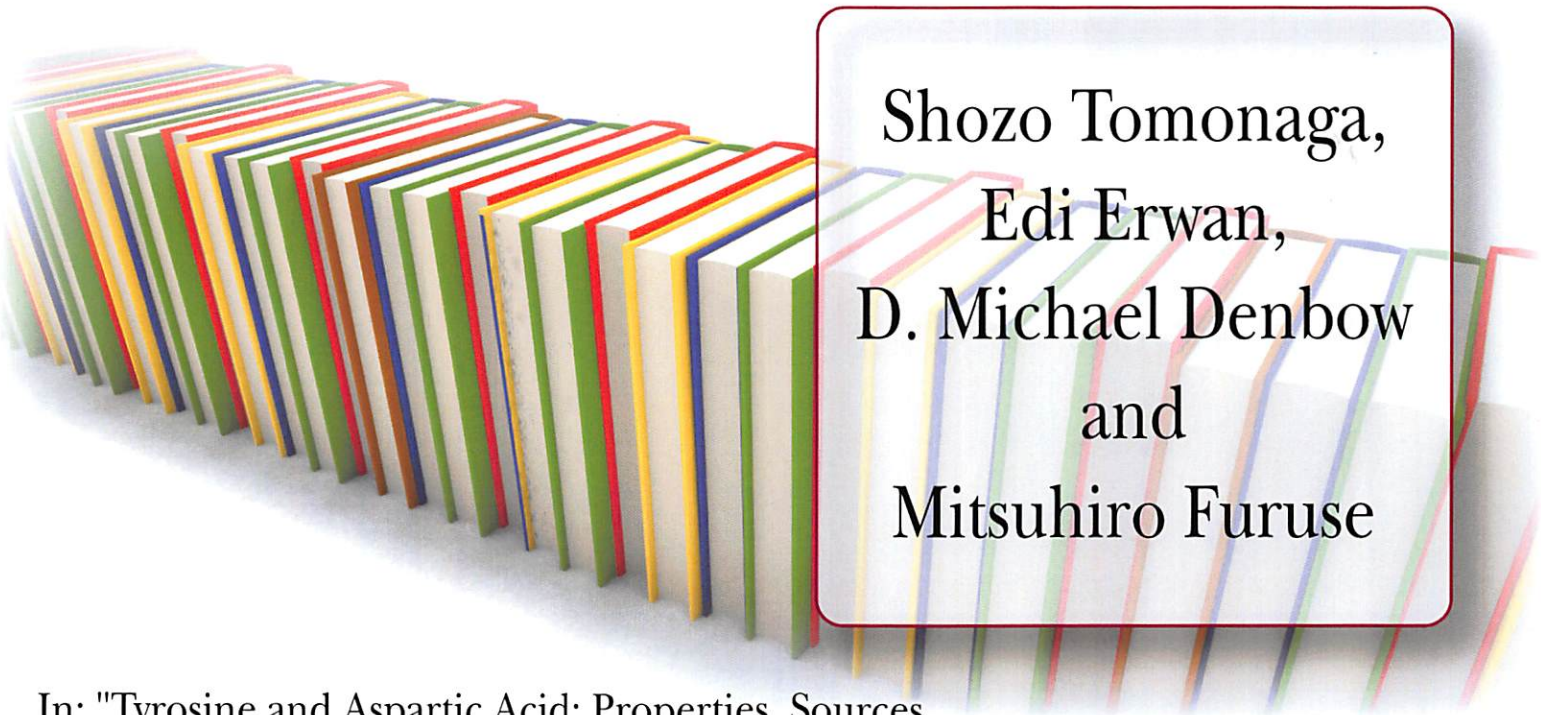
www.novapublishers.com

NOVA SCIENCE PUBLISHERS, INC.

400 Oser Avenue, Suite 1600, Hauppauge, New York 11788-3619, USA
Phone (631) 231-7269 Fax (631) 231-8175 E-mail: main@novapublishers.com

FUNCTIONS OF BRAIN L-ASPARTATE AND ITS DERIVATIVES ON STRESS RESPONSES



Shozo Tomonaga,
Edi Erwan,
D. Michael Denbow
and
Mitsuhiro Furuse

In: "Tyrosine and Aspartic Acid: Properties, Sources and Health Benefits"

Editors: Janice E. Jones and Danielle M. Morano

ISBN: 978-1-62100-752-4

2012

In: Tyrosine and Aspartic Acid
Editors: J. E. Jones and D. M. Morano

ISBN: 978-1-62100-752-4

Chapter 9

FUNCTIONS OF BRAIN L-ASPARTATE AND ITS DERIVATIVES ON STRESS RESPONSES

Shozo Tomonaga[1], Edi Erwan[2], D. Michael Denbow[3] and Mitsuhiro Furuse[2,*]

[1]Laboratory of Advanced Animal and Marine Bioresources, Faculty of Agriculture, Kyushu University, Fukuoka, Japan

[2]Laboratory of Regulation in Metabolism and Behavior, Faculty of Agriculture, Kyushu University, Fukuoka, Japan

[3]Department of Animal and Poultry Sciences, Virginia Tech, Blacksburg, VA, US

ABSTRACT

L-Aspartate (L-Asp) functions as a neurotransmitter to stimulate the N-methyl-D-aspartate (NMDA) receptor (NMDA-R), one of the ionotropic L-glutamate receptors, even though its binding capacity for NMDA-R is weaker than L-glutamate. The amino acid L-Asp, as well as its enantiomer D-aspartate (D-Asp), occurs in the central nervous system of various species including chickens, pigeons, rats, mice and humans. D-Asp, synthesized from L-Asp by aspartate racemase, can also directly stimulate the NMDA-R. Furthermore, D-Asp may indirectly stimulate NMDA-R because it is a substrate for endogenous NMDA. Central injection of L-Asp and L-asparagine derived from L-Asp via transamidation decreased social separation-induced stress responses. In addition, central injection of NMDA can attenuate the stress responses while some reports indicate that stimulation of the NMDA-R has negative impacts on stress responses. Central L-Asp may act on stress responses not only directly but also via its metabolites, and the effects may depend on the type of stressors and/or brain regions.

