

《Research Note》

Intracerebroventricular Injection of L-Pipecolic Acid Exerts Hypnotic Effects Without Activating NMDA Receptors in Neonatal Chicks under Social Isolation-induced Stress

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L-Pipecolic acid is an intermediate of L-lysine catabolism. Its central injection exerted a hypnotic effect on the brain, which was partially mediated by the activation of γ -aminobutyric acid-A and γ -aminobutyric acid-B receptors. L-Proline has also been shown to exert a similar effect on N-methyl-D-aspartate receptors. Furthermore, L-pipecolic acid is known as L-homoproline, and both L-pipecolic acid and L-proline belong to the imino acid group; therefore, it is plausible that they share certain commonalities, including similar functions. However, the role of N-methyl-D-aspartate receptors with respect to the effects of L-pipecolic acid has not been examined yet. In the present study, the relationship between N-methyl-D-aspartate receptors and the central function of L-pipecolic acid was investigated in neonatal chicks. The behavioral postures for active wakefulness and standing/sitting motionless with eyes opened were significantly affected after intracerebroventricular injection of L-pipecolic acid; whereas, sitting motionless with head drooped (sleeping posture) was significantly enhanced. However, the N-methyl-D-aspartate receptor antagonist, MK-801, did not affect these changes. In conclusion, the central administration of L-pipecolic acid did not exert hypnotic effects through the activation of N-methyl-D-aspartate receptors in neonatal chicks. These results suggest that the imino group is not a determinant for activating N-methyl-D-aspartate receptors.

Key words: brain, chicks, hypnotic effect, L-pipecolic acid, NMDA receptor

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Introduction

Several environmental and artificial factors act as stressors to decrease the quality and quantity of domestic animals reared for economic purposes; therefore, it is important to understand the regulation of stressors and the stress responses of these animals. Chicks feel comfortable when reared in crowds, but experience acute stress demonstrated by an increase in spontaneous activity and distress vocalization when isolated (Saito *et al.*, 2004). Such behavioral changes have been used to investigate the effects of several drugs (Feltenstein *et al.*, 2003, 2004).

Amino acids act not only as components of proteins, but also as regulators of various physiological functions. Several studies have investigated the ability of amino acids, including L-arginine (Suenaga *et al.*, 2008a), L-ornithine (Suenaga *et al.*, 2008b), L-aspartic acid (Yamane *et al.*, 2009), and L-serine (Asechi *et al.*, 2006) to regulate stress response behaviors, and each of them exerted sedative and/or associated effects in neonatal chicks under social isolation-induced stress. These effects of amino acids and their metabolites were recently reviewed (Furuse, 2015) and they were found to be similar. For instance, L-lysine (L-Lys) is metabolized through different pathways between central and peripheral tissues (Fig. 1), with L-saccharopine being the major metabolite of L-Lys in the peripheral tissue (Hutzler and Dancis, 1968) and L-pipecolic acid (L-PA) in the brain (Schmidt-Glenewinkel *et al.*, 1977; Nomura *et al.*, 1978; Giacobini *et al.*, 1980). L-PA is known to exert a hypnotic effect (Takagi *et al.*, 2001). According to Takagi *et al.* (2003), L-PA partially activates γ -aminobutyric acid (GABA)-A and GABA-B receptors, both of which contribute to sleep-like behavior.

Similarly, L-proline (L-Pro) has been shown to exert a

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hypnotic effect on neonatal chicks under social isolation-induced stress (Hamasu *et al.*, 2009). However, this effect could be mediated by the activation of N-methyl-D-aspartate (NMDA) receptors because the NMDA receptor antagonist MK-801 attenuated the effect of L-Pro to the level of that in the control group (Hamasu *et al.*, 2010). Furthermore, L-PA is also known as L-homoproline, and both L-PA and L-Pro belong to the imino acid group, as shown in Fig. 2. Therefore, it is plausible that they share certain commonalities, including similar functions. However, the role of NMDA receptors with respect to the effects of L-PA has not been examined yet.

In the present study, we investigated whether NMDA receptors contribute to the central function of L-PA, and whether the imino acid group is a determinant of the activation of NMDA receptors in neonatal chicks.

Materials and Methods

Animals and Feeding

One-day-old male layer chicks (Julia) were purchased from a local hatchery (Murata Hatchery, Fukuoka, Japan), and were housed in a room at a temperature of $30 \pm 1^\circ\text{C}$. The chicks were fed on a commercial starter diet (AX, Toyohashi

Feed and Mills Co Ltd., Aichi, Japan) and water, and were reared with feeding until the penultimate day of the experiment for acclimatization (approximately 20 chicks per cage). Five-day-old chicks were used for the experiment. All experimental procedures used in this study were performed under the guideline 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 Government.

