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Intracerebroventricular Injection of L-Proline Modifies Food Intake in Neonatal Chicks

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Abstract: Although, the central function of amino acids on food intake has been investigated, little information is available on the role of the amino acid L-proline. To clarify the central effect, several doses (0, 0.25, 0.5 and 1.0 mg) of L-proline were intracerebroventricularly (i.c.v.) injected into chicks under fasting (3 h) or ad libitum feeding conditions. Food intake was determined through 60 min post injection. Under fasting conditions, the following regression equation was obtained: food intake (g) = 3.047 + 3.496x – 5.332 x^2 (x in mg of L-proline, R^2 = 0.466, RMS = 1.056). Similarly, the regression equation was obtained under ad libitum conditions as follows: food intake (g) = 0.479 (SE 0.164) + 2.130 (SE 0.815)x – 2.452 (0.747)x^2 (R^2 = 0.313, RMS = 0.487). These results indicated that food intake was mildly stimulated by low levels of L-proline, but was suppressed by high levels in chicks. It is suggested that L-proline may act in the central nervous system to differentially regulate food intake, depending upon dose.

Key words: L-Proline, food intake, feeding condition, intracerebroventricular injection, neonatal chick

INTRODUCTION

More than 20 amino acids serve as the molecular building blocks of proteins. According to one accepted classification, some amino acids are termed essential amino acids (sometimes called indispensable), meaning that they must be supplied via the diet. Nine amino acids (histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine) are generally regarded as essential for humans. The others, which used to be classified as nonessential, are now more correctly termed dispensable amino acids (L-alanine, L-aspartic acid, L-cystine, L-glutamic acid and L-serine) or conditionally indispensable (L-arginine, L-cysteine, glycine and L-tyrosine), meaning they are not normally required in the diet, but must be supplied exogenously to specific populations that do not synthesize it in adequate amounts. The central function of both dispensable and indispensable amino acids on behavior has been clarified. Those amino acids that can activate the glycine receptor including glycine (Asechi *et al*., 2006), β-alanine (Tomonaga *et al*., 2004), L-alanine (Kurauchi *et al*., 2006) and L-serine (Koutoku *et al*., 2005; Asechi *et al*., 2006) have been shown to have sedative and/or hypnotic effects. However, information regarding L-proline, activating the glycine receptor, is limited.

L-Proline also has an aliphatic side chain, but it differs from other amino acids in that its side chain is bonded to both nitrogen and á-carbon atoms. Therefore, L-proline is actually an imino acid. L-Pipecolic acid, a major metabolic intermediate of L-lysine, is also an imine and has a unique function in the brain for the regulation of food intake. Under fasting conditions, central L-pipecolic acid is an anorexigenic factor (Takagi *et al*., 2001, 2003a). However, it has the opposite effect under ad libitum feeding conditions in which brain injections of L-pipecolic acid act as an orexigenic factor (Takagi *et al*., 2003b).

In the present study, the central effects of L-proline on food intake were investigated under both fasting and ad libitum feeding conditions in neonatal chicks.

MATERIALS AND METHODS

One-day-old male layer chicks 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

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continuously for 24 h. The birds were given free access to a commercial starter diet (Toyohashi Feed and Mills Co. Ltd., Aichi, Japan) and water. Chicks were housed individually beginning one day before the start of the experiment. On the experimental day, chicks were distributed into 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.

L-Proline, a gift from Kyowa Hakko Kogyo (Tokyo, Japan), was dissolved in 0.85 % saline containing 0.1 % Evans Blue solution and intracerebroventricularly (i.c.v.) injected into the lateral ventricle of chicks using a microcyringe according to the method of Davis *et al.* (1979). Control groups were given the saline solution containing Evans Blue. At the end of the experiment, the birds were sacrificed by an overdose of sodium pentobarbital and the site of injection verified. Data were deleted from individuals in which the presence of Evans Blue dye in the lateral ventricle was not verified.

Six-day-old layer chicks were fasted for 3 h but given free access to water in Experiment 1. The birds were injected i.c.v. with either 0.25, 0.5 or 1.0 mg 10^{-1} μL of L-proline or saline and then given the diet. Food intake was measured for 60 min after injection. In Experiments 2, similar trials were done without fasting.

Regression equations were fitted to the data using a commercially available package StatView (version 5, SAS Institute, Cary, USA, 1998). The results are presented as means±SEM.

RESULTS AND DISCUSSION

Figure 1 shows the effect of i.c.v. injection of L-proline on food intake of chicks after food deprivation for 3 h. The following regression equation was obtained: food intake (g) = 3.047 (SE 0.339) + 3.496 (SE 1.765)x – 5.332 (1.654)x^2 (x in mg of L-proline, R^2 = 0.466, RMS = 1.056). Figure 2 shows the effect of i.c.v. injection of L-proline on food intake of chicks under an ad libitum feeding condition. The regression equation obtained was: food intake (g) = 0.479 (SE 0.164) + 2.130 (SE 0.815)x – 2.452 (0.747)x^2 (R^2 = 0.313, RMS = 0.487).

