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Intraventricular Administration of Cyclo(His-Pro), a Metabolite of Thyrotropin-Releasing Hormone (TRH), Decreases Water Intake in the Rat[1] (42052)

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Abstract. We explored the effect of cyclo(His-Pro) on drinking in water-deprived rats. Intraventricular administration of cyclo(His-Pro) significantly reduced water intake in these rats in a dose-dependent fashion (3×10^{-10}–3×10^{-8} mole), whereas its intraperitoneal injection did not cause any effect on water intake. The inhibitory potency of cyclo(His-Pro) was found to be similar to that of thyrotropin-releasing hormone (TRH), but cyclo(His-Pro) did not significantly interfere with TRH binding in the rat brain. The data indicate that cyclo(His-Pro) action was not mediated through the TRH counterpart. These results suggest that cyclo(His-Pro) may play a potential role in the regulation of water intake in the rat brain.

Thyrotropin-releasing hormone (TRH) is composed of three amino acids, pyroglutamyl-histidyl-proline amide, and undergoes proteolytic cleavage by pyroglutamate aminopeptidase to produce histidyl-proline amide that is unstable and converted rapidly to histidyl-proline diketopiperazine [cyclo(His-Pro)] (1). Cyclo(His-Pro) is known to be distributed ubiquitously throughout the brain in the rat, mouse, monkey, and human (2–4). A substantial body of evidence has accumulated to categorize cyclo(His-Pro) as a neuroactive substance in the central nervous system (CNS) (5–7), which may be a neurotransmitter or neuromodulator. However, in spite of its numerous and evident biological activities, the physiological significance of cyclo(His-Pro) still remains to be elucidated.

In recent years, TRH has been shown to possess an antidipsogenic action as well as a satiety activity when injected intraventricularly (8–10). In conjunction with evidence that cyclo(His-Pro) shares partly with TRH the same spectrum of biological activities, including suppression of feeding (6, 7), a concept that TRH may produce its neurophysiological actions via the formation of an active metabolite in the CNS (11, 12) had led us to the possibility that cyclo(His-Pro) may also affect water intake in the rat. Data herein, showing that cyclo(His-Pro) exhibited a potent inhibitory action for water intake in the rat brain, form the basis of the present report.

Materials and Methods. Adult male rats of Wistar strain, each weighing 300–350 g, were fed a Purina laboratory chow diet and given tap water to drink. Animals were individually caged for longer than 1 week before experiments in a temperature-controlled room (22 ± 1°C) with light going on at 6:30 AM and off at 6:30 PM. All experiments were done between 8:00 and 11:00 AM. TRH and cyclo(His-Pro) were supplied by Tanabe Pharmaceutical Company, Osaka, Japan, and dissolved in saline.

Experiment 1. Under sodium pentobarbital anesthesia, stainless-steel cannulae were implanted stereotaxically in the lateral ventricles according to the atlas of Paxinos and Watson (13) (posterior = 0.8 mm behind bregma; lateral = 1.5 mm from the mid line; vertical = 3.0 mm from the skull surface). All animals in the single-injection experiments were allowed at least 5 days to recover from surgery before testing. The animals were deprived of water for 48 hr, but allowed free access to food. The cannula insert was connected to a 10-μl Hamilton microsyringe by a PE 10 polyethylene tube. Five microliters of cyclo(His-Pro) or TRH in each of the doses of

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3×10^{-11}–3×10^{-8} mole was flowed in by gravity without anesthesia. As the control group, 5 μl of saline was injected in the same manner. After injection, the animals were placed in their own cages without food and given a preweighed bottle containing tap water. Water intake was determined using a balance (Shimadzu Model LU-T 160 D, Tokyo, Japan) that can accurately detect 0.01 g, and was expressed as milliliter per 100 g body wt.

Experiment 2. The rats were exposed to water deprivation for 48 hr as mentioned above, and given an intraperitoneal injection of cyclo(His-Pro) or TRH in each of the doses of 3×10^{-7}–3×10^{-6} mole/kg body wt. After injection, water intake was measured in the same manner.

