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Central Functions of Amino Acids for the Stress Response in Chicks*

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ABSTRACT : The nutritional significance of essential amino acids, as well as non-essential amino acids, is well documented in poultry production with regards to growth performance and protein accretion. However, the function of amino acids in the stress response is still unclear. L-Pipecolic acid, a L-lysine metabolite in the brain, induced a hypnotic and sedative effect acting via the γ-aminobutyric acid receptors. L-Arginine also induced a sedative effect via its metabolism to L-ornithine. In addition, three-carbon non-essential amino acids like L-alanine, L-serine and L-cysteine also induced sedative effects. These facts suggest that the requirement for amino acids in both essential and non-essential types may require reconsideration to add the concept of stress amelioration in the future.
(**Key Words :** Amino Acids, Sedation, Hypnosis, Stress, Chick)

INTRODUCTION

In addition to functioning as substrates for protein biosynthesis or as an energy source, amino acids also act as physiological messengers in the body. Amino acids may be classified based on the physical and chemical properties of their R groups such as acidic, basic, aromatic, sulfur, uncharged hydrophilic or inactive hydrophobic amino acids. In addition, L-proline has a special structure. Basic amino acids include L-lysine (L-Lys), L-arginine (L-Arg) and L-histidine (L-His), in chickens, these three amino acids are nutritionally essential and are involved in several metabolic pathways.

The major pathway for L-Lys metabolism in the liver occurs through the intermediate saccharopine (Hutzler and Dancis, 1968). However, L-Lys is metabolized into L-pipecolic acid (L-PA) after entering the brain (Chang 1978, 1982; Giacobini et al., 1980). L-Arg exerts its metabolic roles through the production of diverse metabolites including nitric oxide (NO), L-ornithine (L-Orn), polyamines, L-proline, L-glutamate, creatine and agmatine (Morris, 2004). L-His is converted to histamine by the enzyme L-histidine decarboxylase. L-His degrades to L-glutamate (Stifel and Herman, 1971).

Amino acids degraded to acetyl CoA or acetoacetyl CoA are termed *ketogenic* amino acids since they can give rise to ketone bodies or fatty acids. On the other hand, amino acids broken down to pyruvate, α-ketoglutarate, succinyl CoA, fumarate, or oxaloacetate are termed *glucogenic* amino acids. Pyruvate is the entry point of the three-carbon amino acids like L-alanine (L-Ala), L-serine (L-Ser), and L-cysteine (L-Cys) into the metabolic mainstream.

In poultry production, growth is an important parameter and is directly linked to the stress system. The attenuation of stress improves poultry production. Among three basic amino acids, L-Lys and its metabolites (Takagi et al., 2001) and L-Arg and its metabolites (Suenaga et al., 2008a, b) were demonstrated to attenuate the acute stress responses in neonatal chicks. L-His had no effect on acute stress (Tomonaga et al., 2004). Three-carbon amino acids such as L-Ala (Kurauchi et al., 2006), L-Ser (Asechi et al., 2006) and L-Cys (Asechi et al., 2006) attenuated stress responses. While the nutritional significance of essential amino acids on poultry growth and protein accretion has been well investigated, the relationships between the stress response and essential amino acids mainly deficient in vegetable protein have not been well documented. Accordingly, here we summarize the functions of amino acids on the stress

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response in poultry.

L-Lys AND ITS METABOLITE L-PA

A deficiency of L-Lys may cause serious detrimental effects on growth and production. Since most cereals used to feed poultry are deficient in L-Lys, commercial diets are supplemented with L-Lys. A large excess of dietary L-Lys has been also shown to decrease food intake and weight gain (Han and Baker, 1993). The major pathway of L-Lys metabolism in animals occurs through the intermediate saccharopine. This pathway predominates in the liver and is not very active in the brain (Hutzler and Dancis, 1968). However, L-Lys is metabolized into L-pipecolic acid (L-PA) after entering the brain through the blood-brain-barrier in mammals as shown in Figure 1 (Chang, 1978, 1982; Giacobini et al., 1980). Since L-PA is also the main metabolite of L-Lys in the chick brain (Nomura et al., 1978), Takagi et al. (2001) administered L-PA, or its relatives, intracerebroventricularly (i.c.v.) to examine their effect on food intake. While the i.c.v. injection of L-Lys suppressed food intake, it required a higher concentration than L-PA. Part of the L-Lys entering the brain is rapidly converted to L-PA. Consequently L-PA, produced from L-Lys, may be involved in suppression of food intake.