* Correspondence to: Mitsuhiro Furuse, Laboratory of Regulation in Metabolism and Behavior, Faculty of Agriculture Kyushu University, Fukuoka 812-8511, Japan, Tel/Fax: (81) (92) 642-2953, E-mail: furuse@brs.kyushu-u.ac.jp.

1. INTRODUCTION

L-Aspartate (L-Asp) is one of the non-essential amino acids, and it plays various roles not only as a constituent of protein but also as part of bioactive substances. The metabolic pathway linked to L-Asp in animal tissues is shown in Figure 1. L-Asp can be metabolized to oxaloacetate or adenylosuccinate, which can enter the TCA cycle. L-Asp also can be metabolized to L-argininosuccinate and thus contribute to activation of the urea cycle. On the other hand, L-aspagine (L-Asn) is synthesized from L-Asp by asparagine synthetase. In this chapter, we mainly focused on the other pathway, starting from the synthesis of D-aspartate (D-Asp) by aspartate racemase. D-Asp occurs in the central nervous system of various species including chickens, pigeons, rats, mice and humans (Hashimoto et al., 1993, 1995; Kera et al., 1996; Morikawa et al., 2001; Neidle & Dunlop, 1990).

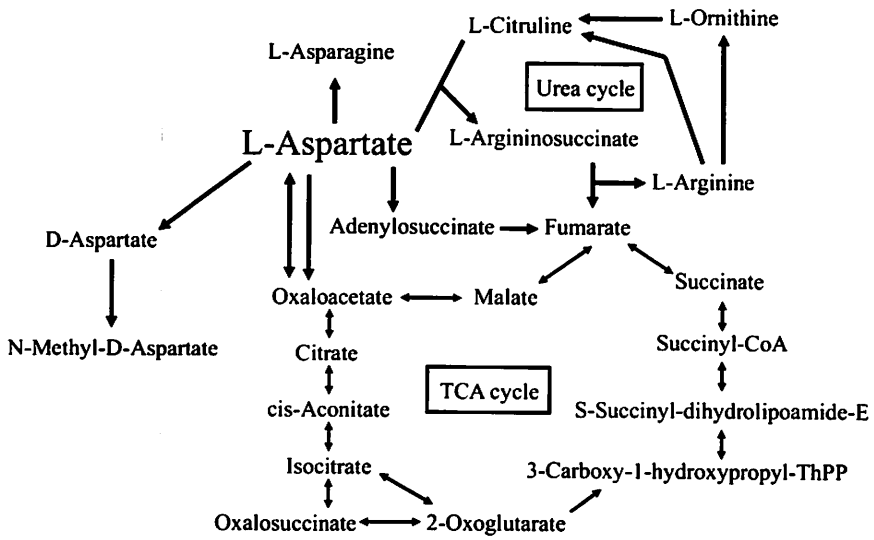


Figure 1. Metabolic pathways linked to L-aspartate.

Furthermore, D-Asp can be metabolized to N-methyl-D-aspartate (NMDA) (D'Aniello et al., 2000). L-Asp, D-Asp and NMDA can activate the NMDA-R in the brain (Kiskin et al., 1990). According to Hamasu et al. (2009), several amino acids including L-Asp and L-Asn were decreased in the diencephalon of neonatal chicks exposed to both restraint with isolation and fasting stress. This fact suggests that central free amino acids have an important role on the stress response. Furthermore, during stress the endocrine stress pathway involving the hypothalamic-pituitary-adrenal (HPA) axis is activated, and this activation is linked to excitatory neurotransmission involving the NMDA receptor (NMDA-R) (Zelena et al., 2005). However, the functions and mechanisms of this pathway have not been fully clarified.

Firstly, we discuss our study showing that L-Asp and L-Asn in the brain can attenuate acute stress responses in neonatal chicks (Yamane et al., 2009b). Then, we discuss the function of NMDA which had similar effects as observed with L-Asp (Yamane et al., 2009a) during the stress response. In these studies, the social separation stress model was used. This

stress model is frequently used for the study of anxiety. Chicks are comfortable when living in a group, but exhibit anxiety when isolated. Social separation stress increases spontaneous activity and vocalization of chicks. This social separation stress paradigm has been used for developing anti-anxiety agents using vocalization and spontaneous activity as parameters (Feltenstein et al., 2003). Thirdly, we introduce the relationships between endocrine stress responses and the NMDA-R in the brain. Finally, possible roles of D-Asp for stress responses are discussed. The overall perspective of L-Asp and its metabolites for stress responses in the brain will be discussed.

2. Central Functions of L-Asp and L-Asn for Stress Behavior in Chicks

Glutamate and L-Asp function as neurotransmitters and are the most likely candidates for neurotransmitter action at excitatory amino acid receptors. There are two types of glutamate receptors, i.e., ionotropic and metabotropic. Agonists acting at ionotropic glutamate receptors increase the probability that the channel will open. There are three classes of glutamate ionotropic receptors (iGluRs), which are named after their selective agonists: NMDA, α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) and kainate (KA).

Of these receptors, L-Asp appears to recognize only the NMDA-R, being inactive at the AMPA, and probably the KA receptor (Dingledine & McBain 1999). The action of L-Asp on the NMDA-R was weaker than that of glutamate (Chen et al., 2005). We investigated the central effect of L-Asp, as well as L-Asn derived from L-Asp via transamidation, on the stress response.