Experiment 3. TRH binding study was performed by the method of Burt and Snyder (14). The whole brains excluding cerebellum were homogenized in 10 vol of 0.32 *M* sucrose and centrifuged at 1000*g* for 10 min. The supernatants were centrifuged at 27,000*g* for 20 min and the resulting precipitates were resuspended in 50 m*M* Tris–HCl buffer, pH 7.4, containing 0.1% bovine serum albumin (THB buffer). A reaction mixture consisting of 100 μl of 2 pmole [^{3}H]TRH (specific activity = 40 Ci/mmole, New England Nuclear, Boston, Mass.), 100 μl of the tissue preparation in an amount equivalent to about 1.0 mg protein, 100 μl of THB buffer, and 100 μl of unlabeled TRH or cyclo(His-Pro) in the doses of 10^{-11}–10^{-5} mole per tube was incubated at 4°C for 120 min. After incubation, ice-cold THB buffer (1 ml) was added, and the mixture was passed rapidly by suction through a glass filter (GF/C Whatman, Inc., Clifton, N.J.; 25 mm in diameter). The filter was washed two times with ice-cold saline. All binding experiments were performed in triplicate together with triplicate samples each containing 2 nmole of unlabeled TRH for determination of nonspecific binding. The filters were placed in vials containing Econofluor (New England Nuclear), and the radioactivity was determined by liquid scintillation spectrometer (Beckman Model LS 7500). Specific binding was defined as the total minus the nonspecific binding.

Statistical analysis was carried out by Student's or Dunnett's (15) *t* test.

Results. As shown in Fig. 1, under the water-deprived condition the rats receiving intraventricularly 3×10^{-9} mole cyclo(His-Pro) had a significant decreased water intake up to 60 min, when compared with that of the control group injected with saline [a cyclo(His-Pro) group (n = 10), 2.73 ± 0.41 for 30 min and 4.85 ± 0.46 ml/100 g body wt for 60 min vs a saline group (n = 10), 4.81 ± 0.27 for 30 min and 6.02 ± 0.31 ml/100 g body wt for 60 min, $P < 0.001$ and $P < 0.05$, respectively]. Three hours later, the drinking consumption was significantly less in the cyclo(His-Pro)-injected group than in the saline-injected group (7.11 ± 0.50 vs 9.22 ± 0.79 ml/100 g body wt, $P < 0.05$). Because the inhibitory effect of cyclo(His-Pro) on water intake was predominent for the first 30 min, and its effect observed 3 hr later was not related to a dose–effect relationship, in the following experiments the consumption of water for the first 30 min was investigated.

Table I shows the effects of intraventricular administration of varying doses of cyclo(His-Pro) on drinking in water-deprived rats. Cyclo(His-Pro) in the doses of 3×10^{-10}–3×10^{-8} mole significantly decreased water intake, and its influence was observed in the dose-dependent fashion when expressed as percentage of control. When TRH was administered into the lateral ventricles in the

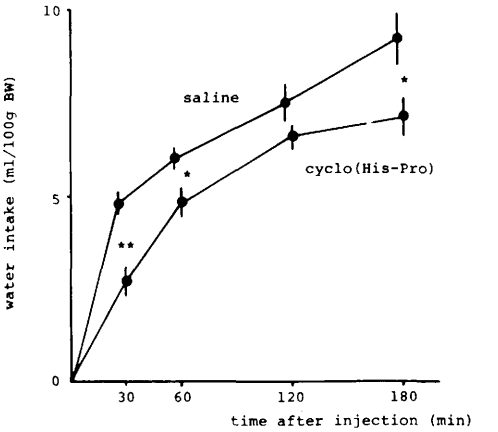

FIG. 1. Cumulative water intake in water-deprived rats following intraventricular injection of saline and 3×10^{-9} mole cyclo(His-Pro). *$P < 0.05$ and **$P < 0.001$ compared with the saline-injected group (Student's and Dunnett's *t* tests).