Takagi et al. (2001) further studied the effect of L-PA on several behaviors since sleep-like behavior was observed following the i.c.v. injection of L-PA. Takagi et al. (2003) investigated whether the induction of sleep-like behaviors by L-PA is associated with γ-aminobutyric acid (GABA) neurotransmission, and which GABA subtype receptor activation mediates this effect. L-PA increased sleeping

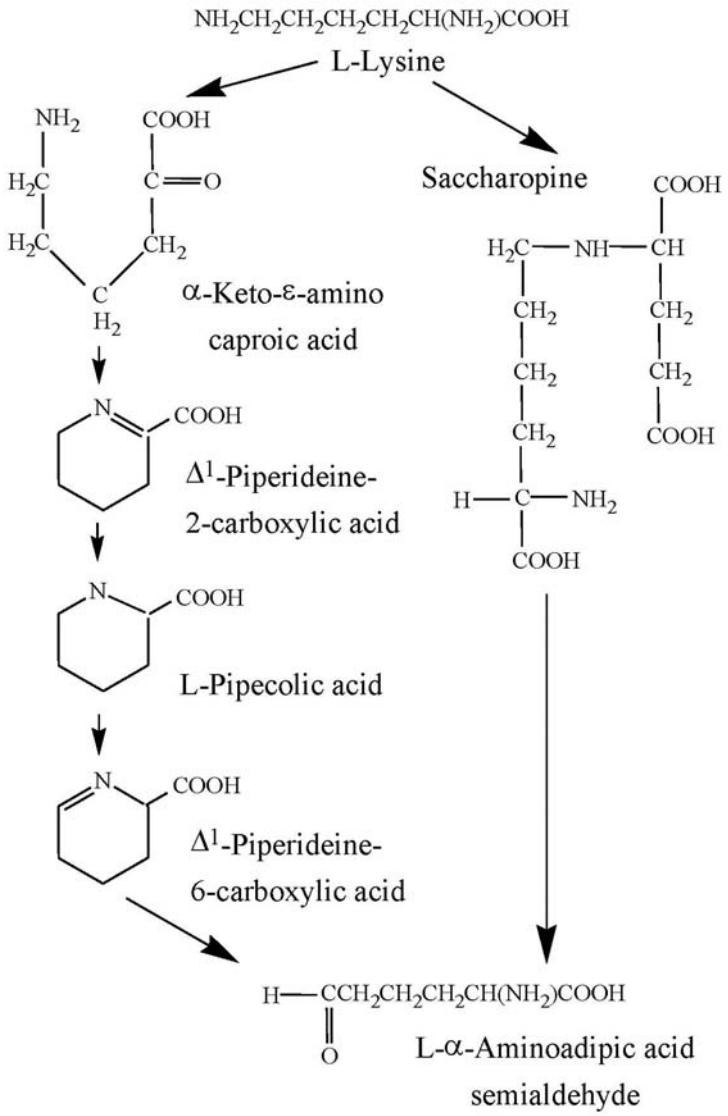

Figure 1. Metaboilc fates of L-Lys in the body.

posture and decreased waking posture, and the GABA-A antagonist picrotoxin attenuated the effects. Similarly, the GABA-B antagonist CGP54626 also attenuated the effects of L-PA. Taken together, induction of sleep-like behavior by L-PA involved the GABAergic system in neonatal chicks. The mechanism by which L-PA induced a hypnotic effect is described elsewhere (Furuse et al., 2006).

L-Arg AND ITS METABOLITES

Research into the biochemistry and physiology of L-Arg during the last 100 years has documented its diverse physiological functions in mammals. L-Arg is classified as an essential amino acid for birds, carnivores and young mammals, and a conditionally essential amino acid for adults. L-Arg itself can stimulate growth hormone release when infused intravenously or orally administered in human (Chromiak and Antonio, 2002). Birds lack carbamyl phosphate synthetase, one of the urea cycle enzymes necessary for the synthesis of citrulline from ornithine in the liver and kidney (Tamir and Ratner, 1963). Therefore, it is impossible to synthesize L-Arg in birds. Therefore, the role of L-Arg in the central nervous system (CNS) under stressful conditions has not been investigated in birds which, unlike mammals, lack a urea cycle.