Preparation of Drugs

L-PA and MK-801 were purchased from Wako Pure Chemical Industries (Osaka, Japan) and were dissolved in 0.85% saline containing 0.1% Evans Blue solution. The suspension was stirred using a vortex mixer and sonicated using a bath sonicator for 10 min.

Experimental Procedure

Intracerebroventricular (i.c.v.) injections, each of $10\ \mu\text{l}$ volume, were performed using a microsyringe according to the method described by Davis *et al.* (1979) and Koutoku *et al.* (2005). The stress and pain suffered by this method is minimal as described by Koutoku *et al.* (2005).

The chicks were administered i.c.v. injections of saline only, L-PA ($1.0\ \text{mg}/10\ \mu\text{l}$), and MK-801 ($0.5\ \text{nmol}/10\ \mu\text{l}$), or

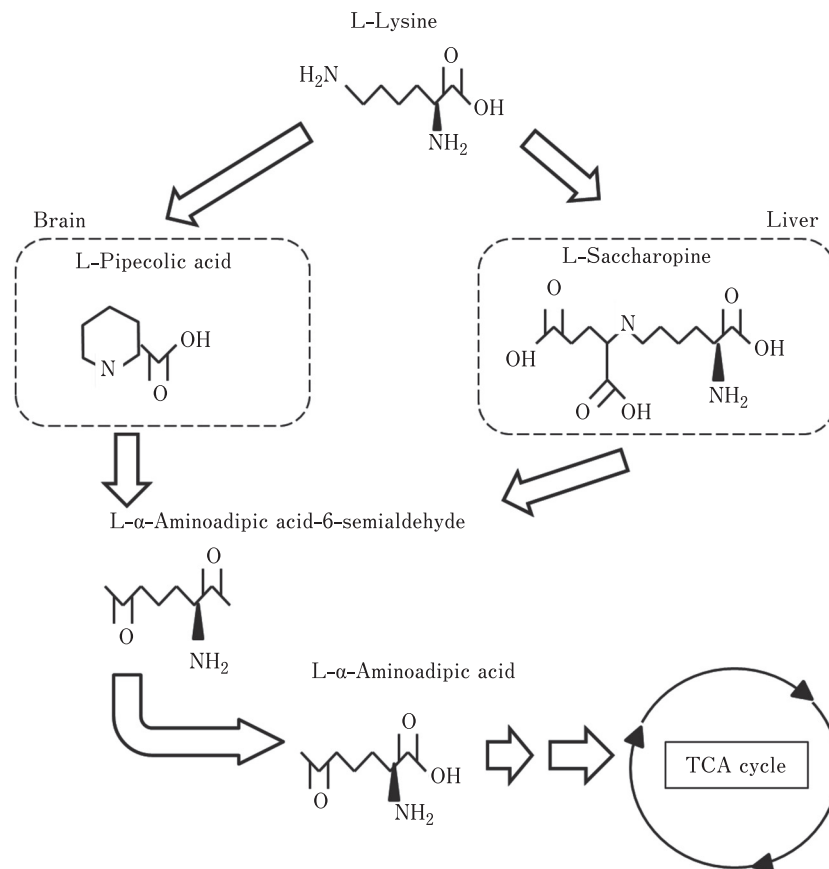


Fig. 1. L-Lysine metabolic pathways in central (L-pipecolic pathway in the brain) and peripheral tissues (L-saccharopine pathway in the liver).

co-injection of both L-PA and MK-801. Thereafter, they were immediately placed individually into an acrylic monitoring cage (40 cm×30 cm×20 cm) to monitor their behavior over a period of 10 min using monitoring systems that were set up in a separate room to avoid disturbing the animals. During this period, the chicks were deprived of food and water, and three video cameras in different positions were used to record their behavior on digital versatile discs. Based on the method described by van Luijelaar *et al.* (1987), chick 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. At the conclusion of this experiment, the chicks were euthanized following anesthesia with isoflurane (Mylan Inc., Japan), following which their brains were removed and the localization of the Evans Blue dye was confirmed. The data of chicks without dye in their lateral ventricles were excluded from the study.

Statistical Analysis

Based on the results of the Bartlett test, square root transformation of some behavioral parameters was performed to obtain an approximation of a normal distribution before analysis. Data were statistically analyzed by two-way analysis of variance (ANOVA), using a statistical program Statview Version 5.0 (SAS Institute, Cary, USA, 1998). Values

are presented as the mean±S.E.M.

