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# The Dipeptide Lys-Pro Attenuates Interleukin-1β–Induced Anorexia

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UEHARA, Y., H. SHIMIZU, N. SATO, Y. SHIMO MURA AND M. MORI. *The dipeptide Lys-Pro attenuates interleukin-1β-induced anorexia.* PEPTIDES 14(2) 175–178, 1993.—The carboxyl-terminal tripeptide of α-MSH(1–13), Lys-Pro-Val, antagonizes anorexia induced by interleukin-1β (IL-1). The present studies were undertaken to determine if the Lys-Pro dipeptide portion of this tripeptide likewise antagonizes anorexia induced by ICV administration of 0.5 pmol IL-1 in rats previously deprived of food. This dose of Lys-Pro did significantly attenuate the IL-1–induced anorexia, but only for 1 h after infusion. Simultaneous administration of a larger dose (5.0 pmol) of Lys-Pro reversed the IL-1–induced anorexia during both the 0–1-h and 2–4-h periods. In addition, both 0.5 and 5.0 pmol of the D-substitute tripeptide, Lys-D-Pro-Thr (LDPT), similarly attenuated the IL-1–induced anorexia. The ICV administration of 5.0 pmol Lys-Pro also did not have any significant effects on food consumption. These results indicate that the dipeptide Lys-Pro may have a short-term antagonistic action against the anorexia induced by IL-1, and it is possible that this action may be partially mediated by the blockade of IL-1 on its own receptor.

Interleukin  Lysine  Proline  Feeding behavior

INTERLEUKIN-1β (IL-1) is thought to play an important role in the febrile response and in the anorexia of severe infection (1,14). The concentration of circulating α-MSH is increased by intracerebroventricular (ICV) administration of crude IL-1 and of endotoxin (5). α-MSH antagonizes the febrile response and anorexia induced by IL-1 (3,5,15,17). The carboxyl-terminal tripeptide of α-MSH [α-MSH(11–13)] antagonizes the effects of endogenous pyrogen and IL-1 on body temperature and food intake (13,15). α-MSH(11–13) is believed to be the primary message sequence responsible for the antipyretic and anti-inflammatory activities of α-MSH(1–13) (8).

In addition, Mugridge et al. (11) demonstrated that α-MSH(11–13) potently and selectively reduced [$^{125}$I]-IL-1 binding to the IL-1 type I (80 kDa) receptor-binding, T-cell subclone EL4-6.1. Therefore, it appears that α-MSH(11–13) exerts its antagonistic effects through blocking IL-1 binding to the type I receptor.

The amino acid sequence of IL-1 was recently obtained from cDNA (9). Furthermore, Mizuno et al. demonstrated an important binding site of IL-1 (positions 82 to 102 of the mature protein, consisting of 153 amino acids) by using monoclonal antibodies (10). Since two regions of Lys-Pro sequence were found in the presumed binding site, this dipeptide may play a role in the binding of IL-1 to its own receptor. In addition, these dipeptides are also found in the carboxyl-terminal tripeptide of α-MSH(1–13), a peptide that is known to antagonize some actions of IL-1. We believe that Lys-Pro may antagonize the actions of IL-1 by the blockade of IL-1 binding to its receptors.

The present studies were undertaken to examine the hypothesis that the dipeptide Lys-Pro may antagonize the anorexia caused by ICV injection of IL-1 in rats deprived of food. In addition, the effects of a putative antagonist of IL-1, 193–195 analogue Lys-D-Pro-Thr (2), were also investigated in fasted animals.

## METHOD

### *Animals*

Thirty male Wistar rats were obtained from Imai Animal Laboratory (Saitama, Japan). The animals were individually housed in a temperature-controlled room (23 ± 1°C) with 10-h dark/14-h light cycle (illumination from 0500 to 1900). The animals were adapted to powdered Purina laboratory chow (Oriental, Osaka, Japan) for at least 1 week before infusion. The feeding test was repeated two or three times in each animal. The animals were used after postinfusion recovery for 7 days.