L-Arg exerts its metabolic roles through the production of diverse metabolites including nitric oxide (NO), L-ornithine (L-Orn), polyamines, L-proline, L-glutamate, creatine and agmatine (Morris, 2004). The pathway of L-Arg metabolism is shown in Figure 2.

Suenaga et al. (2008a), using the social separation stress model in neonatal chicks, demonstrated the effect of central L-Arg. The social separation stress model is frequently used for the study of anxiety because chicks are comfortable when living in a group, but exhibit anxiety when isolated. Social separation stress increases spontaneous activity and vocalization of chicks (Feltenstein et al., 2003). This social separation stress paradigm has been used for developing anti-anxiety agents using vocalization and spontaneous activity as parameters. Additionally, this model has a high utility since chicks are inexpensive to purchase and maintain, and they require small quantities of drugs in the screening process (Watson et al., 1999).

According to Suenaga et al. (2008a), L-Arg clearly attenuated spontaneous activity and the number of vocalizations compared with the control under social

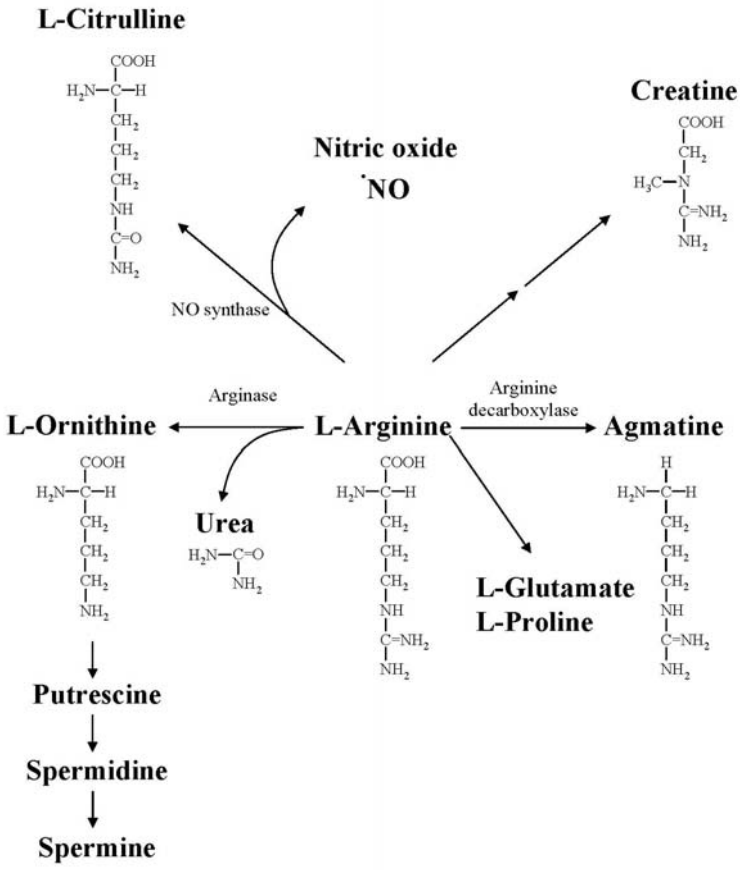

Figure 2. Metabolic fates of L-Arg in the body.

separation stress. In addition, L-Arg increased the time spent in sleeping posture. These results suggest that L-Arg has sedative and hypnotic effects. These results might suggest that a metabolite of L-Arg participates in the central function. To determine the mechanism of the response to L-Arg, Suenaga et al. (2008a) gave i.c.v. co-injections of N^G-nitro-L-arginine methyl estel HCl (L-NAME), a non-selective NO synthase (NOS) inhibitor, with L-Arg. The decrease in total spontaneous activity and vocalizations and the increase in the time of sleeping posture induced by L-Arg were attenuated to the control level by co-injection of L-NAME. Therefore, NO, produced through NOS, could be involved in the sedative and hypnotic effects of L-Arg in chicks under social separation stress. The inactive isomer of the NOS inhibitor, N^G-nitro-D-arginine methyl ester HCl (D-NAME), did not attenuate L-Arg-induced sedation, although it did attenuate the L-Arg-induced sleep-like behavior.