TABLE I. EFFECTS OF INTRAVENTRICULAR INJECTION OF CYCLO(His-Pro) ON WATER INTAKE IN RATS

	ml/100 g body wt	(%)		ml/100 g body wt	(%)
Saline (n = 8)	3.48 ± 0.40	100.0 ± 11.6	Cyclo(His-Pro) 3×10^{-11} mole (n = 8)	2.93 ± 0.17	84.3 ± 4.9
Saline (n = 12)	4.00 ± 0.18	100.0 ± 4.4	Cyclo(His-Pro) 3×10^{-10} mole (n = 9)	2.98 ± 0.24*	74.4 ± 5.9*
Saline (n = 17)	4.77 ± 0.29	100.0 ± 6.0	Cyclo(His-Pro) 3×10^{-9} mole (n = 17)	3.03 ± 0.41*	63.5 ± 8.5*
Saline (n = 16)	4.67 ± 0.30	100.0 ± 6.5	Cyclo(His-Pro) 3×10^{-8} mole (n = 13)	2.47 ± 0.42*	49.5 ± 9.1*

Note. Varying doses of cyclo(His-Pro) were intraventricularly administered to water-deprived rats, as mentioned under Materials and Methods. Water consumption was measured for 30 min after injection, and expressed as ml/100 g body wt and percentage of saline-injected control values. Parentheses indicate the number of animals used. *$P < 0.05$ compared with the saline group (Student's t test).

same manner as was cyclo(His-Pro), its inhibitory activity for drinking was found to be dose related and was similar to that of cyclo(His-Pro) as shown in Fig. 2. In contrast, intraperitoneal injection of cyclo(His-Pro) and TRH in the doses of 3×10^{-7}–3×10^{-6} mole/kg did not significantly decrease water intake in the water-deprived rats (Fig. 3).

Figure 4 illustrates that the crude plasma membrane of brain had a specific receptor site for TRH. [^{3}H]TRH binding in the brain membrane was decreased by adding with 10^{-10} mole of unlabeled TRH, but was not significantly inhibited by cyclo(His-Pro) even in the dose of 10^{-5} mole.

Discussion. The present study demonstrated that intraventricular administration of cyclo(His-Pro) decreased water intake stimulated by water deprivation in rats. Although cyclo(His-Pro) has been shown to

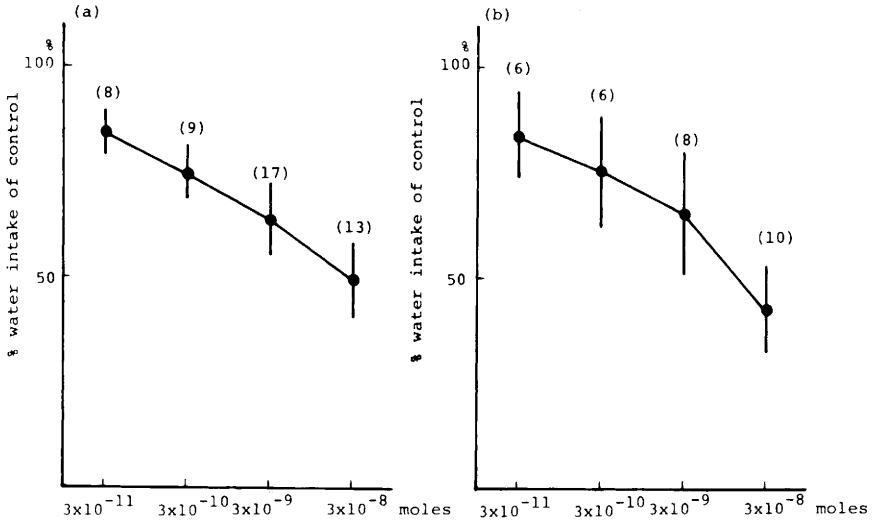

FIG. 2. Comparison of effects of cyclo(His-Pro) (a) and TRH (b) on water intake in rats. Water-deprived rats were intraventricularly injected with varying doses of cyclo(His-Pro) and TRH, as mentioned under Materials and Methods. Water consumption was measured for 30 min after injection and expressed as percentage of the saline-injected control values. Parentheses indicate the number of used animals. The values between cyclo(His-Pro) and TRH in the corresponding doses were statistically similar (Dunnett's t test).

