Amino Acids (2009) 37:377–382
DOI 10.1007/s00726-008-0164-0

ORIGINAL ARTICLE

L-Proline is a sedative regulator of acute stress in the brain of neonatal chicks

K. Hamasu · T. Haraguchi · Y. Kabuki · N. Adachi · S. Tomonaga · H. Sato · D. M. Denbow · M. Furuse

Received: 15 May 2008 / Accepted: 28 July 2008 / Published online: 12 August 2008

Abstract The purpose of the present study was to clarify the central nervous system function of amino acids during acute stress. In Experiment 1, changes in free amino acid pattern were investigated in the brain of neonatal chicks exposed to either restraint with isolation-induced or fasting stress. L-Proline and L-arginine were decreased in the telencephalon and diencephalon under any stress. Since the central nervous system functions of L-arginine during the stress response has recently been reported, in Experiment 2, the effect of intracerebroventricular injection of L-proline (0.5, 1.0, 2.0 μmol) during isolation-induced stress was investigated. L-Proline induced sedative and hypnotic effects in a dose-dependent manner. It is suggested that L-proline may have an important role to attenuate the stress response in the central nervous system of chicks.

Keywords L-Proline · Amino acid · Chick · Stress · Brain · i.c.v. injection

K. Hamasu · T. Haraguchi · Y. Kabuki · N. Adachi ·
S. Tomonaga · M. Furuse (✉)
Laboratory of Advanced Animal and Marine Bioresources,
Graduate School of Bioresource and Bioenvironmental Sciences,
Kyushu University, Fukuoka 812-8581, Japan
e-mail: furuse@brs.kyushu-u.ac.jp

H. Sato
Ajinomoto Co., Inc, Kawasaki-ku, Kawasaki 210-8681, Japan

D. M. Denbow
Department of Animal and Poultry Sciences,
Virginia Polytechnic Institute and State University,
Blacksburg, VA 24061-0306, USA

Introduction

In the central nervous system (CNS), some amino acids have very important functions as neurotransmitters. In particular, the roles of L-glutamate and γ-amino butyric acid (GABA) in the stress response have received much attention. Knowledge of the involvement of amino acids in neural regulation is still expanding (Boehning et al. 2003). For example, sedative and hypnotic effects of some amino acids such as L-serine, L-cysteine, glycine (Asechi et al. 2006), L-alanine (Kurauchi et al. 2006), L-arginine (Suenaga et al. 2008a), L-ornithine (Suenaga et al. 2008b) and β-alanine (Tomonaga et al. 2004) under an acute stressful condition were reported in neonatal chicks. However, these results were obtained using exogenous amino acid adminstration, since the responses were confirmed by intracerebroventricular (i.c.v.) injection of amino acids. Therefore, whether or not endogenous amino acid metabolism in the brain is affected by stress was still unclear.

Previously, the behaviors of chicks were investigated under stressful conditions (Feltenstein et al. 2003, Panksepp et al. 1980, Sahley et al. 1981). For instance, chicks feel comfortable when they are in crowds, but feel acute stress when they are isolated. The isolation-induced stress increases spontaneous activity and vocalization. This isolation-induced stress paradigm has been used to objectively screen anxiolytic drugs, measuring vocalization and spontaneous activity as the index of behaviors induced by stressor.

In the present study, we firstly focused on a survey of amino acids which are rapidly metabolized under stressful conditions, since we believe such amino acids are required to attenuate stress responses in the brain. Since L-proline concentration was rapidly reduced in the brain under several stress conditions in the first experiment, we investigated the central effect of L-proline on the stress response under isolation-induced stress.

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. One was a combination of restraint with isolation-induced stress and another was fasting stress. In the restraint with isolation-induced stress, body and legs of each chick were bound with rubber bands and placed in an acrylic cage for 0.5 h separated from the group. For the fasting stress, chicks were fasted for 3 or 6 h. During the stress treatments, food was removed for 0.5 h from the control group.