In a previous study, D-NAME, even though it had been widely used as an inactive isomer of the NOS inhibitor, was able to inhibit laminarin-stimulated NO production in primary haemocytes from the snail *Lymnaea stagnalis* (Wright et al., 2006). Therefore, there remains a possibility that D-NAME could also produce unexpected actions such as NOS inhibition. On the other hand, 3-morpholinosylnomine hydrochloride (SIN-1), an NO donor, did not induce sedative and hypnotic effects, although it did alter vocalizations (Suenaga et al., 2008a). Results of co-injection of L-NAME with L-Arg were inconsistent with that of SIN-1, and NOx concentration in several brain sites were not significantly increased by i.c.v. injection of L-Arg (Suenaga et al., 2008a). Even when L-Arg was not injected, the chick brain could produce NO. This endogenous NO production may be inhibited by L-NAME, since an adequate amount of NO was not released by exogenous L-Arg.

L-Arg can be catabolized by four sets of enzymes in mammalian cells resulting ultimately in the production of urea, L-proline, L-glutamate, polyamines, NO, creatine, or agmatine (Morris, 2004). Creatine has sedative and hypnotic effects in the CNS under social separation stress in neonatal chicks. This action of creatine was mediated by the activation of GABA-A receptors (Koga et al., 2005). Therefore, it is possible that creatine, made from L-Arg, acts in the CNS or that creatine, which has a similar structure to L-Arg, might act synergistically to induce these effects. In addition, L-Arg is metabolized by arginase to yield L-Orn. L-Orn may regulate the synthesis of polyamines, which are essential for cell proliferation and differentiation processes. The metabolic roles of L-Orn are likely cell- and tissue-specific.

Therefore, Suenaga et al. (2008b) investigated whether L-Arg attenuated the acute stress response in neonatal chicks via its conversion to other metabolites. The i.c.v. injection of agmatine did not induce sedative and hypnotic effects. Agmatine has a guanidino component. Guanidino compounds are known to have a relation to GABA-A receptors (Neu et al., 2002). Creatine, which has a guanidino component, attenuates the acute stress response by acting through GABA-A receptors (Koga et al., 2005). However, the effect of i.c.v. injection of agmatine differed from these reports. On the other hand, the i.c.v. injection of L-Orn (Suenaga et al., 2008b), which does not have a guanidino component, induced sedative and hypnotic effects. These data suggest that the function of L-Arg might not be associated with GABA-A receptor activation by guanidine compounds. Furthermore, L-Arg did not modify brain GABA concentrations (Suenaga et al., 2008b). Accordingly, sedative and hypnotic effects of central L-Arg may not be associated with the GABAergic system.

L-Orn attenuated spontaneous activity and the number of vocalizations, and slightly increased the time of sleeping posture beginning 5 min after i.c.v. injection (Suenaga et al., 2008b). Accordingly, L-Orn itself may have an important role for the induction of sedative and hypnotic effects. L-Orn is rapidly metabolized. The i.c.v. injection of putrescine, an L-Orn metabolite, produced antidepressant-like effects in mice that seem to be mediated through its interaction with the polyamine-site of the NMDA receptor (Zomkowski et al., 2006). The structure of L-Orn is similar to that of putrescine. This suggests the possibility that L-Orn might act on the polyamine-site of NMDA receptors if it is metabolized quickly enough.

The i.c.v. injected L-Arg increased both L-Arg and L-Orn concentrations in the telencephalon and diencephalon of chicks 10 min post-injection (Suenaga et al., 2008b). In addition, L-Orn concentration was proportionally increased by L-Arg injection suggesting that L-Arg was metabolized by arginase in the brain. These results support the chick behavior results observed following the i.c.v. injection of L-Orn under social separation stress, indicating that the sedative and hypnotic effects of L-Arg were mainly caused by L-Orn. However, other amino acids are increased in the telencephalon following L-Arg injection in additioin to L-Arg and L-Orn. Increased amino acids including L-Ala (Kurauchi et al., 2006), L-proline (Hamasu et al., 2009) and L-glutamic acid (Yamane et al., 2009) have been observed to have sedative or hypnotic effects. These results suggest that the effects of L-Arg might be mediated by these amino acids.