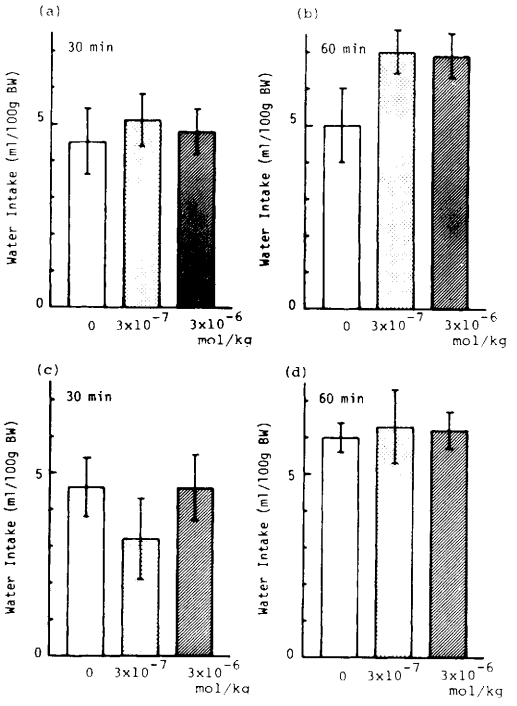

FIG. 3. Effect of intraperitoneal injection of cyclo(His-Pro) and TRH on water intake. Cyclo(His-Pro) and TRH were intraperitoneally administered to water-deprived rats. Water intake was determined for 30 and 60 min after injection. Each group consisted of seven animals. (a) and (b), the TRH-injected group; (c) and (d), the cyclo(His-Pro)-injected group. Any values were not statistically different from the saline-injected control values (Dunnett's *t* test).

possess a number of other biological activities, its physiological role is still unknown. The minimum dose required to produce a significant inhibition of drinking was 3×10^{-10} mole which was apparently less than the *in vivo* administered dose of cyclo(His-Pro) necessary to inhibit feeding (10^{-8} mole) (11) and induce hypothermia (10^{-7} mole/100 g body wt) (16). Furthermore, 50% suppression of the control water intake was observed by injection of 3×10^{-8} mole of cyclo(His-Pro), whereas 10^{-7} mole/100 g body wt of the dipeptide was required to attenuate the ethanol-induced sleeping time by 50% of control (17). These results imply that action of cyclo(His-Pro) for adipsia is more specific than any other biological activity of the dipeptide, suggesting a pivotal role of cyclo(His-Pro) in the regulation of water intake.

The inhibitory action of cyclo(His-Pro) on water intake was unlikely to be attributed to a consequence of behavioral alteration. Although intraventricular administration of this dipeptide was observed to elicit an enhancement of stereotypic behavior (18) and an inhibition of opiate-dependent abstinence (19), these behavioral changes were produced by the dose of cyclo(His-Pro) larger than 2×10^{-8} mole, whereas inhibition of water intake was observed in doses up to 100 times less.

The mechanism(s) by which cyclo(His-Pro) inhibited water intake remains unknown. If cyclo(His-Pro) is the active metabolite of TRH in the brain, it should exhibit the same biological responses as does TRH. However, some activities of cyclo(His-Pro) were observed to be either opposite to those of TRH or completely unrelated to those of TRH (e.g., induction of hypothermia, inhibition of *in vitro* prolactin secretion, and disturbance of dopamine uptake) (16, 20, 21). In addition, there are known TRH-related biological functions where cyclo(His-Pro) has been observed to be inactive (6). On the other hand, the present study revealed that inhibitory response of cyclo(His-Pro) with respect to water intake was quite similar to that of TRH. Therefore, a possible mechanism for the inhibitory action of cyclo(His-Pro) is the antagonism of TRH interactions with brain TRH receptors. However, this possibility is not likely, because cyclo(His-Pro) did not significantly interfere with TRH binding in the rat brain. Because cyclo(His-Pro), as well as TRH, is found to be present outside of the brain (22), the effects of these neuropeptides on water intake were examined by intraperitoneal injection. Intraperitoneal administration of neither cyclo(His-Pro) nor TRH was observed to produce any effect on water intake. These data imply that the neuropeptide action was not derived from any peripheral input produced by the peptides, compatible with the fact that the control of water intake is central in origin (23). Although it remains to be seen whether cyclo(His-Pro) may affect other brain dipsogenic and antidipsogenic factors such as vasopressin, angiotensin II, substance P, nerve growth factor, opioid peptides, and biogenic amines, evidence showing the ubiquitous presence of

