for 30 min. For the fasting stress, chicks were fasted for 180 or 360 min. During the stress treatments, food was removed for 30 min from the control group.

Amino acid analysis: After animals were stressed, blood was collected from the jugular vein into heparinized syringes at the conclusion of the behavioral tests. The blood was centrifuged at 4°C and 10,000× g for 4 min and the plasma was collected and stored at -30°C until analysis. Free amino acids in the plasma were determined with High-Performance Liquid Chromatography (HPLC) (Pico-Tag™, Waters, Milford, MA, USA) using the Pico Tag method (Rubio, 2003).

The plasma was deproteinized by filtration through a 10,000 dalton molecular weight cut-off filter (Millipore, Bedford, United States) via centrifugation at 10,000 g for 20 min. The samples (40 μL) were then completely dried under reduced pressure. Dried residues were disolved with 20 μL of 1 M sodium acetate-methanol-triethylamine (2:2:1) solution. Samples were re-dried and dissolved in 40 μL of derivatization solution [methanol-water-triethylamine-phenylisothiocyanate (7:1:1:1)]. A period of 20 min at room temperature was allowed for the reaction of phenylisothiocyanate with amino groups to produce phenylthiocarbamyl amino acid residues. The samples were dried again. The dried samples were dissolved with 100 μL of Pico-Tag Diluent (Waters, Milford, United States). These diluted samples were filtered through a 0.45 μm filter (Millipore, Bedford, United States). The same method was applied to standard solutions prepared by diluting a commercially available L-amino acid solution (type AN II and type B; Wako, Osaka, Japan) with distilled water. These derivatized samples were applied to a Waters HPLC system (Pico-Tag free amino acid analysis column (3.9×300 mm), Alliance 2690 separation module, 2487 dual-wavelength UV detector and Millennium 32 chromatography manager; Waters, Milford, United States). They were equilibrated with buffer A [70 mM sodium acetate (pH 6.45 with 10% acetic acid)-acetonitrile (975:25)] and eluted with a linear gradient of 0, 3, 6, 9, 40 and 100%buffer B [water-acetonitrile-methanol (40:45:15)] at a flow rate of 1 mL min^{-1} at 46°C. The absorbance at 254 nm was measured. The following amino acids and related compounds were examined in this study: L-alanine, GABA, L-arginine, L-asparagine, L-aspartic acid, β-alanine, L-citrulline, L-cystine, L-glutamic acid, L-glutamine, L-histidine, L-hydroxyproline, L-isoleucine, L-leucine, L-lysine, L-methionine, L-ornithine, L-phenylalanine, L-proline, L-serine, L-taurine, L-threonine, L-tryptophan, L-tyrosine and L-valine. Triethylamine and sodium acetate trihydrate were purchased from Wako (Osaka, Japan), while, other drugs for which, no manufacturer is noted were purchased from Sigma (St. Louis, United States).

Statistical analysis: Data for restraint with isolation-induced stress were statistically analyzed by student's t-test. Regression analysis was done for fasting stress. Statistical analysis was conducted using a commercially available package StatView (version 5, SAS Institute, Cary, USA, 1998).

RESULTS

Table 1 shows, the effect of restraint with isolation-induced stress on plasma free amino acids in chicks. Plasma L-hydroxyproline, L-serine, L-asparagine, β-alanine, L-histidine, L-alanine, L-arginine, L-proline, L-methionine, L-leucine, L-phenylalanine and L-ornithine decreased with stress. Other amino acids determined (L-aspartate, L-glutamate, L-serine, L-glutamine, taurine, GABA, L-citrulline, L-threonine, L-tyrosine, L-valine, L-cystine, L-isoleucine, L-tryptophan and L-lysine) were not influenced.

Table 2 shows the effect of fasting stress on plasma free amino acids in chicks. L-Asparagine, β-alanine, L-histidine, GABA, L-threonine, L-arginine, L-proline, L-methionine, L-leucine, L-phenylalanine and L-ornithine linearly decreased during fasting stress. The reverse was true for L-tryptophan, which increased with time. No correlation was detected for L-aspartate, L-glutamate, L-serine, L-glutamine, taurine, L-citrulline, L-alanine, L-tyrosine, L-valine, L-cystine, L-isoleucine and L-lysine.