THREE-CARBON AMINO ACIDS

When investigating whether centrally administered phosphatidylserine (PS) could modify the behavior of chicks under isolation-induced stress, Koutoku et al. (2005)

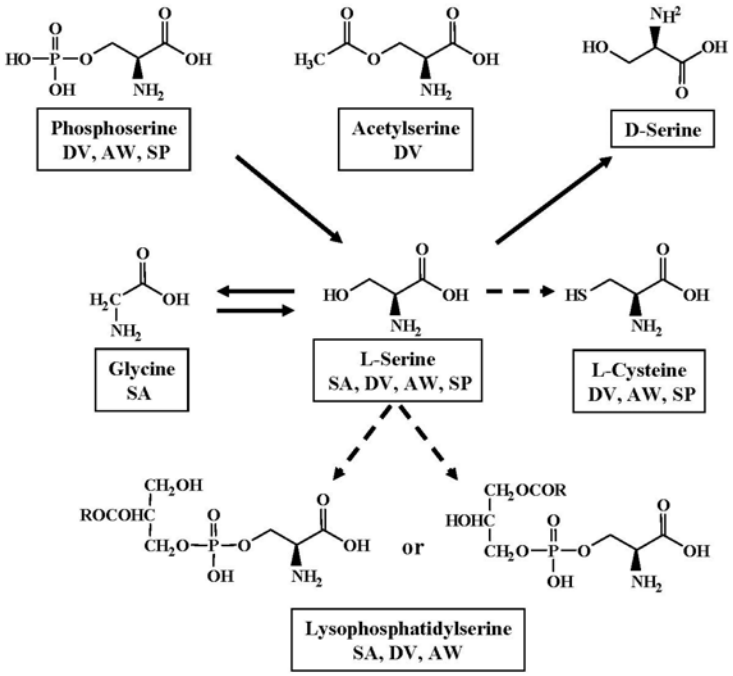

Figure 3. Sedative and hypnotic effects of L-Ser and its analogs and derivatives. Solid and dashed arrows indicate the possible direct and indirect metabolic routes, respectively. When spontaneous activity and distress vocalizations were decreased, SA and DV were appeared under chemical names, respectively. Similarly, AW and SP indicated that the decrease in the time for active wakefulness and increase in the time for sleeping posture, respectively.

compared with the effect of PS to other phospholipids or L-Ser, a constituent of PS. Phosphatidylcholine had no effect on these behavior, but phosphatidylethanolamine significantly increased vocalizations and spontaneous activity compared with PS. Interestingly, L-Ser similarly decreased isolation-induced vocalizations and spontaneous activity.

Later, Asechi et al. (2006) revealed the novel functions of L-Ser and its analogs and derivatives (D-Ser, glycine, acetylserine, phosphoserine, L-Cys and lysophosphatidylserine) under an acute stressful condition in neonatal chicks. First, L-Ser attenuated social-separation stress-induced behaviors during the 10 min post injection period, but had no effect on plasma corticosterone concentration. Therefore, L-Ser might have little or no ability to directly suppress the secretion of corticosterone from the adrenal glands through the hypothalamic-pituitary-adrenal (HPA) axis in response to stress. In addition, the sedative effect of L-Ser was mediated by GABA-A receptors (Shigemi et al., 2008). Although analogs of L-Ser, except for D-Ser, also attenuated stress-induced behaviors as well as L-Ser, there were some differential effects on spontaneous activity and distress vocalizations as shown in Figure 3. The results may indicate differences between the attenuation system for spontaneous activity and distress vocalizations in the chicks. Additionally, the high dose of L-Ser (0.84 μmol) and the analogs and derivatives of L-Ser, except for D-Ser, induced sleeping posture during the 10 min post injection period. These facts suggest that the central administration of L-Ser and its analogs and derivatives may have sedative and hypnotic effects. van Luijtelaar et al. (1987) found that sleeping posture and sitting/motionless with eyes closed were nearly always associated with electrophysiological sleep (90.5% of the time). Therefore, the results for sleeping posture would indicate electrophysiological sleep in neonatal chicks.

In contrast, the i.c.v. injection of D-Ser, proposed as an endogenous ligand for the NMDA receptor-related glycine site, had no sedative effect in chicks (Asechi et al., 2006). Although it was demonstrated that D-Ser blocked stereotypic behavior and ataxia induced by NMDA receptor antagonists (Contreras, 1990; Hashimoto et al., 2005), there might be no interaction between D-Ser and the sedative and hypnotic effects observed immediately after the i.c.v. injection of L-Ser. Alternatively, the subtle indication that spontaneous activity and the number of distress vocalizations increased might be due to the excitatory response by NMDA receptor-mediated D-Ser transmission. A glycine binding site on the NMDA receptor differs from the glycine receptor in that it is strychnine-insensitive. Both the glycine and glutamate sites must be occupied by ligands for receptor activation to occur (McBain and Mayer, 1994).