Amino acid analysis

Free amino acids in the telencephalon and diencephalon were determined with high-performance liquid chromatography (HPLC) (Pico-Tag^{TM}, Waters, Milford, MA, USA) using the Pico Tag method (Rubio 2003). After animals were stressed, they were killed by cervical dislocation. The brains were quickly removed and weighed, and then stored at −80°C until they were analyzed. The tissue was homogenized in 0.2 M perchloric acid and stored in ice for 30 min. The homogenate was centrifuged at 4°C at 15,000×g for 15 min. The supernatant was adjusted pH 7 with 1 M sodium hydroxide and 20 μl dried under reduced pressure. The dried samples were added with the mixed solution containing 40% of 1 M sodium acetate, 40% of methanol and 20% of triethylamine, and then, dried under reduced pressure. After drying, the derivatization was done with the reaction solution that consisted of 70% methanol, 10% triethylamine, 10% phenylisothiocyanate and 10% ultrapure water. After the 20 min reaction, the substances were dried under reduced pressure. Prior to the analysis, the dried samples were dissolved in Pico-tag sample diluent (Waters, Milford, MA, USA) and centrifuged with a centrifuge-filtration unit (Ultra Free C3-GV, Millipore, Bedford, MA, USA) at 10,000×g for 2 min at 4°C to remove the solid contents. Then, 5 μl of the filtrate was applied to the HPLC system for the measurement of amino acids contents. For estimation of relative concentration, and time of elution of various amino acids, a standard amino acids mixture (Type AN II and Type B; Wako, Osaka, Japan) was used, to which glutamine, asparagine, and tryptophan were added.

Preparation of drug

L-Proline was a gift from Kyowa Hakko Kogyo (Tokyo, Japan). L-Proline was dissolved in 0.85% saline containing 0.1% Evans Blue solution and control groups were given the saline solution. At the end of the experiment, the birds were sacrificed with an overdose of sodium pentobarbital after which the location of the injection was verified. Data from individuals not having Evans Blue dye present in the lateral ventricle were deleted.

Experimental procedure

The i.c.v. injections were made using a microsyringe according to the method of Davis et al. (1979). In Experiment 2, chicks were injected i.c.v. with 0.5, 1.0 or 2.0 μmol/10 μl of L-proline, or saline as the control. After the injection, chicks were placed in a acrylic glass monitoring cage (40 cm × 30 cm × 20 cm) with paper on the floor and behavioral observations were made for 10 min at a constant temperature of 30 ± 1°C. The monitoring systems were set in a separate room to avoid disturbing the animals. 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.). The number of distress vocalizations, which are shrill and intensecalls, was simultaneously recorded and counted, using a computer with Gretchen software (Excla Inc., Japan). Chicks were recorded by three video cameras positioned in different directions. Based on the method by van Luijtelaar et al. (1987), the behaviors were classified into four categories: (1) active wakefulness; (2) standing/sitting motionless with eyes opened; (3) standing motionless with eyes closed; and (4) sitting motionless with head drooped (sleeping posture) by watching the videotapes. They demonstrated the correlation between sleeping posture and electrophysiological sleep with EEG measurement (van Luijtelaar et al. 1987). During the monitoring period, chicks were not given food or water.

These behavioral experiments were applied in other studies by Asechi et al. (2006, 2008), Kurauchi et al. (2006, 2008) and Suenaga et al. (2008a, b).

Statistical analysis

Data for restraint with isolation-induced stress in Experiment 1 were statistically analyzed by student's *t*-test. Fasting stress in Experiment 1 and data in Experiment 2 were statistically analyzed by one-way analysis of variance (ANOVA) and Tukey–Kramer test was done as a post hoc test. Statistical analysis was conducted using a commercially available package StatView (version 5, SAS Institute, Cary, USA 1998).

Results

Experiment 1

The following amino acids and related compounds were examined in this study: L-alanine, GABA, anserine, L-arginine, L-asparagine, L-aspartic acid, β-alanine, carnosine, 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. Among them, amino acids in which significant effects of stress were detected are listed in the Tables.