Under acute social separation stress, the intracerebroventricular (i.c.v.) injection of L-Asp and L-Asn caused a sedative effect in neonatal chicks (Yamane et al., 2009b; Figure 2, Table 1). As described in the next section, i.c.v. injection of NMDA can also induce sedative effects. L-Asp acts at the NMDA-R (Dingledine & McBain 1999) and it acts as a selective NMDA-R agonist in avians (Kubrusly et al., 1998). Therefore, the effects of L-Asp may be mediated by the NMDA-R. Since L-Asn can be hydrolyzed by asparaginase to NH_4^+ and L-Asp, it is possible that L-Asn may have induced this effect either following chemical modification or by directly binding to the NMDA-R. This remains to be investigated.

L-Asp attenuated social-separation stress-induced behaviors during the 10 min post injection period, but enhanced plasma corticosterone concentration (Figure 3). Therefore, L-Asp might have little or no ability to suppress the secretion of corticosterone from the adrenal glands through the HPA axis in response to stress. According to Zhang et al. (2004), i.c.v. injection of serotonin increased plasma corticosterone. Their report suggested that serotonin stimulates the HPA axis. Furthermore, Zhang et al. (2004) reported that serotonin dose-dependently decreased locomotor activities of chicks following the central administration of 0.1 μg of corticotrophin-releasing factor (CRF). Kim et al. (1998) suggested that glutamatergic neurotransmission may modulate serotonergic function suggesting that the response to L-Asp may be mediated via a serotonergic pathway.

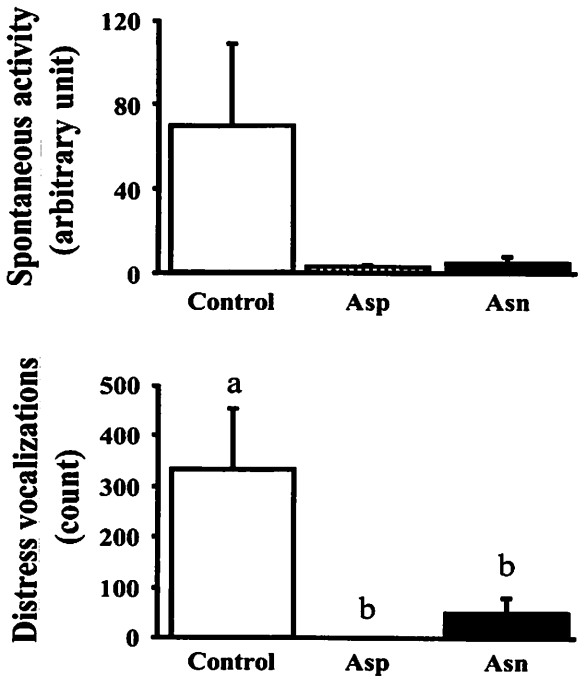


Figure 2. Effect of i.c.v. injection of saline, Asp and Asn on total spontaneous activity (upper panel) and distress vocalizations (lower panel) during a 10 min social-isolation period in 6-day-old layer chicks. Results are expressed as means ± SEM. The number of chicks used in each group was 9 in control, 7 in Asp and 8 in Asn groups. Groups with different letters are significantly different ($P<0.05$) Reproduced from Yamane, H., Asechi, M., Tsuneyoshi, Y., Kurauchi, I., Denbow, D.M., Furuse, M. (2009b) Intracerebroventricular injection of L-aspartic acid and L-asparagine induces sedative effects under an acute stressful condition in neonatal chicks. Animal Science Journal, 80:286-290, with permission from John Wiley & Sons.

Table 1. Effect of i.c.v. injection of saline, L-aspartic acid (Asp) and L-asparagine (Asn) on various behavioral categories of 6-day-old chicks during the 10 min post injection

	Control	Asp	Asn
Active wakefulness	298±96[a]	2±2[b]	95±57[ab]
Standing/sitting motionless with eyes opened	67±34	129±35	145±50
Standing motionless with eyes closed	1±1	0±0	1±1
Sitting motionless with head drooped (sleeping posture)	234±93	469±37	359±72
Total	600	600	600

Values are means ± SEM in seconds. The number of chicks used in each group was 9 in control, 7 in Asp and 8 in Asn groups. Groups with different letters are significantly different ($P<0.05$). Reproduced from Yamane, H., Asechi, M., Tsuneyoshi, Y., Kurauchi, I., Denbow, D.M., Furuse, M. (2009b) Intracerebroventricular injection of L-aspartic acid and L-asparagine induces sedative effects under an acute stressful condition in neonatal chicks. Animal Science Journal, 80:286-290, with permission from John Wiley & Sons.

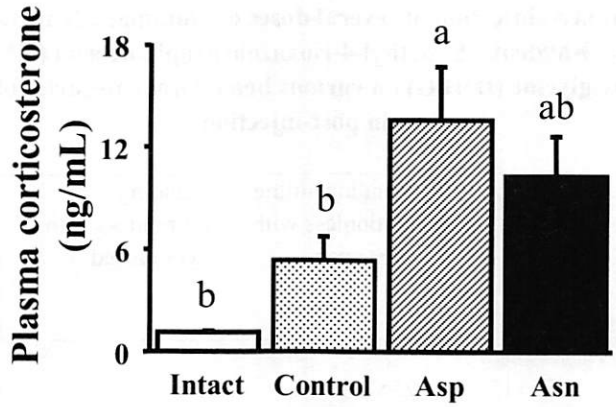


Figure 3. Effect of i.c.v. injection of saline, Asp and Asn on plasma corticosterone concentration following a 10 min social-isolation in 6-day-old layer chicks. Results are expressed as means ± SEM. The number of chicks used in each group was 5 in intact, 9 in control, 7 in Asp and 8 in Asn groups. Groups with different letters are significantly different ($P<0.05$). Reproduced from Yamane, H., Asechi, M., Tsuneyoshi, Y., Kurauchi, I., Denbow, D.M., Furuse, M. (2009b) Intracerebroventricular injection of L-aspartic acid and L-asparagine induces sedative effects under an acute stressful condition in neonatal chicks. Animal Science Journal. 2009 80:286-290, with permission from John Wiley & Sons

In conclusion, central L-Asp, as well as L-Asn, has a sedative effect under an acute stressful condition in neonatal chicks.