For this reason, glutamate and glycine are termed coagonists. Other potential coagonists are D-Ala and D-Ser, which bind to the same site as glycine (Kemp and Leeson, 1993). Glycine showed a similar sedative effect as L-Ser. Recently, however, Shigemi et al. (2008) revealed that the function of glycine was directly mediated via the glycine receptor. Co-injection of glycine and strychnine, a glycine receptor antagonist, inhibited the effect of glycine. Therefore, it is suggested that the sedative effect of glycine may be mainly mediated by the glycine receptor, and not the NMDA receptor glycine binding site. Hence, the lack of function of D-Ser on the stress response may be due to its function as a coagonist on the NMDA receptor.

The mechanism of i.c.v. injection L-Ser and its analogs and derivatives on the behavior of chicks is still obscure. The conversion of derivatives into L-Ser, or a structural L-Ser residue might be related to sedation and hypnotic effect. Moreover, based on the fact that L-Cys inhibits the activity of serine racemase (Cook et al., 2002), which converts L-Ser to D-Ser and was purified from the mammalian brain (Wolosker et al., 1999; Konno, 2003), it is likely that the sedative and hypnotic effects of L-Cys are partly due to inhibition of the synthesis of D-Ser, which might trigger excitatory responses mediated by NMDA receptor. However, as mentioned above, D-Ser itself is not directly involved with sedative and hypnotic effects.

Additionally, it has been shown that systemic administration of L-Ser increased the concentration of L-Ser in several brain areas in infant and adult rats (Hashimoto, 2002), and that oral supplementation of L-Ser could restore the serine-deficiency syndrome (Jaeken et al., 1996; de Koning et al., 2002, 2004). Therefore, the application of L-Ser may be helpful for human and animal health.

Asechi et al. (2008) further investigated the contribution of pyruvate, since L-Ser is metabolized to pyruvate, and finally glucose. Pyruvate metabolism in the rat brain using ^{13}C-labelled pyruvate and glucose has been investigated (Hassel, 2001; Gonzalez et al., 2005). Intravenous (i.v.) injection of 2.25-18 mmol/kg of [3-^{13}C] pyruvate and 1.125, 2.25, 4.5, and 9 mmol/kg of [1-^{13}C] glucose lead to dose-dependent labeling of brain Ala and GABA (Gonzalez et al., 2005). The i.c.v. dose of pyruvate in the study of Asechi et al. (2008), 0.84 μmol/10 μl, could be converted to 0.012 mmol/kg body weight based on the fact that the average body weight of the chicks was 70 g. Although it is difficult to compare directly because of difference in administration route, the i.c.v. dose used in the study of Asechi et al. (2008) might be too low to trigger neurotransmission and behavioral changes in chicks.

Pyruvate appeared to have little involvement with the sedative effect (Asechi et al., 2008). On the other hand, sedation and induction of sleep-like behavior were seen in the chicks given D-glucose, and its potency was as strong as that of L-Ser. The i.c.v. injection of glucose attenuated stress-related behavior of chicks, and appeared to induce a hypnotic effect and inhibit plasma corticosterone release. These effects seem to be almost identical to L-Ser. I.v. administration of [1-^{13}C] glucose increased related amino acids labeled dose-dependently, and this reaction was very similar to that of [3-^{13}C] pyruvate (Gonzalez et al., 2005). Since sedative effects were observed following the i.c.v. administration of glucose, but not pyruvate, it is possible that an increase in brain ketone bodies resulting from hyperglycemia might trigger the sedative effect. However, this seems unlikely since the synthesis of ketone bodies from glucose involves exchange to pyruvate. In addition, the place for ketone production is limited at the liver. The immediate sedative effect of glucose after the i.c.v. injection further argues against a role for ketone bodies since the amount of glucose administered was too small to produce ketone bodies in the liver. Furthermore, if a large amount of glucose was administered to the brain, it would take a relatively long time for it to reach the liver and come back to the brain through the blood stream.

Taken together, it was suggested that the metabolic pathway from L-Ser to glucose via pyruvate has less involved in the sedative and hypnotic effect of L-Ser, since the intermediate product pyruvate had no influence on the stress-related behaviors and plasma corticosterone level. Further, the sedative effect of glucose seems independent of that of L-Ser. This can be explained by the fact that pyruvate itself is the precursor of glucose as well as L-Ser.

