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Re-Evaluation of Histidyl-Proline Diketopiperazine [Cyclo(His-Pro)] Effects on Food Intake in the Rat

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BOWDEN, C. R., C. D. KARKANIAS AND A. J. BEAN. *Re-evaluation of histidyl-proline diketopiperazine [cyclo(His-Pro)] effects on food intake in the rat.* PHARMACOL BIOCHEM BEHAV **29**(2) 357–363, 1988.—Histidyl-proline diketopiperazine [cyclo(His-Pro)], a metabolite of thyrotropin releasing hormone (TRH), has been reported to decrease food intake of rats in a variety of feeding models following intracerebroventricular (ICV) injection. We have re-evaluated the anorectic effects of cyclo(His-Pro) on food deprivation-induced and spontaneous feeding. When injected ICV at the end of the light period into ad lib fed rats, neither the naturally occurring cyclo(L-His-L-Pro) isomer (14 to 1000 nmole/rat) nor any of the four cyclo(D,L-His-D,L-Pro) stereoisomers (100 nmole/rat) significantly suppressed food intake at any hour for up to 12- or 24-hr post-injection. Bombesin (0.6 nmole/rat ICV) decreased food intake in the same model by up to 86% with anorexia still apparent 15-hr post-injection (71%, $p<0.001$). In two food deprivation-induced feeding models, cyclo(L-His-L-Pro) (100 and 1000 nmole/rat ICV) did not cause anorexia while TRH (10 and 1000 nmole/rat ICV) maximally suppressed food intake by 74% ($p<0.02$) and 50% ($p<0.01$). Occasional transient increases of food consumption were observed in cyclo(His-Pro)-treated rats during both spontaneous and induced feeding. Cyclo(L-His-L-Pro) was also without effect on food intake when intraperitoneally administered at 12.5 and 30 μmole/kg to schedule fed rats. TRH at 30 μmole/kg IP transiently suppressed food intake of schedule fed rats ($p<0.005$). These findings indicate that cyclo(His-Pro) does not exhibit anorectic activity in the rat and cast doubt on the concept that TRH-induced anorexia results from conversion of TRH to an active cyclo(His-Pro) metabolite.

Histidyl-proline diketopiperazine Cyclo(His-Pro) Stereoisomers Thyrotropin releasing hormone Bombesin Food intake Anorexia Intracerebroventricular Rat

HISTIDYL-proline diketopiperazine [cyclo(histidyl-proline), cyclo(His-Pro)] is an endogenous cyclic dipeptide reported to exhibit numerous biological effects involving such diverse processes as prolactin secretion [5], cholesterol biosynthesis [9], thermoregulation [26], water intake [11] and (Na^+-K^+) ATPase activity [3] (see [24,25] for review). Among these activities, a potent, long-lasting anorectic effect has been reported for cyclo(His-Pro) in stress-induced, starvation-induced and spontaneous feeding models [22]. The most robust effect of cyclo(His-Pro) on food intake was seen in free-feeding rats in which a 10 nmole intracerebroventricular (ICV) dose suppressed spontaneous food intake by over 80% through 10 hours [22]. Similar anorectic activity has been observed with Thyrotropin Releasing Hormone (TRH) [19], a cyclo(His-Pro) precursor [27], leading to the proposal that TRH acts as a pro-hormone for cyclo(His-Pro) [22]. These observations, coupled with demonstrations of fasting and feeding induced alterations of hypothalamic immunoreactive cyclo(His-Pro) concentrations in both normal and genetically obese rodents [18,31], suggest that cyclo(His-Pro) might serve as an endogenous neuroendocrine modulator of food intake.

We could not, however, confirm the anorectic activity of cyclo(His-Pro) using a spontaneous-feeding paradigm in which each of the four individually synthesized stereoisomers of cyclo(D,L-His-D,L-Pro) were tested at 100 nmole ICV [14]. In the present studies, we have tested the naturally occurring isomer, cyclo(L-His-L-Pro), for anorectic effects in multiple feeding models and expanded stereoisomeric testing. In no case were we able to demonstrate an anorectic effect for cyclo(His-Pro).

METHOD

Male, CD strain Sprague-Dawley rats (Charles River Breeding Laboratories) were individually housed in stainless steel cages at 22–24°C on a 12 hour light-dark cycle (0600 hr

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to 1800 hr). Rats had ad lib access to food (diet specified under each experimental protocol) and tap water throughout the experiments unless otherwise noted.

For studies requiring intracerebroventricular (ICV) injection, rats were anesthetized with sodium pentobarbital (Nembutal, Harvey Labs, 65 mg/kg IP) and 21 gauge stainless steel lateral ventricular guide cannulae were implanted using a Kopf stereotaxic instrument (AP=−1.0, Lat=−1.5 from Bregma, lowered 2.5 mm ventral to skulltop) such that the cannula tip was ~1 mm above the roof of the lateral ventricle. The cannula was secured to the skull using two stainless steel screws and dental cement. An obturator was inserted to occlude the guide cannula and a minimum of five days was allowed for surgical recovery before studies were performed.