Table 1 shows the effect of restraint with isolation-induced stress on free amino acids and related compounds in the telencephalon and diencephalon of chicks. In the telencephalon, L-glutamine was significantly higher ($P < 0.05$), whereas GABA ($P < 0.05$), L-arginine ($P < 0.05$), and L-proline ($P < 0.001$) were significantly lower than those of the control group. In the diencephalon, L-alanine ($P < 0.05$), L-arginine ($P < 0.05$), L-asparagine ($P < 0.05$), L-aspartic acid ($P < 0.05$), L-phenylalanine ($P < 0.05$), L-proline ($P < 0.01$), L-serine ($P < 0.05$), and L-tyrosine ($P < 0.05$) of restraint with isolation-induced stress group were significantly lower than those of the control group.

Table 1 Effect of restraint with isolation stress on free amino acids and related compounds in the telencephalon and diencephalon of 5- or 6-day-old chicks

	Control	Restraint with isolation-induced stress
Telencephalon		
L-Glutamine	16641 ± 774	19512 ± 571 *
GABA	2617 ± 98	2379 ± 22*
L-Arginine	170 ± 17	128 ± 5*
L-Proline	141 ± 4	117 ± 3***
Diencephalons		
L-Alanine	317 ± 14	258 ± 16*
L-Arginine	129 ± 7	103 ± 5*
L-Asparagine	152 ± 8	126 ± 6*
L-Aspartic acid	1671 ± 51	1475 ± 43*
L-Phenylalanine	44 ± 2	24 ± 2*
L-Proline	91 ± 4	68 ± 3**
L-Serine	384 ± 19	322 ± 15*
L-Tyrosine	47 ± 4	36 ± 3*

Vales are expressed as mean ± SEM in nmol/g wet tissue. The number of chicks used in each group was six

* $P < 0.05$; **$P < 0.01$, *** $P < 0.001$

Table 2 Effect of fasting stress on free amino acids and related compounds in the telencephalon and diencephalon of 5- or 6-day-old chicks

	Control	3 h	6 h
Telencephalon			
L-Tyrosine	69 ± 5^{a}	72 ± 8^{a}	105 ± 6^{b}
L-Valine	88 ± 4^{a}	97 ± 3^{a}	108 ± 7^{b}
L-Arginine	170 ± 17^{a}	127 ± 5^{b}	114 ± 6^{b}
L-Histidine	54 ± 4^{a}	31 ± 5^{b}	25 ± 3^{b}
L-Proline	141 ± 4^{a}	99 ± 8^{b}	86 ± 3^{b}
Diencephalons			
L-Tyrosine	47 ± 4^{a}	41 ± 4^{a}	61 ± 4^{b}
L-Asparagine	152 ± 8^{a}	111 ± 7^{b}	123 ± 4^{b}
L-Aspartic acid	1671 ± 51^{a}	1411 ± 56^{b}	1428 ± 67^{b}
L-Arginine	129 ± 7^{a}	95 ± 2^{b}	91 ± 5^{b}
L-Glutamine	11312 ± 509^{a}	9177 ± 646^{b}	9393 ± 470^{b}
L-Histidine	32 ± 4^{a}	16 ± 3^{b}	12 ± 2^{b}
L-Phenylalanine	44 ± 2^{a}	36 ± 2^{b}	34 ± 2^{b}
L-Proline	91 ± 4^{a}	52 ± 3^{b}	51 ± 4^{b}

Vales are expressed as mean ± SEM in nmol/g wet tissue. The number of chicks used in each group was seven. Groups with different letters are significantly different ($P < 0.05$)

