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Experiments with a Reported Anorexigenic Tripeptide: Pyro-Glu-His-Gly-OH[1]

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NANCE, D. M., D. H. COY AND A. J. KASTIN. *Experiments with a reported anorexigenic tripeptide: Pyro-Glu-His-Gly-OH.* PHARMAC. BIOCHEM. BEHAV. 11(6) 733–735, 1979.—A newly described tripeptide, pyro-Glu-His-Gly-Oh, which was reported to produce profound and long-term anorexia and weight loss in female mice, was initially tested in female rats. After total does of either 8, 16, or 32 μg SC of the peptide, administered across an eight day interval, there was no detectable effect on food intake, body weight, or estrous cycles of female rats. In a second study, we attempted to verify the anorexigenic potency of this peptide in mice. Total doses of 3.4 and 6.8 μg, injected across a 20 day period, had no effect on the food intake or body weight of S/W albino female mice. Thus, the anorexigenic potency of pyro-Glu-His-Gly-OH has yet to be established.

Anorexia Food intake Body weight Peptide Rats Mice

AS early as 1964, Stevenson *et al.* [2] isolated a polypeptide from the urine of fasted rats that had fat mobilizing activity, but even more interesting was the observation that this polypeptide had a potent anorexic effect on food intake when injected into normal rats. The anorexia and weight reducing effects of this substance were even greater in animals made obese by hypothalamic lesions. Since there are major similarities between hypothalamic-lesioned animals and obese humans, these laboratory findings may be of importance with regard to our current lack of effective long-term therapeutics for human obesity. Continuation of this exciting work was apparently limited by the lack of complete knowledge of the structure of this polypeptide. However, a recent report by Trygstad and associates indicates that a new tripeptide isolated from the urine of female patients with anorexia nervosa may be of clinical and experimental interest. They reported that injecting a total dose of 3.4 μg/animal across a 20 day period produced a *chronic* reduction in food intake and body weight of female mice that lasted for 6 months. The sequence of the anorexigenic tripeptide was found to be pyro-Glu-His-Gly-OH [1,3]. It should be noted that the structure of this peptide is somewhat similar to the structure of an established hypothalamic tripeptide, TRH, which is pyro-Glu-His-Pro-NH_2. In the present two experiments, we have examined the anorexigenic potency of this new peptide in both rats and mice.

EXPERIMENT 1
EFFECTS OF PYRO-GLU-HIS-GLY-OH ON THE FOOD INTAKE AND BODY WEIGHT OF FEMALE RATS

To be of general interest, this peptide should be effective across species as shown for several other peptides. If it were effective in rats, a more complete conceptual framework and body of literature would be available to explain the results.

Method

Twenty-four Sprague/Dawley female rats (200–220 g) were purhcased from Simonsen Laboratories (Gilroy, CA) and maintained in individual stainless steel cages with ad lib access to food and water throughout the experiment. The animal room was illuminated from 7:00 a.m. to 7:00 p.m. and daily room entires were limited to 10:00–11:00 a.m. Consumption of powdered Purina rat chow (presented in special feeding cups) was measured daily. At the same time, all animals were weighed and vaginal secretions sampled with an eyedropper for examination of the smear.

The tripeptide was synthesized by standard solid phase procedures and purified to homogeneity. Purity was checked by amino acid analysis and verified by four systems of thin layer chromotography. The peptide was dissolved in an acidified saline solution (pH 4.1) in a concentration of 40 μg/ml.