Compounds for ICV injection were dissolved in sterile physiological saline and the appropriate dose administered intracerebroventricularly in a volume of 10 μl over a one minute interval. Injections were given using a Hamilton syringe and a 26 gauge needle that was passed through the guide cannula into the lateral ventricle. A 1 mm collar of PE 20 tubing was attached to the injection needle to achieve proper insertion depth.

After feeding studies were completed, cannula patency was pharmacologically assessed by the drinking response to an ICV injection of angiotensin II (500 ng) and cannula placement was verified post-mortem by examination of brain sections after cresyl violet injection. Rats which either did not drink >5 ml of water in ten min after angiotensin II injection or did not have dye widespread throughout the ventricles were excluded from data analysis.

Spontaneous Feeding Studies

Rats were habituated to metabolism cages equipped with automated feeding monitors (Coulbourn Instruments) for at least one week and guide cannulae for ICV injections were then implanted. Food was provided as 45 mg pellets (Bio-Serv No. 0021) which was singly delivered into a feeding trough. A photodetector sensed removal of the pellet, which triggered delivery of another food pellet, and the number of pellets delivered over a specified time interval was recorded on a ten channel printing counter. Injections were given just prior to the onset of the dark period (1800 hr) and food intake monitored hourly for up to 24 hours post-dosing. The maximum number of spilled pellets, which were recovered on a plastic mesh under each cage, was 5/24 hr (<0.25 g/day) and no correction to food intake was made.

In Experiment 1, rats (n=9, mean±SEM body weight=375±10 g) were randomly divided into two groups, injected ICV with either saline (n=4) or 14 nmole/rat cyclo(L-His-L-Pro) (n=5) and food intake monitored. Seven and twelve days later, rats were rerandomized and the procedure repeated with 72 (n=5) and 145 nmole/rat (n=4) cyclo(L-His-L-Pro), respectively.

In Experiment 2, rats (n=8, 461±21 g b.wt.) received either saline or 1 μmole cyclo(L-His-L-Pro) ICV (n=4 per group) and food intake monitored through 12 hours. The protocol was repeated six days later with treatments reversed giving a total of n=8 per group.

In Experiment 3, each rat received a saline injection and one or two test compound injections ICV (100 nmole of a cyclo(His-Pro) stereoisomer or 1 μg=0.6 nmole bombesin) with tests separated by at least five days. A total of 33 rats (421±10 g b.wt.) were used yielding group sizes of: saline, n=33; each cyclo(His-Pro) stereoisomer, n=8; bombesin, n=7.

Induced Feeding Studies

In Experiment 4, rats (n=6, 338±12 g b.wt.) implanted with ventricular guide cannulae and habituated to automated Bio-Serv pellet feeding were used. Animals were fasted in individual cages without feeding troughs to avoid loss of pellet eating behavior. After 24 hr food deprivation, rats were injected ICV at 1800 hr with either sterile saline, TRH (10 nmole) or cyclo(L-His-L-Pro) (100 nmole), replaced into their original feeding trough-equipped cages and food intake recorded at indicated times. Each rat received all three treatments with at least one week between tests and treatment order was randomized giving a total of n=6 per group.

In Experiment 5, rats (n=7, 496±11 g b.wt.) were allowed to recover from guide cannula implantation with continuous access to pelleted Purina Rat Chow® (No. 5002) and water. Rats were then food deprived for 24 hr, injected ICV at 1600 hr with either sterile saline, TRH (1 μmole/rat) or cyclo(L-His-L-Pro) (1 μmole/rat) and given access to a single nugget (~10 g) of Purina Rat Chow®. Food intake, corrected for spillage, was measured after 30 min. Each rat received all three treatments with each test separated by one week and treatment order randomized giving a total of n=7 per group.

In Experiment 6, rats (n=16, 320±4 g b.wt.) were accustomed to eating powdered Purina Rat Chow® (No. 5001) on a 6 hr-fed:18 hr-fasted schedule for 10 days, with food jars placed in cages at 0900 hr. After being randomly assigned to treatment groups, rats were intraperitoneally injected at 0830 hr with either vehicle (0.5% methylcellulose, n=6), cyclo(L-His-L-Pro) (12.5 μmole/kg=3 mg/kg, n=5) or d-amphetamine sulfate (1 mg/kg on a salt-free basis, n=5). Thirty minutes post-injection, food jars were placed in cages and food intake, corrected for spillage, measured after 1, 2 and 6 hr of feeding.

In Experiment 7, rats (n=15, 317±7 g b.wt.) were trained to schedule-feeding as in experiment 6 and randomly allocated to two groups. Groups were intraperitoneally injected with either saline (n=8) or cyclo(L-His-L-Pro) (30 μmole/kg=7 mg/kg, n=7) and food jars immediately placed in cages. Food intake corrected for spillage was measured after 30, 60 and 120 min of feeding. One week later, the groups were reversed, injected with either saline or TRH (30 μmole/kg=11 mg/kg, n=8) and the study repeated.