Table 2 shows the effect of fasting stress on free amino acids and related compounds in the telencephalon and diencephalon of chicks. In the telencephalon, L-tyrosine ($F(2, 15) = 9.654$, $P < 0.01$) and L-valine ($F(2, 15) = 4.017$, $P < 0.05$) were significantly increased at 6 h after fasting, whereas L-arginine ($F(2, 15) = 7.600$, $P < 0.01$), L-histidine ($F(2, 15) = 13.134$, $P < 0.001$), and L-proline ($F(2, 15) = 29.541$, $P < 0.0001$) were significantly decreased after 3 h fasting compared with the control group. In the diencephalon, L-tyrosine ($F(2, 15) = 7.408$, $P < 0.01$) was significantly increased at 6 h fasting, whereas L-arginine ($F(2, 15) = 16.568$, $P < 0.001$), L-asparagine ($F(2, 15) = 9.735$, $P < 0.01$), L-aspartic acid ($F(2, 15) = 6.230$, $P < 0.05$), L-glutamine ($F(2, 15) = 4.618$, $P < 0.05$), L-histidine ($F(2, 13) = 14.557$, $P < 0.001$), L-phenylalanine ($F(2, 15) = 7.831$, $P < 0.01$), and L-proline ($F(2, 15) = 40.057$, $P < 0.0001$) were significantly decreased after 3 h fasting compared to the control group.

Experiment 2

Figure 1 shows the effect of i.c.v. injection of several doses of L-proline on spontaneous activity (upper panel) and distress vocalizations (lower panel) during the 10 min isolation-induced stress. Significant effects on total spontaneous activity ($F(3, 24) = 7.596$, $P < 0.001$) and total distress vocalizations ($F(3, 24) = 8.543$, $P < 0.001$) were detected. Negative correlations between the dose of L-proline and spontaneous activity and total distress vocalization were detected.

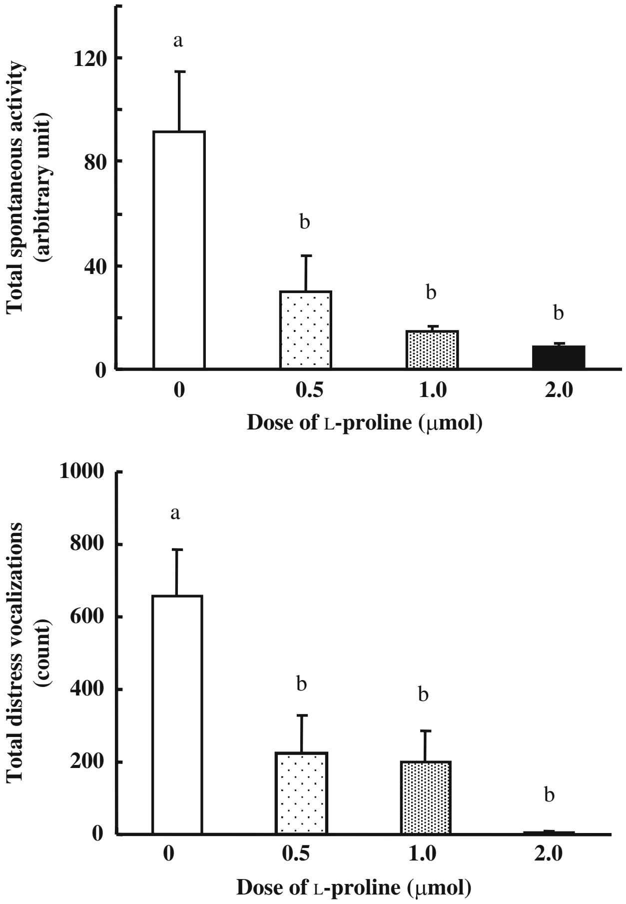

Fig. 1 Effect of i.c.v. injection of several doses of L-proline on total spontaneous activity (*upper panel*) and distress vocalizations (*lower panel*) during 10 min isolation in 5- or 6-day-old layer chicks. Results are expressed as means ± SEM. The number of chicks used in each group was seven. Groups with *different letters* are significantly different ($P < 0.05$)

Table 3 shows the effect of i.c.v. injection of L-proline on various behavioral categories of chicks during the 10 min behavior observation under isolation stress. The time for active wakefulness was reduced with increasing dose of L-proline ($F(3, 24) = 25.117$, $P < 0.0001$). Negative correlations between the dose of L-proline and the time for active wakefulness were detected. Additionally, L-proline increased the time for standing or sitting motionless with eyes open ($F(3, 24) = 6.132$) and sleeping posture ($F(3, 24) = 14.103$, $P < 0.0001$).