### *Chemicals*

Recombinant human interleukin-1β (IL-1) was kindly provided by Dr. Y. Hirai (Otsuka Pharmaceuticals, Ltd., Tokushima, Japan). The biological activity of IL-1 was estimated by

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the mouse thymocyte [$^3$H]-thymidine incorporation assay (lymphocyte activating factor activity), as $2 \times 10^7$ half-maximal units/mg protein (6). The material has been judged to exhibit a purity of at least 99%, based on analysis by high performance liquid chromatography and polyacrylamide gel electrophresis. The IL-1 contains the identical amino acid sequence predicted by the cDNA sequence (12).

Lys-Pro and Lys-D-Pro-Thr (LDPT) were obtained from Novabiochem (Switzerland) and Peninsula Laboratories, Inc. (Belmont, CA), respectively. These peptides were dissolved in physiological saline.

*Cannula Implantation*

Under pentobarbital anesthesia (40 mg/kg, IP), a guide cannula of 23-gauge stainless steel tubing was stereotaxically implanted into the third ventricle 1 week before the infusion. The coordinates were chosen from the atlas of König and Klippel (7): AP +2.5 mm with respect to the bregma; midline; −10.0 mm from the surface of the skull. The cannula was fixed to the skull with stainless steel screws and dental cement.

*Microinfusion Technique*

The perfusion was conducted in the early morning. A 29-gauge stainless steel cannula connected via a long polyethylene tube to a 25-μl Hamilton microsyringe was inserted into the guide cannula. From our previous studies, 0.5 pmol of IL-1 inhibits food consumption in rats previously deprived of food (14), and this dose was used in the following experiments. Peptides, alone or combined with IL-1, were infused into the third ventricle over a few minutes in rats previously deprived of food for 18 h. The injection cannula was kept in the guide cannula for at least 3 min to prevent reflux of chemicals. After microinfusion, the animals were immediately returned to their own cages, and changes in food consumption over times were measured to the nearest 0.1 g.

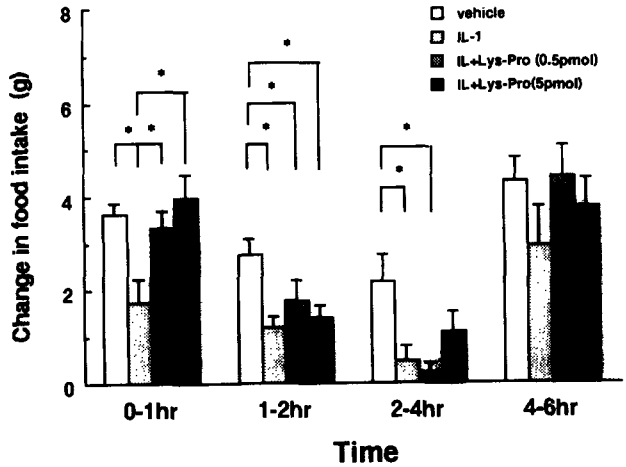


FIG. 1. The antagonistic effects of intraventricularly administered 0.5 and 5.0 pmol Lys-Pro on interleukin-1β (IL-1)–induced anorexia in the rats previously deprived of food for 18 h. $n = 6$ (control), 5 (IL-1), 5 (0.5 pmol Lys-Pro), 5 (5.0 pmol Lys-Pro). A (treatment) = 11.425, $p < 0.01$, B (time) = 20.248, $p < 0.01$, A × B (interaction) = 1.432, NS. Asterisks show significant ($p < 0.05$) differences between two groups.