According to Davis *et al.* (1979), the i.c.v. injection of C^{14}-1abeled L-proline in neonatal chicks resulted in its distribution in periventricular tissue including the septum, hippocampus and hypothalamus 1 min after injection. This fact suggests that i.c.v. injected L-proline quickly distributes within the brain.

Under fasting conditions, food intake was mildly increased and then decreased severely in a dose dependent manner. The most efficacious dose of L-proline under fasting conditions was calculated as 0.12 mg from the curve. In contrast, under ad libitum conditions the most efficacious dose was 0.35 mg. These facts imply that the addition of small amounts of L-proline to the diet may improve food intake. On the other hand, L-proline does not cross the blood-brain barrier readily (Davis *et al.*, 1979), therefore, the dietary effect of L-proline on food intake remains to be determined.

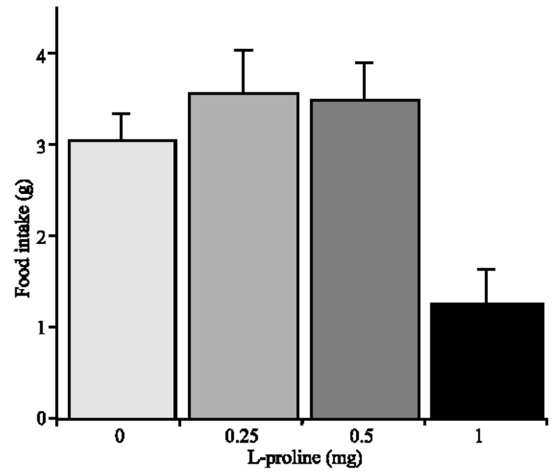

Fig. 1: Effect of i.c.v. injection of L-proline on food intake of chicks under a fasting condition. The number of chicks in each group was as follows: 0 mg, 9; 0.25 mg, 8; 0.5 mg, 8; and 1.0 mg, eight. Data are expressed as means±S.E.M

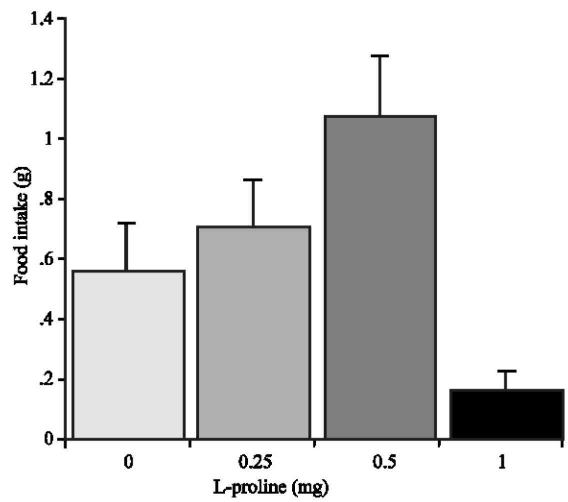

Fig. 2: Effect of i.c.v. injection of L-proline on food intake of chicks under an ad libitum feeding condition. The number of chicks in each group was as follows: 0 mg, 7; 0.25 mg, 9; 0.5 mg, 9; and 1.0 mg, 9. Data are expressed as means±S.E.M

The present data suggest that a large amount of L-proline in the brain is toxic to chicks. However, this response in ad libitum feeding conditions was different from the effect of L-pipecolic acid, since central administration of L-pipecolic acid enhanced food intake at i.c.v. levels of 0.5 and 1 mg (Takagi *et al*., 2003b). The response under fasting conditions was similar between L-proline and L-pipecolic acid (Takagi *et al*., 2001, 2003a) in that 1 mg of both L-proline in the present study and L-pipecolic acid (Takagi *et al*., 2001, 2003a) severely suppressed food intake.

While we hypothesized that imines such as L-proline and L-pipecolic acid would have a similar function on feeding, it appears that their functions are different depending on the feeding conditions. The difference may be explained by the following reason. Takagi *et al*. (2003a, b) revealed that L-pipecolic acid worked through γ-amino Butyric Acid (GABA) receptors whereas L-proline binds to glycine receptor as well as glutamate receptors (Henzi *et al*., 1992). The difference in the receptor may influence food intake. On the hand, further functions of L-proline on behaviors should be studied since L-pipecolic acid induced sleep-like behavior (Takagi *et al*., 2001).

CONCLUSION

L-proline can act in the chick brain to modulate food intake, its effect being dependent on the dose.

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