Discussion

In order to examine the relationships between amino acid metabolism in the brain and stress response, we investigated changes in the content of amino acids in the telencephalon and diencephalon of chicks after stress in Experiment 1. We showed that the contents of several amino acids were modified under stressful conditions. It was clear that the response of amino acids during the stress was mainly a reduction of their contents. Among them, L-arginine and L-proline were reduced in both the telencephalon and diencephalon under both restraint with isolation-induced stress and fasting stresses. Both amino acids may be rapidly metabolized in the brain under stressful conditions. For L-arginine, Suenaga et al. (2008a) reported that central L-arginine functions to attenuate stress responses. Furthermore, the mechanism of sedation induced by L-arginine was revealed by the same group. The sedative effect of L-arginine was mainly mediated by L-ornithine and by cooperation with other free amino acids having sedative and hypnotic effects in the CNS (Suenaga et al. 2008b). L-Ornithine is a metabolite of L-arginine. L-Arginine might be converted to L-ornithine for sedative and hypnotic effects under stressful conditions. Therefore, brain L-arginine of the stress groups was significantly lower than in the control group.

The function of L-proline during the stress response had not been investigated. Therefore, the i.c.v. injection of L-proline in Experiment 2 examined whether centrally administered L-proline could modify the behavior of neonatal chicks under isolation-induced stress.

L-Proline is one of the non-essential amino acids involved in numerous physiologic processes including glucogenesis and lipogenesis (Baquet et al. 1991, Sugden et al. 1984). In addition, its effects on various physiologic activities has been reported in past studies. For example, it produces a depressor response when microinjected into the caudal ventrolateral medulla of anesthetized rats (Takemoto 2005). In Experiment 2, spontaneous activity and

Table 3 Influence of i.c.v. injections of several doses of L-proline on various behavioral categories of chicks 10 min post-injection

L-Proline	0	0.5	1.0	2.0
Active wakefulness	509 ± 38^{a}	132 ± 45^{b}	143 ± 63^{b}	12 ± 5^{b}
Standing/sitting motionless with eyes open	88 ± 36^{a}	255 ± 30^{b}	231 ± 40^{b}	133 ± 16^{ab}
Standing motionless with eyes closed	3 ± 3	3 ± 3	59 ± 40	2 ± 2
Sitting motionless with head drooped (sleeping posture)	0 ± 0^{a}	210 ± 67^{b}	167 ± 72^{ab}	453 ± 17^{c}
Total (second)	600	600	600	600

Values are means ± SEM in seconds. The number of chicks used in each group was seven

vocalization of chicks were significantly decreased and sleeping posture was increased in a does-dependent manner by L-proline. These results revealed the novel function that L-proline has sedative and hypnotic effects under an acute stressful condition in neonatal chicks.

First, these effects of i.c.v. L-proline may have a considerable link to several mechanisms of neurotransmission in the CNS. L-Proline has been suggested as a neuronal modulator or transmitter candidate in the CNS for many years (Snyder et al. 1973). In addition, Henzi et al. (1992) revealed that L-proline binds to glycine and glutamate receptors. Glycine (Asechi et al. 2006) and L-glutamate (Yamane et al., unpublished data) cause a sedative and hypnotic effect, as observed with L-proline. So, the sedative and hypnotic effects of L-proline were possibly associated with the modulation of these receptor functions in the brain. Second, metabolites of L-proline might be responsible for sedative and hypnotic effects. L-Proline metabolism involves two other amino acids, L-glutamate and L-ornithine. This tri-amino-acid system also links with three other essential metabolic systems, the TCA cycle, urea cycle, and pentose phosphate pathway (Hu et al. 2008). Namely, L-proline and metabolites might affect these pathway and induce sedative and hypnotic effects. However, the detailed mechanism of i.c.v. injection of L-proline on the behavior in the chicks is still obscure. Further experiments will be needed to clarify the precise mechanism in the brain.

Other than L-proline and L-arginie, several amino acids were modified by stress. In fasting stress, interestingly, amino acids that increased were altered at 6 h, but those that decreased were altered at 3 h. Furthermore, the amounts by which amino acids decreased was much higher than the amount by which other amino acids increased. These facts implied that amino acids were quickly catabolized and/or consumed under stressful conditions.