Regarding plasma corticosterone levels, the i.c.v. injection of L-Ser decreased its levels (Asechi et al., 2008). This result was in contrast to the results of the previous report that demonstrated a poor ability for L-Ser to attenuate the release of corticosterone from adrenal glands through the HPA axis (Asechi et al., 2006). Although it was confirmed that social separation stress increases plasma corticosterone concentration, the intensity sometimes fluctuant with each experiment. Considering that plasma corticosterone concentrations in the L-Ser-treated groups tended to be lower than that in the control groups in our previous work, it seems possible that the i.c.v. injection of L-Ser attenuates release of corticosterone from the adrenal glands.

L-Ala is an α-amino acid and is one of the 20 proteinogenic amino acids, i.e. the building blocks of proteins. β-Alanine (β-Ala) 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-Ala and β-Ala are greatly different. However, both L-Ala and β-Ala can activate the glycine

receptor (Olsen and DeLorey, 1999). Under an isolation-induced stress, i.c.v. injection of glycine significantly decreased spontaneous activity in chicks (Asechi et al., 2006; Shigemi et al., 2008). β-Ala induced hypoactivity with lower spontaneous activity and less vocalization manifested as sleep-like behavior (Tomonaga et al., 2004). Therefore, Kurauchi et al. (2006) investigated the central effect of L-Ala on the stress response and showed that i.c.v. injection of L-Ala had a similar effect to that of L-Ser and L-Cys (Asechi et al., 2006) in its ability to reduced social stress-induced vocalizations. As a result, it is clear that all three-carbon nonessential amino acids have a sedative function.

AMINO ACIDS DEFICIENT IN VEGETABLE PROTEIN

Amino acids have recently been shown to affect behavior by acting within the brain. The i.c.v. injection of nonessential amino acids such as L-Ser, L-Cys (Asechi et al., 2006) and L-Ala (Kurauchi et al., 2006) induced sedative and hypnotic effects in chicks under an acute stress. However, information is limited on central functions of essential amino acids on the stress response. Kurauchi et al. (2007) investigated whether the i.c.v. administration of the first three major limiting amino acids L-Lys, L-tryptophan (L-Trp) and L-methionine (L-Met) can attenuate behavioral changes caused by social separation stress in neonatal chicks. For L-Lys and its metabolite L-PA, we have already discussed some effects on feeding behavior. However, whether or not L-Lys has a sedative effect was not investigated. Kurauchi et al. (2007) applied 0.8 μmol of L-Lys, a level was similar to that used for L-Ser and L-Cys, but no improvement for stress response was obtained.

The trans-sulfuration pathway entails the transfer of the sulfur atom of L-Met to L-Ser with the ultimate formation of L-Cys (Yudkoff, 1999). L-Ser and L-Cys have a sedative effect (Asechi et al., 2006), but L-Met did not show any attenuation of acute stress. While 0.8 μmol of L-Met was used in this study, this level may be too low to convert to enough L-Ser and L-Cys and/or the 10 min time may have been too short to allow for sufficient conversion.

L-Trp is the only amino acid that can be converted to serotonin, and serotonin, in turn is converted into melatonin. Centrally administered DL-Trp showed similar effects on spontaneous activity and vocalization to that of L-Ser, L-Cys (Asechi et al., 2006) and L-Ala (Kurauchi et al., 2006). L-Ala is one of the L-Trp metabolites. Accordingly, part of the actions of L-Trp may be due to its conversion to L-Ala rather than serotonin or melatonin. Kurauchi et al. (2007) used DL-Trp methyl ester hydrochloride due to the problem of solubility, but not the L type. Thus, while the total amount of tryptophan was 0.8 μmol, L-Trp itself was half (0.4 μmol) this amount. In spite of this low dose, the effectiveness of tryptophan in the stress responses was clear compared to L-Lys and L-Met. On the other hand, D forms of amino acid including D-PA, and their metabolites, have central functions. D-PA has similar functions as observed with L-PA (Takagi et al., 2001). Thus, D-Trp may have a sedative function.

CONCLUSION

At present, it is well known that if diets for animal production containing vegetable proteins are supplemented with specific essential amino acids, the overall biological value of that protein improves. The concept of limiting essential amino acids has been heavily applied in animal production. In the present review, some of essential amino acids, as well as some of the non essential amino acids, and their metabolite attenuated the stress response through a central action in neonatal chicks. The requirement for amino acids may need to be reconsidered to add the concept for stress in the future.

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