3. Central Functions of NMDA and Related Compounds for Stress Behavior in Chicks

Excitatory amino acids, including glutamate and L-Asp, act as neurotransmitters in the central nervous system. They can induce neuronal activity with powerful stimulatory effects (Monaghan et al., 1989). As stated above, there are three classes of iGluRs. The NMDA-R is usually blocked by Mg^{2+}. When the synaptic membrane is slightly depolarized, e.g., by previous activation of AMPA and KA receptors, the Mg^{2+} block of the NMDA-R is removed. The metabotropic glutamate receptors (mGluRs) are coupled to GTP-binding proteins, and regulate the production of intracellular messengers. The mGluRs have eight members (mGluR1-8), categorized into three groups based on sequence homology, second messenger coupling and pharmacology. Group I includes mGluR1 and 5, group II is mGluR2 and 3 and group III is mGluR4, 6, 7 and 8. Group I mGluRs are localized postsynaptically and predominantly activate phospholipase C. Group II and III mGluRs are localized in presynaptic densities and inhibit adenylyl cyclase activity. They contribute to the regulation of synaptic plasticity and transmission (De Blasi et al., 2001; Kew & Kemp, 2005).

The i.c.v. injection of NMDA, glutamate and AMPA attenuated total distress vocalizations and induced sedation in neonatal chicks (Yamane et al., 2009a: Figure 4, Table 2).

Table 2. Effect of i.c.v. injection of several doses of glutamate, N-methyhl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA), kainate and 5-dehydroxyphenylglycine (DHPG) on various behavioral categories of chicks during 10 min post-injection

Drugs	Active wakefulness	Standing/sitting motionless with eyes open	Standing motionless with eyes closed	Sitting motionless with head drooped (sleeping posture)
L-Glutamate				
0 μmol	575±15	25±15	0±0	0±0
0.2 μmol	382±85*	120±39	11±11	87±53
0.4 μmol	159±46*	205±44*	29±29	207±69*
0.8 μmol	19±15*	134±23	10±10	437±26*
NMDA				
0 nmol	560±13	40±13	0±0	0±0
0.5 nmol	490±54	110±54	0±0	0±0
1 nmol	238±57*	262±41*	0±0	100±52
2 nmol	134±46*	314±53*	0±0	152±71
AMPA				
0 pmol	551±16	49±16	0±0	0±0
50 pmol	568±23	32±23	0±0	0±0
100 pmol	471±49	129±49	0±0	0±0
150 pmol	281±101*	102±34	10±7	207±79*
Kainate				
0 nmol	468±54	75±22	21±21	43±43
0.25 nmol	404±61	86±32	19±12	91±43
0.5 nmol	454±62	68±24	19±19	60±40
1 nmol	558±20	31±13	0±0	11±11
DHPG				
0 nmol	591±4	9±4	0±0	0±0
0.01 nmol	585±4	15±4	0±0	0±0
0.1 nmol	589±9	11±9	0±0	0±0
1 nmol	593±4	7±4	0±0	0±0

Values are means ± SEM in seconds. *Significantly different when compared with control at $P < 0.05$. L-Glutamate; Active wakefulness (second/10min) = 526 (SE= 41) – 692 (SE= 89) X (R^2= 0.701, $P < 0.0001$) and sleeping posture (second/10 min) = -11 (SE= 34) + 553 (SE= 74) X (R^2= 0.684, $P < 0.0001$).

NMDA; Active wakefulness (second/10 min) = 552 (SE= 38) - 223 (SE= 32) X (R^2= 0.640, $P < 0.0001$) and standing/sitting motionless with eyes open (second/10min) = 58 (SE = 35) + 140 (SE = 30) X (R^2 = 0.450, $P < 0.0001$).

AMPA; Active wakefulness (second/10min) = 600 (SE= 51) – 2 (SE= 1) X (R^2= 0.309, $P < 0.01$) and sleeping posture (second/10 min) = -40 (SE= 38) + 1 (SE= 0.4) X (R^2= 0.274, $P < 0.01$). Reproduced from Yamane, H., Tsuneyoshi, Y., Denbow, D.M., Furuse, M. (2009a) N-Methyl-D-aspartate and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate receptors involved in the induction of sedative effects under an acute stress in neonatal chicks. Amino Acids, 37:733-739.Erratum, 37:767, with permission from Springer-Verlag.