A large fraction of L-glutamate released from nerve terminals is probably taken up into glial cells, where it is converted to L-glutamine (Ottersen et al. 1992). γ-amino butyric acid is formed from L-glutamate via the action of glutamate decarboxylase. L-Glutamate is also the precursor of two other nonessential amino acids, L-proline and L-arginine. In restraint with isolation-induced stress, L-glutamine was increased, but GABA, L-arginine and L-proline levels were decreased. Presumably, L-glutamate was mainly metabolized to glutamine in the telencephalon. On the other hand, responses in the diencephalon were somewhat different from the telencephalon even under the same restraint with isolation-induced stress. Among the reduced amino acids, as well as L-arginine and L-proline, L-serine (Asechi et al. 2006) and L-alanine (Kurauchi et al. 2006) function to attenuate stress responses. Recently, Yamane et al. (unpublished data) found that L-aspartate and L-aspargine had a sedative effect under stressful conditions. These data suggest that the diencephalon was protected by several amino acids when the host is exposed to a stressor.

Under fasting stress, L-histidine was significantly lower than in the control group in both the telencephalon and diencephalon (Table 2). L-Histamine synthesis is suspected as the cause. Neuronal L-histamine may crucially regulate glycogenolysis, and brain L-histamine systems seem to comprise an essential regulatory mechanism involved in energy balance (Sakata et al. 1994). Additively, the L-histamine system in the brain is activated by a decrease in glucose (Nishibori et al. 1986). Therefore, it is inferred that L-histidine was used as substrate to synthesis L-histamine when chicks were fasted, and thus decreased in the brain. However, Tomonaga et al. (2004) reported that i.c.v. injection of L-histidine did not attenuate the stress response of chicks under isolation-induced stress. In contrast, L-tyrosine was increased in both the telencephalon and diencephalon. Tyrosine hydroxylase catalyses the conversion of L-tyrosine to L-dopa, which is the first, rate-limiting step in the biosynthesis of catecholamines (dopamine, noradrenaline and adrenaline), the central and sympathetic neurotransmitters and adrenomedullary hormones. We determined the plasma concentration of L-tyrosine among control, 3 and 6 h fasted groups, but no significant changes were observed (Hamasu et al., unpublished data). Thus, the increase in L-tyrosine under fasting stress may have occurred by the inhibition of brain tyrosine hydroxylase. The mechanism was unclear, but these results indicate that changes in brain amino acid metabolism are altered by the type of stressor, and brain areas.

In conclusion, we found L-proline was significantly decreased in the telencephalon and diencephalon of neonatal chicks when several types of stress were applied. Furthermore, the i.c.v. injection of L-proline had sedative and hypnotic effects under an acute stressful condition in neonatal chicks. From these results, it is expected that L-proline can contribute to improve anxiety disorders or sleep disorders induced by stressor.

Acknowledgments This work was supported by a Grant-in-Aid for Scientific Research from Japan Society for the Promotion of Science (No. 18208023) and the SKYLARK Food Science Institute. The authors are grateful to the staffs and students of Laboratory of Chemistry and Technology of Animal Products in Kyushu University for their assistance in analysis of amino acids.

References

Asechi M, Tomonaga S, Tachibana T, Han L, Hayamizu K, Denbow DM, Furuse M (2006) Intracerebroventricular injection of L-serine analogs and derivatives induces sedative and hypnotic effects under an acute stressful condition in neonatal chicks. Behav Brain Res 170:71–77

Asechi M, Kurauchi I, Tomonaga S, Yamane H, Suenaga R, Tsuneyoshi Y, Denbow DM, Furuse M (2008) Relationships between the sedative and hypnotic effects of intracerebroventricular administration of L -serine and its metabolites, pyruvate and the derivative amino acids contents in the neonatal chicks under acute stressful conditions. Amino Acids 34:55–60

Baquet A, Lavoinne A, Hue L (1991) Comparison of the effects of various amino acids on glycogen synthesis, lipogenesis and ketogenesis in isolated rat hepatocytes. J Biochem 273:57–62