Compounds

Each stereoisomer of cyclo(histidyl-proline) was individually synthesized and characterized by elemental analysis, optical rotation, thin layer chromatography, IR spectra and NMR spectra as previously described [14]. A commercial sample of cyclo(L-His-L-Pro) (used in Experiments 1 and 7) was obtained from Peninsula Laboratories (Lot No. 004503) and also characterized for purity. Bombesin (Lot No. 003748) and TRH (Lot No. 011640) were purchased from Peninsula Laboratories, synthetic angiotensin II was obtained from Calbiochem-Bering and d-amphetamine sulfate from Sigma.

Statistics

Food intakes were calculated as g per 100 g body weight and statistical comparisons were performed using one way

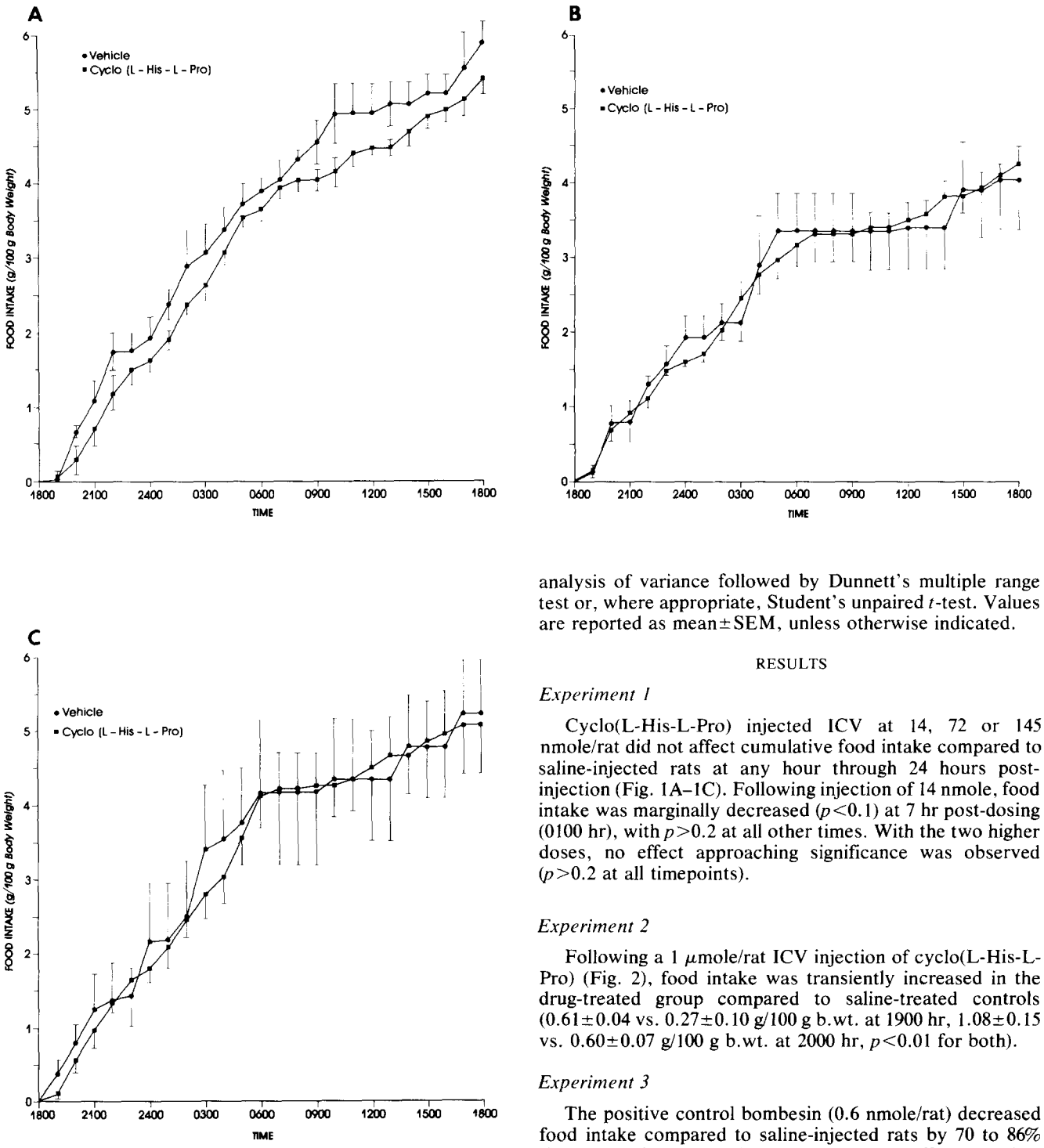

FIG. 1. Effects of centrally administered cyclo(L-His-L-Pro) at three different doses on cumulative hourly food intake of spontaneously feeding rats. Studies were performed as described in the Method section, Experiment 1. (A) Cyclo(L-His-L-Pro), 14.5 nmole/rat, n=5; saline, n=4. (B) Cyclo(L-His-L-Pro), 72.6 nmole/rat, n=5; saline, n=4. (C) Cyclo(L-His-L-Pro), 145 nmole/rat, n=4; saline, n=5. Food intake values are mean±SEM. No cyclo(L-His-L-Pro) values were significantly different compared to simultaneously run saline-treated by ANOVA.

analysis of variance followed by Dunnett's multiple range test or, where appropriate, Student's unpaired t-test. Values are reported as mean±SEM, unless otherwise indicated.