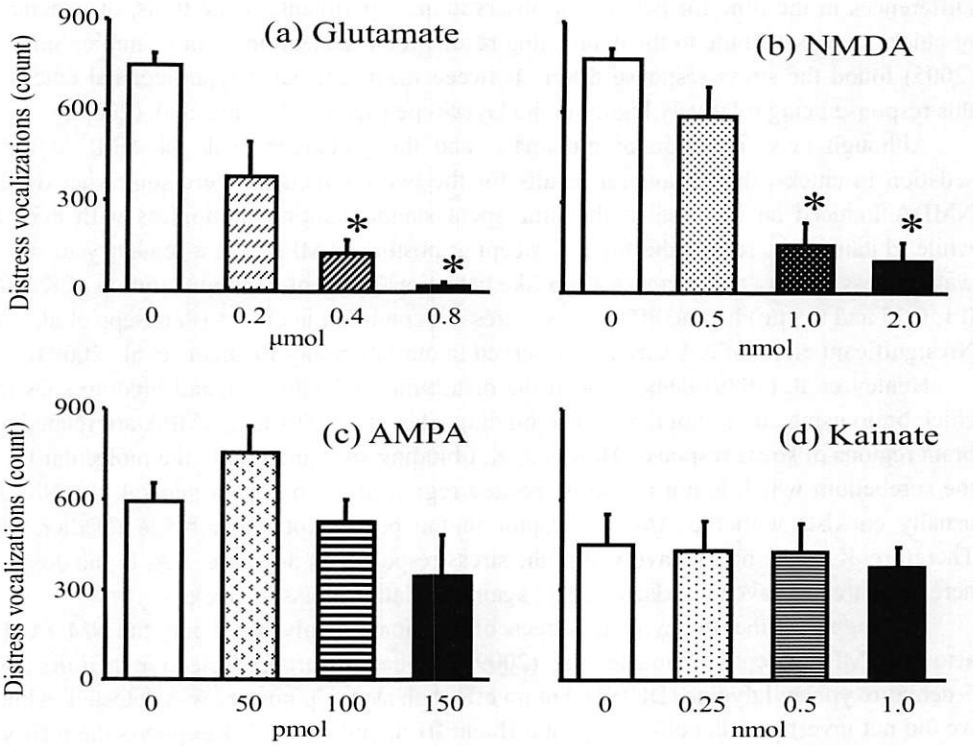


Figure 4. Effect of i.c.v. injection of iGluR agonists on total distress vocalizations during a 10 min isolation period in chicks. Results are expressed as means with S.E.M. Glutamate (count/10 min) = 621 (SE = 62) – 868 (SE = 135) X (R2= 0.613, $P < 0.0001$). NMDA (count/10 min) = 708 (SE = 64) – 342 (SE = 54) X (R2= 0.596, $P < 0.0001$). AMPA (count/10 min) = 691 (SE = 83) – 2 (SE = 1) X (R2= 0.158, $P < 0.05$). *Significant difference when compared with the control at $P < 0.05$. Reproduced from Yamane, H., Tsuneyoshi, Y., Denbow, D.M., Furuse, M. (2009a) N-Methyl-D-aspartate and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate receptors involved in the induction of sedative effects under an acute stress in neonatal chicks. Amino Acids, 37:733-739. Erratum, 37:767, with permission from Springer-Verlag.

In contrast, previous studies showed that localized infusion of glutamate induced vocalizations in cats (Bandler, 1982) and squirrel monkeys (Jürgens & Richter, 1986). Additionally, the injection of NMDA, AMPA and KA into the substantia innominata/lateral preoptic area of rats dose-dependently stimulated locomotion (Shreve & Uretsky, 1988). Lateral hypothalamic injection of KA also increased locomotor activity in rats (Stanley et al., 1993; Hettes et al., 2007). Further, when injected intracranially to restricted brainstem regions, NMDA elicited locomotion in decerebrate geese and ducks (Sholomenko et al., 1991). On the other hand, injection of glutamate into the lateral hypothalamus increased sleeping time (Stanley et al., 1993). The difference between these studies and Yamane et al. (2009a) may be due to species differences or the site of injection. The i.c.v. injection of glutamate dose-dependently decreased distress vocalizations in isolated chicks similarly to that observed by Panksepp et al. (1988) who injected glutamate at doses from 25 to 500 μg. In contrast, Panksepp et al. (1988) reported the administration of NMDA (0.1, 0.25, 0.5 and 1.0 μg) had no effect on the number of vocalizations, which is inconsistent with our results.

Differences in the time for behavioral observation, experimental conditions, or genetic lines of chicks may contribute to the contrasting results for vocalizations. For example, Saito et al. (2005) found the stress response differs between meat- and layer-type neonatal chicks with this response being relatively higher in the layer-type used by Yamane et al. (2009a).

Although i.c.v. injection of glutamate, and the glutamate analogue NMDA, induced sedation in chicks, the behavioral results for the two compounds were somewhat different. NMDA induced an increase in the time spent standing/sitting motionless with eyes open while glutamate increased the time of sleeping posture. AMPA had a tendency to decrease wakefulness activity and increase sleep-like behavior. The central administration of KA (0.05, 0.1, 0.25 and 0.5 μg) had no effect under stressful condition in chicks (Panksepp et al., 1988). No significant effect of KA was also observed in our laboratory (Yamane et al., 2009a).

Henley et al. (1989) demonstrated the distribution of iGluRs ligand binding sites in the chick brain using autoradiography. The binding sites of NMDA and AMPA are related to the brain regions of stress response. However, KA binding sites are mainly the molecular layer of the cerebellum which is not the stress related region in the brain. In general, the NMDA-R usually coexists with the AMPA receptor in the postsynaptic membrane (Nadler, 2007). Therefore, KA may not be involved in the stress response. In any case, KA, in the doses used here, appeared to have no sedative effect against isolation stress in chicks.

It is suggested that the hypnotic effects of glutamate involved not only the NMDA-R, but also the AMPA receptor. Yamane et al. (2009a) applied the group I selective mGluRs agonist 5-dehydroxyphenylglycine (DHPG), but no effect on sleeping posture was detected. Although we did not investigate the effect of group II and III mGluRs, the data supports the theory that the iGluRs NMDA and AMPA, but not KA, are related to sleep-induction in neonatal chicks. Glutamate can bind to both AMPA and KA receptors; its action is probably mediated via NMDA, AMPA and KA receptors. On the other hand, the group I selective mGluRs agonist DHPG had no effect. Thus, it appears likely that the hypnotic and sedative effects of glutamate are induced by interaction with NMDA and AMPA receptors. However, the activity of group II mGluRs regulates the HPA axis (Scaccianoce et al., 2003), and group III mGluRs agonists have anxiolytic- and antidepressant-like effects in rats (Pałucha et al., 2004). Therefore, it is necessary to further investigate the interaction between mGluRs and stress behavior.

In conclusion, the central administration of glutamate attenuates stress-induced behaviors and triggers sleep-like behavior in neonatal chicks. Furthermore, it was demonstrated that NMDA and AMPA, but not KA and DHPG, also have a sedative effect. It is suggested that glutamate induced hypnosis and sedation may be necessary for the interaction between NMDA and AMPA receptors. Therefore, studies involving the co-administration with NMDA or AMPA antagonists are needed to better understand the mechanism of glutamate.