Table 1: Effect of restraint with isolation stress on plasma free amino acids (mM) in 5 or 6 days old chicks

Amino acids	Control	Restraint with isolation-induced stress
β-Alanine	10±0	8±1 *
L-Alanine	587±27	462±18 **
L-Arginine	455±20	346±43 *
L-Asparagine	250±11	162±11 **
L-Histidine	44±2	28±3 **
L-Hydroxyproline	181±11	117±5 **
L-Leucine	336±11	263±19 **
L-Methionine	166±4	126±13 *
L-Ornithine	48±5	33±2 *
L-Phenylalanine	200±8	153±10 *
L-Proline	637±28	464±23 **
L-Serine	661±18	477±26 **

Vales are expressed as mean±SEM. The number of chicks used in each group was 6. *p<0.05, **p<0.01

Table 2: Effect of fasting stress on plasma free amino acids (mM) in 5 or 6 days old chicks

Amino acids	Control	180 min	360 min	Regression equation	r	p-value
β-Alanine	10±0	8±0	9±0	10.027-0.005x	0.524	<0.05
L-Arginine	455±20	347±21	299±19	456.349-0.473x	0.799	<0.0001
L-Asparagine	250±11	176±12	155±13	247.859-0.284x	0.782	<0.0001
GABA	35±2	29±3	26±2	35.036-0.027x	0.519	<0.05
L-Histidine	44±2	21±5	10±2	44.147-0.101x	0.846	<0.0001
L-Leucine	336±11	293±16	256±19	341.039-0.242x	0.671	<0.01
L-Methionine	166±4	130±11	117±16	165.532-0.146x	0.589	<0.05
L-Ornithine	48±5	26±2	17±2	48.376-0.095x	0.849	<0.0001
L-Phenylalanine	200±8	170±6	137±8	205.25-0.192x	0.844	<0.0001
L-Proline	637±28	384±33	308±17	629.121-0.979x	0.863	<0.0001
L-Threonine	848±61	692±73	653±46	840.823-0.579x	0.487	<0.05
L-Tryptophan	37±1	45±4	48±3	37.044 + 0.033x	0.586	<0.05

Vales are expressed as mean±SEM. The number of chicks used in each group was 6. Control was fasting for 30 min and 180 and 360 min were time after fasting

DISCUSSION

In order to examine, the relationships between amino acid metabolism in the blood and from a stress response, changes in plasma amino acids in chicks after two different stresses, i.e., restraint with isolation-induced stress and fasting stress were investigated. Several amino acids were modified under stressful conditions. It was clear that the plasma concentration of most amino acids decreased during stress. In restraint with isolation-induced stress, plasma L-hydroxyproline, L-serine, L-asparagine, β-alanine, L-histidine, L-alanine, L-arginine, L-proline, L-methionine, L-leucine, L-phenylalanine and L-ornithine decreased. According to Hamasu *et al.* (2009a), levels of L-alanine, L-arginine, L-asparagine, L-aspartic acid, L-phenylalanine, L-proline, L-serine and L-tyrosine in the diencephalon of the restraint with isolation-induced stress group were significantly lower than those of the control group. The diencephalon was a key center for the stress response. There are similarities in the changes in plasma amino acids observed in the present study and previous changes noted in the brain (Hamasu *et al.*, 2009a). Rapid and great changes in plasma amino acid may influence brain amino acid metabolism. Not only restraint with isolation-induced stress, but fasting stress greatly influence plasma amino acid concentration. Plasma L-asparagine, β-alanine, L-histidine, GABA, L-threonine, L-arginine, L-proline, L-methionine, L-leucine, L-phenylalanine and L-ornithine decreased after fasting. In the diencephalon, L-arginine, L-asparagine, L-aspartic acid, L-glutamine, L-histidine, L-phenylalanine and L-proline were significantly decreased after 3 h fasting compared to the control group (Hamasu *et al.*, 2009a). Compared with changes in plasma and diencephalon amino acid concentration, responses for some amino acids were different. The permeability for blood brain barrier and metabolism in the brain may concern these differences.

Among amino acids changed after restraint with isolation-induced stress, L-hydroxyproline (Hamasu *et al.*, 2009b), L-serine (Asechi *et al.*, 2006), L-asparagine (Yamane *et al.*, 2009), β-alanine (Tomonaga *et al.*, 2004), L-alanine (Kurauchi *et al.*, 2006), L-arginine (Suenaga *et al.*, 2008a), L-proline (Hamasu *et al.*, 2009a, b) and L-ornithine (Suenaga *et al.*, 2008b) were confirmed to function in the brain as sedative and/or hypnotic regulators under an acute stress. The present study demonstrated that amino acids shown previously to attenuate the stress response also, decrease rapidly in the plasma during stressful conditions. On the other hand, the contribution of L-histidine and L-methionine for the stress was unclear, since L-histidine (Tomonaga *et al.*, 2004) and L-methionine (Kurauchi *et al.*, 2007) had no effect in the central nervous system on the stress response.

CONCLUSION

The present data suggests that possible supplementation of quickly metabolized amino acids in the plasma may be helpful for chicken health just before or after exposure to stressful conditions. Amino acid requirement for stress should be included in the future.

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