RESULTS

Experiment 1

Cyclo(L-His-L-Pro) injected ICV at 14, 72 or 145 nmole/rat did not affect cumulative food intake compared to saline-injected rats at any hour through 24 hours post-injection (Fig. 1A–1C). Following injection of 14 nmole, food intake was marginally decreased ($p<0.1$) at 7 hr post-dosing (0100 hr), with $p>0.2$ at all other times. With the two higher doses, no effect approaching significance was observed ($p>0.2$ at all timepoints).

Experiment 2

Following a 1 μmole/rat ICV injection of cyclo(L-His-L-Pro) (Fig. 2), food intake was transiently increased in the drug-treated group compared to saline-treated controls (0.61±0.04 vs. 0.27±0.10 g/100 g b.wt. at 1900 hr, 1.08±0.15 vs. 0.60±0.07 g/100 g b.wt. at 2000 hr, $p<0.01$ for both).

Experiment 3

The positive control bombesin (0.6 nmole/rat) decreased food intake compared to saline-injected rats by 70 to 86% through 15 hr post-dosing (Fig. 3). These effects reached significance beginning at the 4 hr timepoint, $F(5,66)=3.27$, $p<0.05$, with a 71% reduction still apparent at 15 hr post-injection, $F(5,66)=5.17$, $p<0.001$. No significant alterations of food intake, compared to saline-treated controls, were observed for any of the four stereoisomers of cyclo(His-Pro) injected at 100 nmole ICV (Fig. 3).

Experiment 4

TRH injected at 10 nmole/rat significantly decreased food

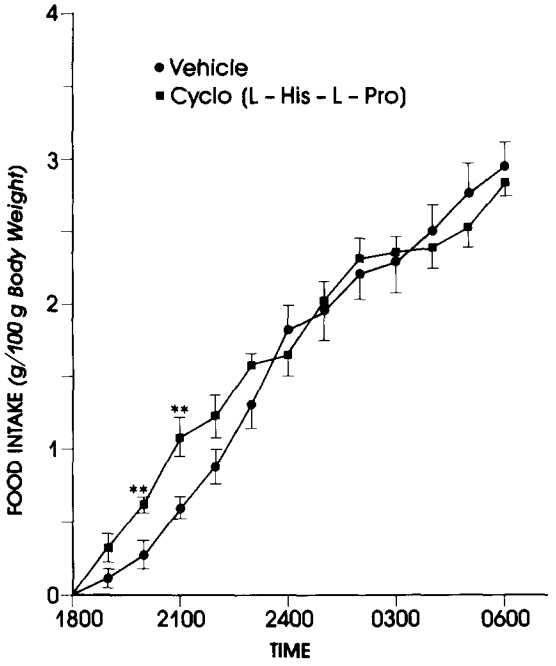

FIG. 2. Effects of centrally administered cyclo(L-His-L-Pro), 1 μmole/rat, on cumulative hourly food intake of spontaneously feeding rats. Studies were performed as described in the Method section, Experiment 2. Food intake values are mean±SEM, n=8 for each group. $^{**}p<0.01$ compared to saline-treated by ANOVA.

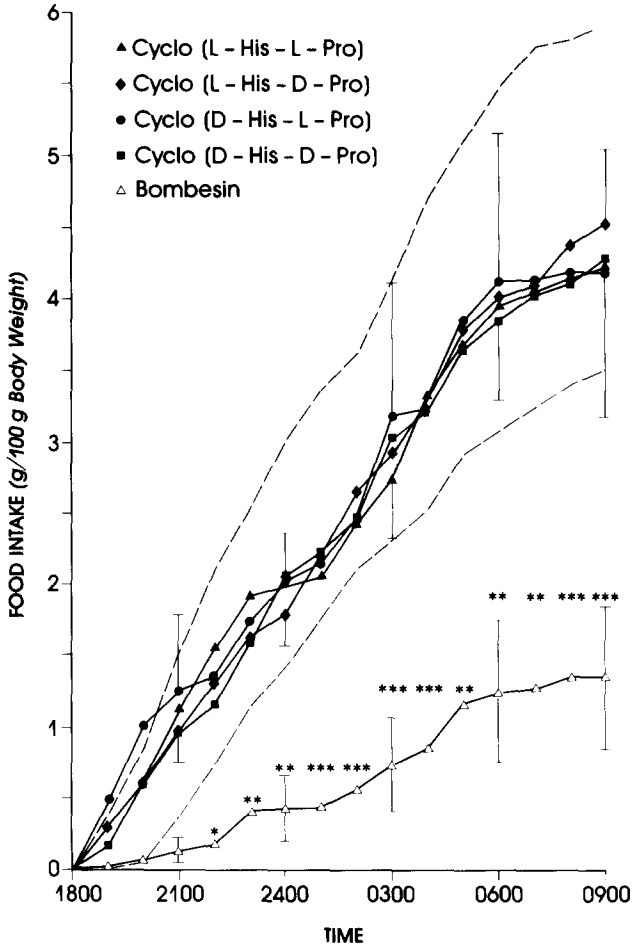

FIG. 3. Effects of centrally administered cyclo(His-Pro) stereoisomers, 100 nmole/rat, and bombesin, 0.6 nmole/rat, on cumulative hourly food intake of spontaneously feeding rats. Studies were performed as described in the Method section, Experiment 3. Food intake values are mean±SEM, with SEM indicated at 3 hr intervals to improve legibility. Broken lines indicate mean±SD for saline treated rats, n=33; n=8 for each cyclo(His-Pro) stereoisomer and n=7 for bombesin. $^{*}p<0.05$, $^{**}p<0.01$ and $^{***}p<0.001$ compared to saline-treated by post-hoc t-test after significant intergroup difference was found by ANOVA.