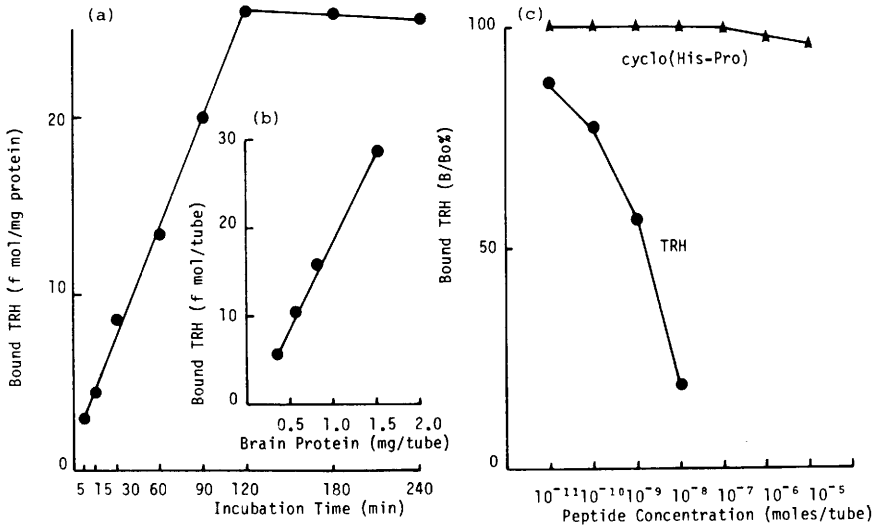

FIG. 4. TRH binding in the rat brains. The crude membrane of brain was incubated in the THB buffer containing [^{3}H]TRH in the presence or absence of unlabeled TRH and cyclo(His-Pro), as mentioned under Materials and Methods. (a) [^{3}H]TRH binding and incubation time; (b) [^{3}H]TRH binding and tissue protein concentrations which were determined by the Lowry method; (c) inhibition curve of [^{3}H]TRH binding by cyclo(His-Pro) and TRH. TRH bound was expressed as percentage inhibited radioactivity (B) of total bound radioactivity (B_0).

cyclo(His-Pro) in the CNS (2–4) brought about the concept that cyclo(His-Pro) may be a candidate for one of the brain factors participating in the control of water intake.

The central site(s) involved in the adipsic action of cyclo(His-Pro) is unknown at present. Drinking behavior is known to be closely related to food intake in the rats (24), but there may be a regional dissociation of anorectic and adipsic actions induced by neuropeptides. Suzuki *et al.* (10) reported that a microinjection of TRH into the medial hypothalamus resulted in decreases in both feeding and drinking, whereas a sole reduction of drinking was observed by injection of TRH into the lateral hypothalamus, in which stimulation and lesion are known to elicit, respectively, drinking and adipsia (25–27). Alternatively, food consumption, but not water intake, was stimulated by lesion in the nuclei accumbence septi (28), in which TRH application produced a decrease in food intake without affecting water intake (10). The present study focused on the effect of neuropeptides solely on water intake, and the resulting data that the dose of cyclo(His-Pro) necessary to decrease water intake was approximately 100 times less than its dose required to reduce food intake (11) were noteworthy. Studies are in progress to elucidate which locus (or loci) in the brain may be responsible for the adipsic action evoked by cyclo(His-Pro).

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