4. Functions of Brain NMDA Receptor on Endocrine Stress Axis

In this section, we briefly reviewed the functions of the NMDA-R on the endocrine stress axis because L-Asp, D-Asp and NMDA can all stimulate the NMDA-R (Kiskin et al., 1990). The HPA axis is a main endocrine stress axis responding to a diverse set of stressful stimuli.

CRF from the paraventricular nucleus (PVN) of the hypothalamus stimulates adrenocorticotropic hormone (ACTH) release from the anterior pituitary into the blood. Then, ACTH goes to the adrenal cortex and stimulates release of glucocorticoids. Anatomical, pharmacological, and electrophysiological studies indicate that glutamate is a major excitatory signal to the HPA axis (Herman et al., 2001). Focusing on the NMDA-R, acute immobilization stress increases NMDA-R1, one of the NMDA-R subunits, mRNA in the hypothalamic PVN (Bartanusz et al., 1995). In contrast, osmotic stress induced by water deprivation does not increase NMDA-R expression in the PVN (Meeker et al., 1994), suggesting that acute psychological stress may have a more profound affect on activating glutamatergic input to the PVN. On the other hand, chronic intermittent stress induces a significant decrease in the NR2B subunit of NMDA-R mRNA expression in the CRF-containing region of the PVN in rats (Ziegler et al., 2005). Zelena et al. (2005) suggested that intravenously administered NMDA (5 mg/kg) elevated ACTH and glucocorticoid levels while PVN lesions had no influence on their basal secretion but blocked NMDA-induced elevations. Therefore, NMDA may activate the HPA axis at a central (PVN) level and not at the level of pituitary or adrenal gland in rats.

5. Possible Functions of Central D-Asp for Stress Responses

D-Amino acids, the enantiomers of L-amino acids, are candidates to be novel physiologically active substances and biomarkers in animals, but D-amino acids demonstrate different actions and mechanisms in the central nervous system. For instance, L-serine might be converted to the enantiomer D-serine by serine racemase, which has been purified from the mammalian brain (Wolosker et al., 1999). D-Serine is present in the brain of several vertebrate species including carp, frog, mice, rat and chick (Nagata et al., 1994), and is in particularly high concentration in the mammalian brain where it acts as an endogenous ligand for the NMDA-R-related glycine site. According to Asechi et al. (2006), L-serine induced sedative and hypnotic effects in chicks under acute stress, but D-serine did not. The ligand for the NMDA-R, glycine, had similar sedative and hypnotic effects under acute stress. Why D-serine did not show sedative and hypnotic effects was unclear, but glycine can act at the glycine receptor. L-Serine is a precursor for the synthesis of other amino acids including glycine and L-cysteine. Asechi et al. (2006) confirmed that L-cysteine, as well as L-serine and glycine, had sedative and hypnotic effects in chicks under acute stress. However, L- and D-cysteine had a different mechanism under acute stress since L-cysteine increased sleep-like behavior while D-cysteine caused abnormal behavior including syncope as well as sleep-like behavior (Yamane et al., 2009c). On the other hand, it was an interesting observation that L- and D-proline functioned through different receptors. L-Proline and D-proline differentially induce sedative and hypnotic effects through NMDA and glycine receptors, respectively (Hamasu et al., 2010).

D-Asp has been detected in various proteins from human tissue samples such as eye lens, brain, skin, bone, teeth and aorta from elderly individuals (Fujii, 2005). The presence of D-Asp is believed to be the result of racemization of L-Asp. However, a chiral reaction field exists in the native higher order structure of protein which induces the inversion of L-Asp to D-Asp residue (Fujii, 2005). Free D-Asp occurs in the central nervous system of various

species including chickens, pigeons, rats, mice and humans (Hashimoto et al., 1993, 1995; Kera et al., 1996; Morikawa et al., 2001; Neidle & Dunlop, 1990). To our knowledge, there are few reports investigating the effects of D-Asp on stress responses. From discussions in the previous sections, it can be easily hypothesized that brain D-Asp has sedative effects under social isolation stress in chicks and it can stimulate the HPA-axis by acting on the NMDA-R not only by itself but also by its metabolite NMDA. On the other hand, recently Molinaro et al. (2010) suggests that D-Asp, but not NMDA, activates mGluR5 receptors coupled to polyphosphoinositide hydrolysis in early postnatal rat brain slices. mGluR5 is highly expressed during the early postnatal brain and is also found in the embryonic brain (Di Giorgi Gerevini et al., 2004). mGluR5 could play some role in the stress response because stress-induced hyperthermia was reduced in mGluR5 knockout mice (Brodkin et al., 2002). Therefore, behavioral and/or endocrine stress responses may be regulated by brain D-Asp to stimulate both NMDA-R and mGluR5 especially in the developmental period. Stress in this period could induce a big impact (both negatively and positively) on brain maturation and/or function such as stress sensitivity in adulthood (Parker et al., 2006; Kikusui & Mori, 2009). Therefore, clarification of the relationship between central D-Asp and stress response in this period may be important to understand the physiological mechanisms of stress response in the brain. This should be investigated in the future.

ACKNOWLEDGMENTS

A part of studies described here was supported by grant-in-aid for scientific research from Japan Society for the Promotion of Science (Nos. 17208023, 18208023 and 23248046) and Uehara Memorial Fundation to MF. This work was also supported by a Research Fellowship of the Japan Society for the Promotion of Science for Young Scientist to ST (No. 189592 and 22780240).

REFERENCES

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

Bandler, R. (1982) Induction of 'rage' following microinjections of glutamate into midbrain but not hypothalamus of cat. *Neuroscience Letters,* 30:183-188.

Bartanusz, V., Aubry, J.M., Pagliusi, S., Jezova, D., Baffi, J., Kiss, J.Z. (1995) Stress-induced changes in messenger RNA levels of N-methyl-Daspartate and AMPA receptor subunits in selected regions of the rat hippocampus and hypothalamus. *Neuroscience,* 66:247–252.