intake of 24 hr fasted rats at 10, $F(2,15)=3.71$, $p<0.02$, and 20 min, $F(2,15)=6.32$, $p<0.02$, post-dosing (Fig. 4). This effect was transient as food intake was not significantly different from that of saline-treated animals at 30 through 90 min. Although rats receiving cyclo(L-His-L-Pro) at 100 nmole ICV appeared to eat at a similar rate as saline-treated animals through 20 min post-injection, the drug-treated rats continued eating after control rats had ceased. As a result, food intake for cyclo(L-His-L-Pro)-treated rats was increased by 39% at 30 min, $F(2,15)=8.08$, $p<0.01$, and 42% at 60 min, $F(2,15)=4.86$, $p<0.02$.

Experiment 5

Thirty minute food intake of TRH-treated (1 μmole ICV) rats was decreased by 50% compared to saline-treated animals [0.51±0.10 vs. 1.01±0.11 g/100 g b.wt./30 min, $F(2,18)=11.57$, $p<0.01$] while an equivalent dose of cyclo(L-His-L-Pro) yielded a nonsignificant (1.22±0.12 g/100 g b.wt./30 min, $p>0.2$) increase of food intake (n=7 for each group).

Experiment 6

Injection of d-amphetamine (1 mg/kg IP) essentially abolished 1 hr, $F(2,13)=56.35$, $p<0.001$, and 2 hr, $F(2,13)=54.04$, $p<0.001$, food intake of rats accustomed to 6 hr schedule-feeding (Table 1). The anorectic effect of amphetamine at this dose was short-lived since cumulative 6 h intake was equivalent to that of saline-treated rats. Cyclo(L-His-L-Pro) (3 mg/kg=12.5 μmole/kg IP) did not affect food intake at any timepoint ($p>0.2$).

Experiment 7

Cyclo(L-His-L-Pro), intraperitoneally administered at 30 μmole/kg, did not affect food intake of schedule-fed rats at any time point through two hours post-dosing ($p>0.5$, Table 2). An equimolar injection of TRH significantly reduced 30 min intake by 38%, $F(2,27)=3.89$, $p<0.05$, and 60 min consumption by 44%, $F(2,27)=8.09$, $p<0.005$. The TRH effect was again transient as 120 min food intake was not significantly different than that of saline-treated rats, $F=1.71$.

DISCUSSION

Histidyl-proline diketopiperazine [cyclo(His-Pro)] has been reported to potently decrease food intake following central injection in spontaneous, food deprivation-induced, stress-induced [22] and insulin-induced [17], but not muscimol-induced [20] or norepinephrine-induced [21], feeding models. Although TRH has not to our knowledge been

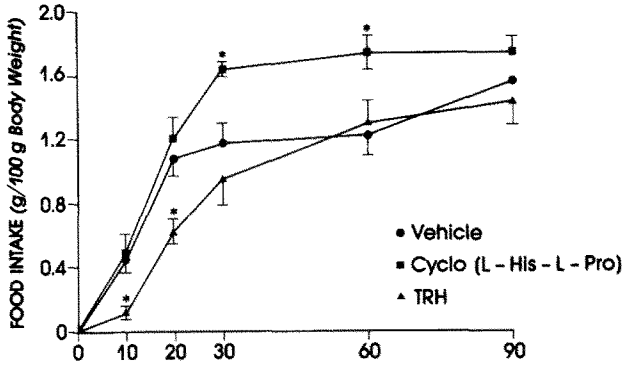

FIG. 4. Effects of centrally administered cyclo(L-His-L-Pro), 100 nmole/rat, and TRH, 10 nmole/rat, on food intake of fasted rats. Studies were performed as described in the Method section, Experiment 4. Food intake values are mean±SEM, n=6 for each group, and abcissa indicates time post-dosing in minutes. *$p<0.05$ compared to saline-treated by post-hoc t-test after significant intergroup difference was found by ANOVA.

TABLE 1

EFFECTS OF INTRAPERITONEALLY ADMINISTERED cyclo(L-His-L-Pro) AND d-AMPHETAMINE SULFATE ON FOOD INTAKE OF SCHEDULE-FED RATS

	Food Intake (g/100 g body weight)		
Treatment	1 hr	2 hr	6 hr
Vehicle	3.09 ± 0.25	4.57 ± 0.45	6.12 ± 0.41
Cyclo(L-His-L-Pro) (3 mg/kg)	2.60 ± 0.29	3.83 ± 0.34	6.03 ± 0.45
d-Amphetamine (1 mg/kg)	0.15 ± 0.09*	0.30 ± 0.15*	6.18 ± 0.53

Rats were trained to a 6 hr fed:18 hr fasted schedule feeding, injected 30 min prior to feeding, and food intake measured at indicated times as described in the Method section, Experiment 6. Food intake values are mean ± SEM, n=6 for vehicle-treated and n=5 for each drug-treated group.