Boehning D, Snyder SH (2003) Novel neural modulators. Annu Rev Neurosci 26:105–131

Davis JL, Masuoka DT, Gerbrandt LK, Cherkin A (1979) Autoradiographic distribution of L-proline in chicks after intracerebral injection. Physiol Behav 22:693–695

Feltenstein MW, Lambdin LC, Ganzera M, Ranjith H, Dharmaratne W, Nanayakkara NP (2003) Anxiolytic properties of Piper methysticum extract samples and fractions in the chick social-separation-stress procedure. Phytother Res 17:210–216

Henzi V, Reichling DB, Helm SW, Macdermott AB (1992) L-Proline activates glutamate and glycine receptors in cultured rat dorsal horn neurons. Mol Pharmacol 41:793–801

Hu CA, Phang JM, Valle D (2008) Proline metabolism in health and disease. Amino Acids (in press)

Kurauchi I, Asechi M, Tachibana T, Han L, Hayamizu K, Denbow DM, Furuse M (2006) Intracerebroventricular injection of L-alanine induces a sedative effect under an acute stressful condition in neonatal chicks. J Poult Sci 43:384–387

Kurauchi I, Yamane H, Tsuneyoshi Y, Denbow DM, Furuse M (2008) Central L-alanine reduces energy expenditure with a hypnotic effect under an acute stressful condition in neonatal chicks. Amino Acids (in press)

Nishibori M, Oishi R, Itoh Y, Saeki K (1986) Glucose modulates the release of histamine from the mouse hypothalamus in vitro. J Neurochem 47:1761–1767

Ottersen OP, Zhang N, Walberg F (1992) Metabolic compartmentation of glutamate and glutamine: morphological evidence obtained by quantitative immunocytochemistry in rat cerebellum. Neuroscience 46:519–534

Panksepp J, Bean NJ, Bishop P, Vilberg T, Sahley TL (1980) Opioid blockade and social comfort in chicks. Pharmacol Biochem Behav 13:673–683

Rubio LA (2003) Determination of diaminopimelic acid in rat feces by high-performance liquid chromatography using the Pico Tag method. J chromatogr B 784:125–129

Sahley TL, Panksepp J, Zolovick AJ (1981) Cholinergic modulation of separation distress in the domestic chick. Eur J Pharmacol 72:261–264

Sakata T, Kurokawa M, Oohara A, Yoshimatsu H (1994) A physiological role of brain histamine during energy deficiency. Brain Res Bull 35:135–139

Snyder SH, Young AB, Bennett JP, Mulder AH (1973) Synaptic biochemistry of amino acids. Fed Proc 32:2039–2047

Suenaga R, Tomonaga S, Yamane H, Kurauchi I, Tsuneyoshi Y, Sato H, Denbow DM, Furuse M (2008a) Intracerebroventricular injection of L-arginine induces sedative and hypnotic effects under an acute stress in neonatal chicks. Amino Acids 35:139–146

Suenaga R, Yamane H, Tomonaga S, Asechi M, Adachi N, Tsuneyoshi Y, Kurauchi I, Sato H, Denbow DM, Furuse M (2008b) Central L-arginine reduced stress responses are mediated by L-ornithine in neonatal chicks. Amino Acids 35:107–113

Sugden MC, Watts DI, West PS, Palmer TN (1984) Proline and hepatic lipogenesis. Biochimica Biophys Acta 798:368–373

Takemoto Y (2005) Depressor responses to L-proline microinjected into the rat ventrolateral medulla are mediated by ionotropic excitatory amino acid receptors. Auton Neurosci-Basic 120:108–112

Tomonaga S, Tachibana T, Takagi T, Saito E, Zhang R, Denbow DM, Furuse M (2004) Effect of central administration of carnosine and its constituents on behaviors in chicks. Brain Res Bull 63:75–82

Van Luijtelaar ELJM, van der Grinten CPM, Blokhuis HJ, Coenen AML (1987) Sleep in the domestic hen (*Gallus domesticus*). Physiol Behav 41:409–414