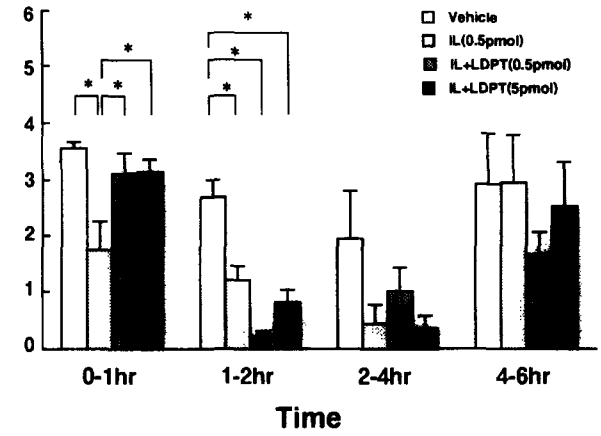


FIG. 2. The effects of intraventricularly administered 0.5 and 5.0 pmol Lys-D-Pro-Thr (LDPT) on interleukin-1β–induced anorexia in the rats previously deprived of food for 18 h. $n = 6$ (control), 5 (IL-1), 5 (0.5 pmol LDPT), 5 (5.0 pmol LDPT). A (treatment) = 8.208, $p < 0.01$, B (time) = 10.017, $p < 0.01$, A × B (interaction) = 1.368, NS. Asterisks show significant ($p < 0.05$) differences between two groups.

*Statistics*

All data were expressed as mean ± SE. The statistical analysis was performed using a two-way analysis of variance with one repeated measurement. Newman–Keuls test was used for the individual comparison of the means.

RESULTS

*Experiment 1: Effects of Lys-Pro on IL-1–Induced Anorexia*

As shown in Fig. 1, IL-1 inhibits feeding during the 4-h period after infusion. Simultaneous administration of 0.5 pmol Lys-Pro attenuated the reduction of food consumption during the first hour after IL-1 infusion. However, anorexia caused by IL-1 was still observed at the 1–2- and 2–4-h periods in 0.5 pmol Lys-Pro–treated rats. Lys-Pro at 5.0 pmol partially reversed the IL-1–induced anorexia in the 0–1- and 2–4-h periods, although the reduced food intake was not affected during the 1–2-h period. Infusion of IL-1 inhibited body weight recovery after starvation for 18 h [control group ($n = 6$): 21.7 ± 1.7 g, IL-1–treated group ($n = 5$): 5.2 ± 5.9 g; $p < 0.01$]. But simultaneous administration of Lys-Pro significantly ($p < 0.05$) attenuated the IL-1–induced inhibition of body weight gain [0.5 pmol Lys-Pro–treated group ($n = 6$): 15.8 ± 2.9 g, 5.0 pmol Lys-Pro–treated group ($n = 6$): 14.8 ± 1.4 g].

*Experiment 2: Effects of LDPT on IL-1–Induced Anorexia*

During the 0–1-h period, LDPT reduced the IL-1 effects on feeding after doses of both 0.5 and 5.0 pmol (Fig. 2). However, these doses of LDPT did not attenuate the reduction of food consumption by IL-1 at the 1–2- and 2–4-h periods.

The IL-1–induced reduction of body weight recovery after starvation [control group ($n = 6$): 20.1 ± 1.0 g, IL-1–treated group ($n = 5$): 5.2 ± 5.9 g; $p < 0.01$] was not decreased by simultaneous administration of LDPT [0.5 pmol LDPT-treated group ($n = 6$): 5.1 ± 1.9 g, 5.0 pmol LDPT-treated group ($n = 6$): 5.4 ± 2.3 g].

*Experiment 3: Effects of ICV Lys-Pro on Feeding Behavior*

The ICV injection of Lys-Pro did not significantly change food consumption at any time (Fig. 3). There was no difference