*$p<0.001$ compared to vehicle-treated by post-hoc t-test after a significant intergroup difference was found by ANOVA.

TABLE 2

EFFECT OF INTRAPERITONEALLY ADMINISTERED cyclo(L-His-L-Pro) AND TRH ON FOOD INTAKE OF SCHEDULE-FED RATS

	Food Intake (g/100 g body weight)		
Treatment	30 min	60 min	120 min
Saline	1.49 ± 0.14	2.64 ± 0.16	3.89 ± 0.26
Cyclo(L-His-L-Pro) (30 μmole/kg)	1.53 ± 0.22	2.68 ± 0.30	4.05 ± 0.32
TRH (30 μmole/kg)	0.93 ± 0.08*	1.49 ± 0.26†	3.13 ± 0.47

Rats were trained to a 6 hr fed:18 hr fasted schedule feeding, injected with either saline (n=15), cyclo(L-His-L-Pro) (n=7) or TRH (n=8) immediately prior to feeding and food intake measured at the indicated times as described in the Method section, Experiment 7. Food intake values are mean ± SEM. *$p<0.05$, †$p<0.005$ compared to saline-treated by post-hoc t-test after a significant intergroup difference was found by ANOVA.

studied for effects on spontaneous or insulin-induced food intake, the anorectic activity of centrally administered TRH in the other models [19–21, 29] parallels that reported for cyclo(His-Pro). The similarity in the anorectic activities of these two compounds, coupled with the demonstration that TRH undergoes metabolism by pyroglutamate aminopeptidase to produce cyclo(His-Pro) [27], has led to the hypothesis that TRH acts as a pro-hormone for cyclo(His-Pro) [22]. In the present studies, we have confirmed the reported anorectic activity of TRH but have been unable to confirm that of cyclo(His-Pro) in either spontaneous or food deprivation-induced feeding. Similar discrepancies exist in the literature concerning the presence or absence of cyclo(His-Pro) activity on prolactin secretion [2, 6, 8], natriuresis [28] and (Na^+-K^+)ATPase activity [25].

Rats treated with cyclo(His-Pro) ICV displayed a spontaneous nocturnal feeding pattern which did not differ from that of saline-treated animals over a dose range of 14 to 1000 nmole/rat. This finding conflicts with the previously reported 80% reduction of spontaneous food intake over a 10 hour interval following a central injection of 10 nmole cyclo(His-Pro) [22]. Our inability to observe this effect cannot be attributed to injection at a site other than intraventricular as each animal was tested for a centrally mediated pharmacological response (angiotensin II induced drinking) and underwent dye injection with post-mortem histological verification of dye presence in the lateral ventricles. Although there are methodological differences between our experiments and the previously reported spontaneous feeding studies (e.g., we used automatic pellet delivery with hourly measurements instead of weighing chow for a single 10 hour measurement, our rats weighed more but were of the same sex and strain as the previous report), it is unlikely that these modest design variations account for the observed difference in effects. Our spontaneous feeding paradigm was certainly capable of detecting anorectic agents, as bombesin yielded significant, long-lasting suppression of food intake comparable in magnitude to that reported by others [7, 10, 15, 16]. In addition, we have successfully tested other such agents in this model (e.g., fenfluramine, data not shown).

An alternative explanation for our disparate findings was that a stereoisomer other than the expected natural L,L isomer was responsible for the originally reported anorectic activity. This seemed unlikely in that both the D,L and L,D stereoisomers were reported to be approximately ten-fold less potent than the L,L isomer in displacing labeled cyclo(His-Pro) binding to adrenal particulate fraction [4]. In addition, the hypothermic effects observed after cyclo(L-His-D-Pro) administration to cold-acclimated rats was less severe than that seen with equimolar cyclo(L-His-L-Pro) treatment [25]. Nonetheless, the greater activity reported for the L,L isomer compared to other stereoisomers did not necessarily extend to control of food intake and the four stereoisomers were thus each individually examined for effects on spontaneous feeding. None of the cyclo(His-Pro) stereoisomers significantly altered food consumption when centrally administered at 100 nmole and we conclude that a cyclo(His-Pro) isomer was not responsible for the anorectic effects initially observed [22]. An objection to this conclusion is that we did not test the originally reported dose of 10 nmole, but rather a tenfold higher dose, and that the response curve for cyclo(His-Pro) may exhibit an "inverted U-shape" (i.e., decreased effect with high doses) as has been reported for other peptides [12,23]. This is quite unlikely as dose-dependent inhibition of stress-induced food intake was reported over the range of 10 to 1000 nmole for cyclo(His-Pro) [22], bracketing the dose used for stereoisomeric testing, with no evidence of a less potent effect at higher doses.