Brodkin, J., Bradbury, M., Busse, C., Warren, N., Bristow, L.J., Varney, M.A. (2002) Reduced stress-induced hyperthermia in mGluR5 knockout mice. *European Journal of Neuroscience,* 16:2241-2244.

Chen, P.E., Geballe, M.T., Stansfeld, P.J., Johnston, A.R., Yuan, H., Jacob, A.L., Snyder, J.P., Traynelis, S.F., Wyllie, D.J.A. (2005) Structural features of the glutamate binding

site in recombinant NR1/NR2A N-methyl-D-aspartate receptors determined by site-directed mutagenesis and molecular modeling. *Molecular Pharmacology,* 67:1470-1484.

D'Aniello, G., Tolino, A., D'Aniello, A., Errico, F., Fisher, G.H., Di Fiore, M.M. (2000) The role of D-aspartic acid and N-methyl-D-aspartic acid in the regulation of prolactin release. *Endocrinology,* 141:3862-3870.

De Blasi, A., Conn, P.J., Pin, J.P., Nicoletti, F. (2001) Molecular determinants of metabotropic glutamate receptor signaling. *Trends in Pharmacological Sciences,* 22:114-120.

Di Giorgi Gerevini, V.D., Caruso, A., Cappuccio, I., Ricci Vitiani, L., Romeo, S., Della Rocca, C., Gradini, R., Melchiorri, D., Nicoletti, F. (2004) The mGlu5 metabotropic glutamate receptor is expressed in zones of active neurogenesis of the embryonic and postnatal brain. *Brain Research, Developmental Brain Research,* 150:17-22.

Dingledine, R., McBain, C.J. (1999) Glutamate and Aspartate. In: Basic Neurochemistry. Molecular, *Cellular and Medical Aspects.* (Siegel G., Agranoff B.W., Albers R.W., Fisher S.K. and Uhler M.D. eds.). Sixth Edition. pp. 315-333. Lippincott-Raven Publishers. New York.

Feltenstein, M.W., Lambdin, L.C., Ganzera, M., Ranjith, H., Dharmaratne, W., Nanayakkara, N.P., Khan, I.A., Sufka, K.J. (2003) Anxiolytic properties of Piper methysticum extract samples and fractions in the chick. *Phytotherapy Research,* 17:210-216.

Fujii, N. (2005) *D-amino Biological & Pharmaceutical Bulletin,* 28:1585-1589.

Hamasu, K., Haraguchi, T., Kabuki, Y., Adachi, N., Tomonaga, S., Sato, H., Denbow, D.M., Furuse, M. (2009) L-Proline is a sedative regulator of acute stress in the brain of neonatal chicks. *Amino Acids,* 37:377-382.

Hamasu, K., Shigemi, K., Tsuneyoshi, Y., Yamane, H., Sato, H., Denbow, D.M., Furuse, M. (2010) Intracerebroventricular injection of L-proline and D-proline induces sedative and hypnotic effects by different mechanisms under an acute stressful condition in chicks. *Amino Acids,* 38:57-64.

Hashimoto, A., Kumashiro, S., Nishikawa, T., Oka, T., Takahashi, K., Mito, T., Takashima, S., Doi, N., Mizutani, Y., Kaneco, T., Ootomo, E. (1993) Embryonic development and postnatal changes in free D-aspartate and D-serine in the human prefrontal cortex. *Journal of Neurochemistry,* 61:348–351.

Hashimoto, A., Oka, T., Nishikawa, T. (1995) Anatomical distribution and postnatal changes in endogenous free D-aspartate and D-serine in rat brain and periphery. *European Journal of Neuroscience,* 7:1657–1663.

Henley, J.M., Moratallo, R., Hunt, S.P., Barnard, E.A. (1989) Localization and quantitative autoradiography of glutamatergic ligand binding sites in chick brain. *European Journal of Nuroscience,* 1:516-523.

Herman, J.P., Tasker, J.G., Ziegler, D.R., Cullinan, W.E. (2001) Paraventricular hypothalamic stress integration: limbic connections with local circuit neurons. *Pharmacology Biochemistry and Behavior,* 71:457–468.

Hettes, S.R., Heyming, W., Stanley, B.G. (2007) Stimulation of lateral hypothalamic kainate receptors selectively elicits feeding behavior. *Brain Research,* 1184:178-185.

Jürgens, U., Richter, K. (1986) Glutamate-induced vocalization in the squirrel monkey. *Brain Research,* 373:349-358.

Kera, Y., Aoyama, H., Watanabe, N., Yamada R.H. (1996) Distribution of D-aspartate oxidase and free D-glutamate and D-aspartate in chicken and pigeon tissues.

Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology, 115:121-126.

Kew, J.N.C., Kemp, J.A. (2005) Ionotropic and metabotropic glutamate receptor structure and pharmacology. *Psychopharmacology* (Berl), 179:4-29.

Kikusui, T., Mori, Y. (2009) Behavioural and neurochemical consequences of early weaning in rodents. Journal of Neuroendocrinology, 21:427-431.

Kim, H.S., Park, I.S., Park, W.K. (1998) NMDA receptor antagonists enhance 5-HT2 receptor-mediated behavior, head-twitch response, in mice. *Life Sciences,* 63:2305-2311.

Kiskin, N.I., Krishtal, O.A., Tsyndrenko, A.Y. (1990) Cross-desensitization reveals pharmacological specificity of excitatory amino acid receptors in isolated hippocampal neurons. *European Journal of Neuroscience,* 2:461-470.

Kubrusly, R.C., de Mello, M.C., de Mello, F.G. (1998) Aspartate as a selective NMDA receptor agonist in cultured cells from the avian retina. *Neurochemistry International,* 32:47-52.

Meeker, R.B., McGinnis, S., Greenwood, R.S., Hayward, J.N. (1994) Increased hypothalamic glutamate receptors induced by water deprivation. *Neuroendocrinology,* 60:477-485.