Results and Discussion

Table 1 shows the effects of i.c.v. injections of L-PA and MK-801 on various behavioral parameters of the five-day-old layer chicks exposed to social isolation stress for 10 min. Both active wakefulness ($F(1, 26)=5.977, P<0.05$) and standing/sitting motionless with eyes opened ($F(1, 26)=0.652, P<0.05$) were significantly decreased after i.c.v. injection of L-PA. Furthermore, sitting motionless with head drooped (sleeping posture) was significantly increased ($F(1, 26)=11.459, P<0.01$) after i.c.v. injection of L-PA. No significant effects of MK-801, as well as of interactions between L-PA and MK-801 were detected for any of the behaviors.

L-PA partially activates GABA-A and GABA-B receptors (Takagi *et al.*, 2003), and L-Pro exerts a hypnotic effect on neonatal chicks under social isolation-induced stress (Hamasu *et al.*, 2009) similar to that exerted by L-PA. L-Pro is an imino acid that is presumed to induce the activation of NMDA receptors, because this activity of L-Pro is strongly attenuated by the NMDA receptor antagonist MK-801 (Hamasu *et al.*, 2010). Therefore, to reveal whether central administration of L-PA exerts sedative and hypnotic effects through NMDA receptors, both L-PA and MK-801 were i.c.v. administered. Only L-PA injected in chicks exerted hypnotic effects in a manner similar to that reported in previous studies. However, the sleeping posture induced by L-PA injection was hardly attenuated by the co-injection of L-PA and MK-801. Based on these results, it appears that the hypnotic effects exerted by L-PA might not be mediated through NMDA receptors, as has been observed with L-Pro.

Some amino acids exert sedative and hypnotic effects through different mechanisms. For instance, D-Pro exerts the same effect as L-Pro; however, D-Pro mediates its function through glycine receptors, not NMDA receptors (Hamasu *et al.*, 2010). Specifically, the central amino acids function using several different mechanisms with GABA, NMDA, and glycine receptors to exert hypnotic effect on the brain of animals. The involvement of NMDA receptors was not con-

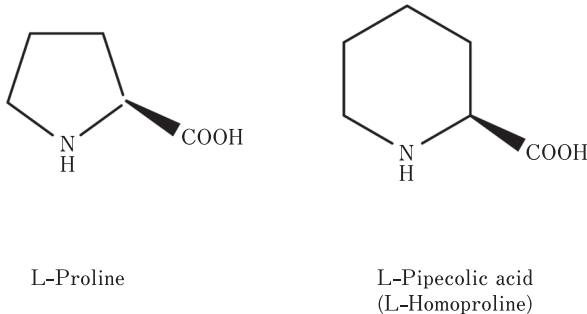


Fig. 2. Structures of L-proline and L-pipecolic acid (L-homoproline).

Table 1. Effects of i.c.v. injection of L-pipecolic acid and MK-801 on various behavioral parameters of 5-day-old layer chicks subjected to social separation for 10 min

| L-PA (mg/10 μ l) | 0 | 0 | 1.0 | 1.0 | Results of ANOVA | | |
|---|--------|--------|--------|--------|------------------|----------|-------------|
| MK-801 (nmol/10 μ l) | 0 | 0.5 | 0 | 0.5 | L-PA | MK-801 | Interaction |
| Active wakefulness | 126±71 | 96±62 | 0 | 20±20 | $P<0.05$ | $P>0.05$ | $P>0.05$ |
| Standing/sitting motionless with eyes open | 215±39 | 257±46 | 133±18 | 161±37 | $P<0.05$ | $P>0.05$ | $P>0.05$ |
| Standing motionless with eyes closed | 16±11 | 0 | 0 | 1±1 | $P>0.05$ | $P>0.05$ | $P>0.05$ |
| Sitting motionless with head drooped (sleeping posture) | 243±60 | 246±62 | 467±18 | 417±55 | $P<0.01$ | $P>0.05$ | $P>0.05$ |
| Total | 600 | 600 | 600 | 600 | | | |

Values are means±S.E.M. in seconds.

The number of chicks used in each group was as follows: vehicle, 9; L-PA, 7; MK-801, 7; and L-PA + MK-801, 7.

sidered with respect to the function of L-PA in the present study; however, other receptors, such as the glycine receptor, may affect the function of L-PA simultaneously with GABA receptors. Further studies would be required to determine the accurate mechanism.

In conclusion, central L-PA did not exert a hypnotic effect by activating NMDA receptors in neonatal chicks. Moreover, our results suggest that the imino acid group is not a determinant of NMDA receptor activation.

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