We also examined cyclo(His-Pro) for suppressive effects on food-deprivation induced feeding. Experiment 5 was replicated from the original report [19,22], however, no effect of 1 μmole cyclo(His-Pro) on food intake was observed while an equimolar dose of TRH substantially decreased food consumption. Similarly, significant decreases of starvation-stimulated food intake were observed in Experiment 4 when TRH was centrally injected at 10 nmole while cyclo(His-Pro) at a ten-fold higher dose did not exhibit an anorectic effect. In both of these studies as well as the high dose spontaneous feeding experiment (Experiment 2), cyclo(His-Pro) treated rats transiently ate more than saline-treated animals with the effect being variably significant. Although the mechanism underlying this inconsistent orexigenic activity is unknown, its existence strongly argues against consideration of cyclo(His-Pro) as an anorectic dipeptide.

The anorectic effects of TRH following either central or peripheral administration to starved-refed rats have previously been described using a 4 hr schedule-fed model [30], in rats acutely deprived of food and water for 20 hr [29] and using 24 hr fasted rats [19]. Our studies using ICV injection in 24 hr acutely-starved and intraperitoneal injection in 6 hr schedule-fed models further confirm TRH's anorectic effects. However, the concept that TRH-induced anorexia is mediated by conversion of TRH to its active metabolite, cyclo(His-Pro), is not supported by our data in fasted rats since ICV cyclo(His-Pro) was not anorectic. Intraperitoneal administration of cyclo(His-Pro) (Experiment 6) also failed to reveal an anorectic effect. The lack of response could have a pharmacokinetic basis as rapid clearance of radioactively labeled cyclo(His-Pro) has been reported following intravenous administration of the dipeptide to rats [13]. For this reason, Experiment 7 was performed with food intake measured over shorter intervals, a higher dose of cyclo(His-Pro) and a direct comparison to TRH. Cyclo(His-Pro) at 30 μmole/kg was without effect on food intake while an equimolar dose of TRH caused a significant depression of food consumption. Anorexia following parenteral TRH at doses equivalent to or smaller than ours has been reported by other groups using food deprivation-induced feeding models [19,30]. Since the $t_{1/2}$ for clearance of TRH is comparable to that of cyclo(His-Pro) [1], these data do not support rapid cyclo(His-Pro) clearance as a reason for the observed inactivity.

Drug effects on stress-induced (tail pinch) feeding were not examined in the current studies. The reported activity, however, was similar to that seen in starvation-induced feeding and less than that on spontaneous feeding [22]. The complete absence of anorectic activity noted in our studies using the latter two models would argue against expecting activity in the tail pinch paradigm.

In summary, we were unable to confirm cyclo(His-Pro)'s effect on either spontaneous or food deprivation-induced feeding using well characterized synthetic and commercial cyclo(L-His-L-Pro). Furthermore, anorectic activity could not be produced by any of the four stereoisomers of cyclo(D,L-His-D,L-Pro) using a spontaneous feeding model. We speculate that the material originally tested [22] contained something other than cyclo(His-Pro). We must conclude that cyclo(His-Pro) does not play a role in the

neuropeptidergic regulation of food intake and these data cast doubt on the concept of TRH-induced anorexia being a result of TRH acting as a pro-hormone which is converted to an active cyclo(His-Pro) metabolite.