Molinaro, G., Pietracupa, S., Di Menna, L., Pescatori, L., Usiello, A., Battaglia, G., Nicoletti, F., Bruno, V. (2010) D-aspartate activates mGlu receptors coupled to polyphosphoinositide hydrolysis in neonate rat brain slices. *Neuroscience Letters,* 478:128-130.

Monaghan, D.T., Bridges, R.J., Cotman, C.W. (1989) The excitatory amino acid receptors: Their classes, pharmacology, and distinct properties in the function of the central nervous system. *Annual Review of Pharmacology and Toxicology,* 29:365-402.

Morikawa, A., Hamase, H., Inoue, T., Konno, R., Niwa, A., Zaitsu, K. (2001) Determination of free D-aspartic acid, D-serine and D-alanine in the brain of mutant mice lacking D-amino acid oxidase activity. *Journal of Chromatography B: Biomedical Sciences and Applications,* 757:119–125.

Nadler, J.V. (2007) Encyclopedia of stress. In: Fink G, McEwen B, Ronald de Kloet E, Rubin R, Chrousos G, Steptoe A, Rose N, Craig I, Feuerstein G (ed) *Excitatory amino acids,* 2nd edn. Elsevier, Australia, pp. 970-975.

Nagata, Y., Horiike, K., Maeda, T. (1994) Distribution of free D-serine in vertebrate brains. *Brain Research,* 634: 291-295.

Neidle, A., Dunlop, D.S. (1990) Developmental changes of free D-aspartic acid in the chicken embryo and in the neonatal rat. *Life Sciences,* 46:1517–1522.

Pałucha, A., Tatarczyńska, E., Brański, P., Szewczyk, B., Wierońska, J.M., Kłak, K., Chojnacka-Wójcik, E., Nowak, G., Pilc, A. (2004) Group III mGlu receptor agonists produce anxiolytic- and antidepressant-like effects after central administration in rats. *Neuropharmacology,* 46:151-159

Panksepp, J., Normasell, L., Herman, B., Bishop, P., Crepeau, L. (1988) The physiological control of mammalian vocalization. In: Newman J.D. (ed) *Neural and neurochemical control of the separation distress call,* Plenum Press, New York, pp. 263-299.

Parker, K.J., Buckmaster, C.L., Sundlass, K., Schatzberg, A.F., Lyons, D.M. (2006) Maternal mediation, stress inoculation, and the development of neuroendocrine stress resistance in primates. *Proceedings of the National Academy of Sciences of the United States of America,* 103:3000-3005.

Saito, S., Tachibana, T., Choi, Y.H., Denbow, D.M., Furuse, M. (2005) ICV CRF and isolation stress differentially enhance plasma corticosterone concentrations in layer- and meat-type neonatal chicks. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, 141:305-309.

Scaccianoce, S., Matrisciano, F., Del Bianco, P., Caricasole, A., Gerevini, V.D.G., Cappuccio, I., Melchiorri, D., Battaglia, G., Nicoletti, F. (2003) Endogenous activation of group-II metabotropic glutamate receptors inhibits the hypothalamic-pituitary-adrenocortical axis. *Neuropharmacology* (Berl), 44:555-561.

Sholomenko, G.N., Funk, G.D., Steeves, J.D. (1991) Avian locomotion activated by brainstem infusion of neurotransmitter agonists and antagonists. I. Acetylcholine, excitatory amino acids and substance P. *Experimental Brain Research,* 85:659-673.

Shreve, P.E., Uretsky, N.J. (1988) AMPA, kainic acid, and N-methyl-D-Aspartic acid stimulate locomotor activity after injection into the substantia innominata/lateral preoptic area. *Pharmacology Biochemistry and Behavior,* 34:101-106.

Stanley, B.G., Ha, L.H., Spears, L.C., Dee, M.G. II (1993) Lateral hypothalamic injections of glutamate, kainic acid, D, L-α-amino-3-hydroxy-5-methyl-isoxazole propionic acid or N-methyl-D-aspartic acid rapidly elicit intense transient eating in rats. *Brain Research,* 613:88-95.

Wolosker, H., Blackshaw, S., Snyder, S.H. (1999) Serine racemase: a glial enzyme synthesizing D-serine to regulate glutamate-N-methyl-D-aspartate neurotransmission. *Proceedings of the National Academy of Sciences of the United States of America,* 96:13409-13414.

Yamane, H., Tsuneyoshi, Y., Denbow, D.M., Furuse, M. (2009a) N-Methyl-D-aspartate and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate receptors involved in the induction of sedative effects under an acute stress in neonatal chicks. *Amino Acids,* 37:733-739.Erratum, 37:767.

Yamane, H., Asechi, M., Tsuneyoshi, Y., Kurauchi, I., Denbow, D.M., Furuse, M. (2009b) Intracerebroventricular injection of L-aspartic acid and L-asparagine induces sedative effects under an acute stressful condition in neonatal chicks. *Animal Science Journal,* 80:286-290.

Yamane, H., Asechi, M., Tsuneyoshi, Y., Denbow, D.M., Furuse, M. (2009c) Central L-cysteine induces sleep, and D-cysteine induces sleep and abnormal behavior during acute stress in neonatal chicks. *Animal Science Journal,* 80:428-432.

Zelena, D., Mergl, Z., Makara, G.B. (2005) Glutamate agonists activate the hypothalamic-pituitary-adrenal axis through hypothalamic paraventricular nucleus but not through vasopressinerg neurons. *Brain Research,* 1031:185-193.

Zhang, R., Tachibana, T., Takagi, T., Koutoku, T., Denbow, D.M., Furuse, M. (2004) Serotonin modifies corticotropin-releasing factor-induced behaviors of chicks. Behavioural Brain Research, 151:47-52.

Ziegler, D.R., Cullinan, W.E., Herman, J.P. (2005) Organization and regulation of paraventricular nucleus glutamate signaling systems: N-methyl-D-aspartate receptors. *Journal of Comparative Neurology*, 484:43-56.