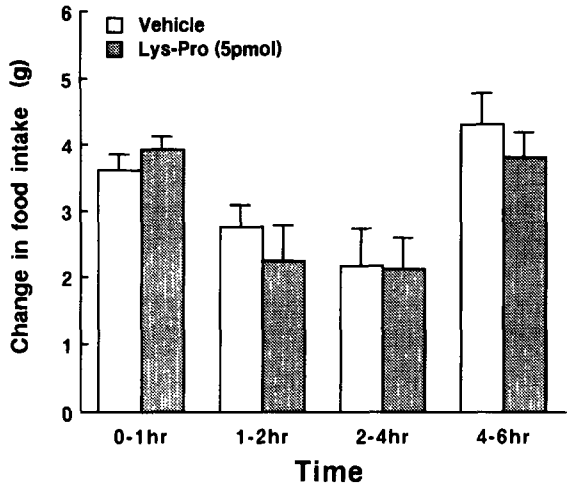


FIG. 3. The effects of intraventricularly administered Lys-Pro on food consumption in the rats previously deprived of food for 18 h. $n = 6$ (control), 5 (Lys-Pro). A (treatment) = 3.476, NS, B (time) = 19.078, $p < 0.01$, A × B (interaction) = 0.275, NS. There was no significant difference between vehicle- and Lys-Pro–treated groups.

in body weight recovery between groups [control group ($n = 6$): 21.7 ± 1.7 g, 5.0 pmol Lys-Pro–treated group ($n = 5$): 22.6 ± 3.2 g; $p < 0.05$].

## DISCUSSION

The present studies demonstrate that simultaneous administration of Lys-Pro reduces acute anorexia caused by IL-1. In addition, LDPT similarly temporarily attenuated IL-1–induced anorexia at the same picomolar doses. However, ICV administration of Lys-Pro alone did not have any effects in the rats deprived of food.

Both Lys-Pro and LDPT delayed the appearance of the IL-1 effects on feeding, and there was no difference between Lys-Pro and LDPT in the antagonistic effects on IL-1–induced anorexia. However, a difference in body weight recovery was observed with these peptides. L-Pro[12] of α-MSH(1–13) has been reported to be essential to its anti-inflammatory effect on picryl chloride-induced edema (4). The present results indicate that both L-Pro (Lys-Pro) and D-Pro (LDPT) can similarly antagonize the IL-1–induced anorexia. Therefore, Pro must be essential to the binding of IL-1 to its receptor and subsequent influence on feeding behavior. However, further studies that prove Lys-Pro does not inhibit cholecystokinin and/or corticotropin-releasing factor should be necessary for demonstrating specificity of this dipeptide. Since a difference in body weight gain existed between animals treated with two peptides, the antagonistic effects on a factor that affects body weight recovery may differ with those peptides.

The antagonistic action of α-MSH is thought to be mediated by an IL-1 type I receptor (5,11). Lys-Pro exists in the presumed binding portion of IL-1 (9,19), and the antagonistic peptide, α-MSH, also has the dipeptide in its carboxyl terminal. The carboxyl-terminal tripeptide that contains Lys-Pro appears to have the antagonistic effects on IL-1 actions that are similar to those exerted by the parent α-MSH(1–13) molecule (15). We hypothesize that the IL-1 effects on feeding are mediated by type I receptors, and the carboxyl-terminal tripeptide of α-MSH inhibits the IL-1 binding to its type I IL-1 receptor. The fact that Lys-Pro and LDPT both antagonize the anorexia caused by IL-1 supports our hypothesis.

Furthermore, the antagonistic action of these two peptides appears to be relatively short, perhaps 1 h. From the present results, dipeptide Lys-Pro can temporarily antagonize the IL-1 binding, but the binding capacity of these peptides appears to be structurally unstable, since the IL-1 effects on feeding behavior became obvious from 1 h after the infusion.

We have recently demonstrated that large dose of LDPT temporarily stimulate food consumption (16). The ICV injection of 5.0 pmol α-MSH alone inhibited feeding behavior, but α-MSH(11–13) alone did not (15). The present study added a finding that picomolar amounts of Lys-Pro alone do not modulate feeding behavior in rats. Small peptides such as α-MSH(11–13), LDPT, and Lys-Pro can only antagonize the anorexic effects of IL-1 at picomolar doses.

In conclusion, the present experiments indicate that dipeptide Lys-Pro has antagonistic actions on the anorexia caused by ICV injection of IL-1. The antagonistic action of Lys-Pro may be mediated by the blockade of IL-1 binding to its own receptor.

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