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REFERENCES

1. Bassiri, R. M. and R. D. Utiger. Metabolism and excretion of exogenous thyrotropin-releasing hormone. *J Clin Invest* **52:** 1616–1619, 1973.
2. Battaini, F., P. S. Dannies and A. Peterkofsky. Unresponsiveness of GH cells to cyclo(histidyl-proline), a metabolite of thyrotropin releasing hormone. *Life Sci* **35:** 2519–2527, 1984.
3. Battaini, F. and A. Peterkofsky. Histidyl-proline diketopiperazine, an endogenous brain peptide that inhibits (Na^+-K^+)-ATPase. *Biochem Biophys Res Commun* **94:** 240–247, 1980.
4. Battaini, F., Y. Koch, Y. Takahara and A. Peterkofsky. Specific binding to adrenal particulate fraction of cyclo(histidyl-proline), a TRH metabolite. *Peptides* **4:** 89–96, 1983.
5. Bauer, K., K. J. Graf, A. Faivre-Bauman, S. Beier, A. Tixier-Vidal and H. Kleinkauf. Inhibition of prolactin secretion by histidyl-proline diketopiperazine. *Nature* **274:** 174–175, 1978.
6. Brewster, D., P. W. Dettmar, A. G. Lynn, G. Metcalf, B. A. Morgan and M. J. Rance. Modification of the proline residue of TRH enhances biological activity and inhibits degradation. *Eur J Pharmacol* **66:** 65–71, 1980.
7. De Caro, G., M. Massi, L. G. Micossi and M. Perfumi. Drinking and feeding inhibition by ICV pulse injection or infusion of bombesin, ranatensin and litorin to rats. *Peptides* **5:** 607–613, 1984.
8. Emerson, C. H., S. Alex, L. E. Braverman and M. S. Safran. A study of the effect of the thyrotropin-releasing hormone metabolite, histidyl-proline diketopiperazine, on prolactin secretion *in vivo*. *Endocrinology* **109:** 1375–1379, 1981.
9. Gebhard, R. L., J. E. Morley, W. F. Prigge and C. Prasad. TRH and cyclo(His-Pro) inhibit cholesterol synthesis in dog intestine. *Peptides* **3:** 475–478, 1981.
10. Grinker, J. A., B. S. Schneider, G. Ball, A. Cohen, A. Strohmayer and J. Hirsch. Cholecystokinin (CCK-8) and bombesin (BBS) intracranial injections and satiety in rats. *Fed Proc* **38:** 501, 1980.
11. Ishihara, J., M. Mori, I. Kobayashi and S. Kobayashi. Intraventricular administration of cyclo(his-pro), a metabolite of thyrotropin-releasing hormone (TRH), decreases water intake in the rat. *Proc Soc Exp Biol Med* **178:** 623–628, 1985.
12. Kastin, A. J., E. L. Scollan, R. H. Ehrensing, A. V. Schally and D. H. Coy. Enkephalin and other peptides reduce passiveness. *Pharmacol Biochem Behav* **9:** 515–519, 1978.
13. Koch, Y., F. Battaini and A. Peterkofsky. [^{3}H]-Cyclo(histidyl-proline) in rat tissues: Distribution, clearance and binding. *Biochem Biophys Res Commun* **104:** 823–829, 1982.
14. Kukla, M. J., H. J. Breslin and C. R. Bowden. Synthesis, characterization and anorectic testing of the four stereoisomers of cyclo(histidyl-proline). *J Med Chem* **28:** 1745–1747, 1985.
15. Kulkosky, P. J., J. Gibbs and G. P. Smith. Behavioral effects of bombesin administration in rats. *Physiol Behav* **28:** 505–512, 1982.
16. Kulkosky, P. J., J. Gibbs and G. P. Smith. Feeding suppression and grooming repeatedly elicited by intraventricular bombesin. *Brain Res* **242:** 194–196, 1982.
17. Levine, A. S. and J. E. Morley. Peptidergic control of insulin-induced feeding. *Peptides* **2:** 261–264, 1981.
18. Mori, M., J. Pegues, C. Prasad and J. F. Wilbur. Fasting and feeding-associated changes in cyclo(his-pro)-like immunoreactivity in the rat brain. *Brain Res* **268:** 181–184, 1983.
19. Morley, J. E. and A. S. Levine. Thyrotropin releasing hormone (TRH) suppresses stress induced eating. *Life Sci* **27:** 269–274, 1980.
20. Morley, J. E., A. S. Levine and J. Kniep. Muscimol induced feeding: A model to study the hypothalamic regulation of appetite. *Life Sci* **29:** 1213–1218, 1981.
21. Morley, J. E., A. S. Levine, S. S. Murray and J. Kniep. Peptidergic regulation of norepinephrine induced feeding. *Pharmacol Biochem Behav* **16:** 225–228, 1982.
22. Morley, J. E., A. S. Levine and C. Prasad. Histidyl-proline diketopiperazine decreases food intake in rats. *Brain Res* **210:** 475–478, 1981.
23. Myers, R. D., J. R. Hepler and W. Holahan. Action of anorexigenic peptide injected into the brain: Dissociation of effect on body weight and feeding in the rat. *Peptides* **4:** 85–88, 1983.
24. Peterkofsky, A. and F. Battaini. The biological activities of the neuropeptide histidyl-proline diketopiperazine. *Neuropeptides* **1:** 105–118, 1980.
25. Peterkofsky, A., F. Battaini, Y. Koch, Y. Takahara and P. Dannies. Histidyl-proline diketopiperazine: Its biological role as a regulatory peptide. *Mol Cell Biochem* **42:** 45–63, 1982.
26. Prasad, C., T. Matsui, J. Williams and A. Peterkofsky. Thermoregulation in rats: Opposing effects of thyrotropin releasing hormone and its metabolite histidyl-proline diketopiperazine. *Biochem Biophys Res Commun* **85:** 1582–1587, 1978.
27. Prasad, C. and A. Peterkofsky. Demonstration of pyroglutamylpeptidase and amidase activities toward thyrotropin releasing hormone in hamster hypothalamus extracts. *J Biol Chem* **251:** 3229–3234, 1976.
28. Reisin, E., J. E. Pearson, C. Prasad and F. M. Gonzalez. Failure of cyclo(his-pro) to exhibit natriuretic activity. *Clin Res* **32:** 875A, 1984.
29. Vijayan, E. and S. M. McCann. Suppression of feeding and drinking activity in rats following intraventricular injection of thyrotropin releasing hormone (TRH). *Endocrinology* **100:** 1727–1730, 1977.
30. Vogel, R. A., B. R. Cooper, T. S. Barlow, A. J. Prange, Jr., R. A. Mueller and G. R. Breese. Effects of thyrotropin-releasing hormone on locomotor activity, operant performance and ingestive behavior. *J Pharmacol Exp Ther* **208:** 161–168, 1979.
31. Wilber, J. F., M. Mori, J. Pegues and C. Prasad. Endogenous histidyl-proline diketopiperazine [cyclo(his-pro)]: A potential satiety neuropeptide in normal and genetically obese rodents. *Trans Assoc Am Physicians* **96:** 131–136, 1983.