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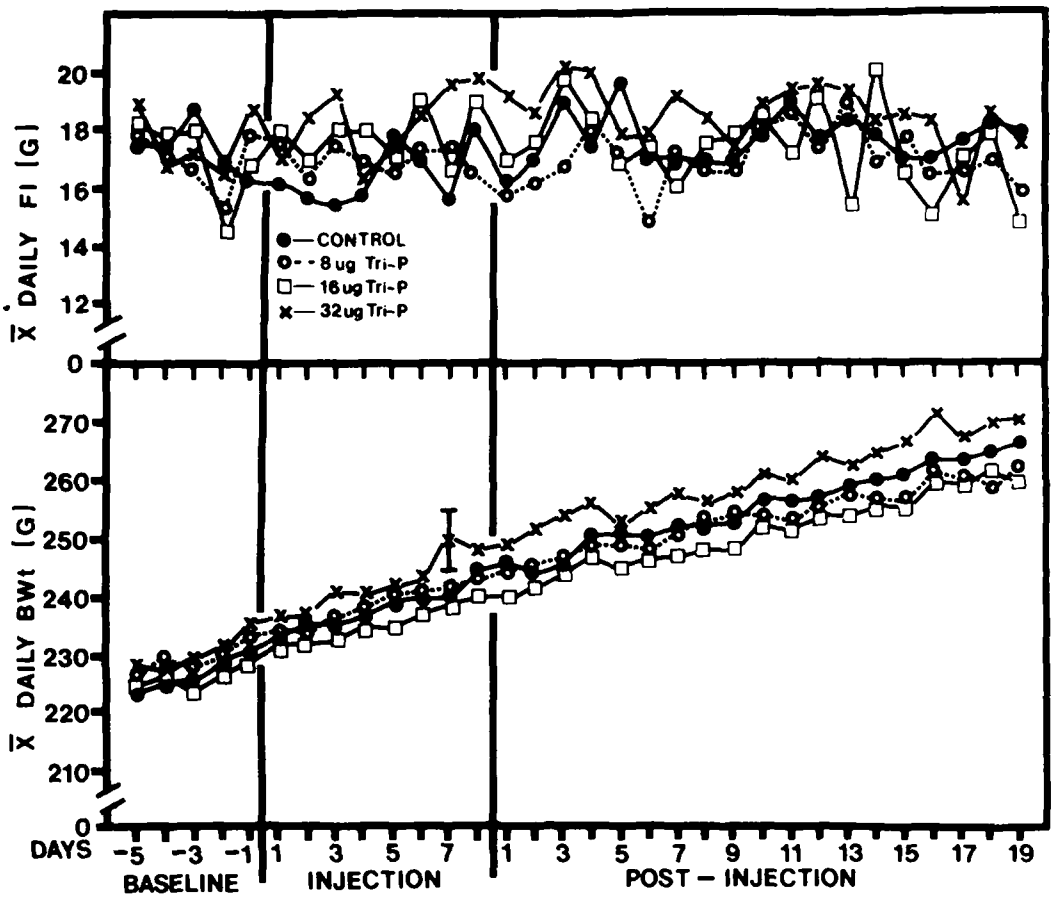

FIG. 1. Mean daily food intake (FI) and body weight (BWt) of four groups of female rats measured five days before (baseline) and 19 days after (Post-injection) an eight day injection period. The groups were injected with either total doses of 0.0 (control), 8.0, 16.0 or 32.0 μg of pyro-Glu-His-Gly-Oh (Tri-P).

After a 12 day baseline period, animals were injected SC with either 0.0 (0.10 ml of the diluent), 1.0, 2.0, or 4.0 μg of the tripeptide each day for eight days. Daily food intake and body weight was measured for an additional 19 days after the termination of the daily injections.

Results and Discussion

Illustrated in Fig. 1. is the mean daily food intake and body weight of the four groups of female rats treated with total doses of either 0, 8, 16 or 32 μg of the tripeptide. No significant group differences were found among the four groups at any time during or after the eight day period of injection. Also, all animals showed regular 4–5 day estrous cycles before, during and after the period of treatment. The most obvious difference between the above experiment and the reports of Trygstad and associates [1,3] which would account for the present negative results is difference in species.

EXPERIMENT 2
EFFECTS OF PYRO-GLU-HIS-GLY-OH ON THE FOOD INTAKE AND BODY WEIGHT OF FEMALE MICE

Although the results of Experiment 1 would tend to decrease the potential experimental utility of the present tripeptide, it was decided to test the anorexigenic potency of this substance in mice as originally reported by Trygstad *et al.*

Method

Forty-eight S/W albino female mice, weighing 30–35 g, were purchased from Simonsen Laboratories (Gilroy, CA) and were housed two per cage under conditions identical to those outlined in Experiment 1. After a seven day baseline period, the animals were divided into four equal groups (six cages per group) and were given either no treatment (uninjected controls) or else injected with total doses of 0.0 (0.1 ml of diluent), 3.4, or 6.8 μg of the peptide across a 20 day interval. The peptide was dissolved in an acidified saline solution (3.4 μg/ml) and injected daily SC. Daily individual body weights and food intakes (per two mice) were measured before and during the 20 day injection period and also during an additional two week postinjection interval.

Results and Discussion

As summarized in Tables 1 and 2, there were no statistically significant differences among any of the treatment groups in terms of body weights or food intakes at any time

TABLE 1

MEAN BODY WEIGHT OF FEMALE MICE BEFORE, FOLLOWING, AND TWO WEEKS AFTER THE TERMINATION OF EITHER NO TREATMENT (UNINJECTED CONTROLS) OR ELSE TOTAL DOSES OF EITHER 0.0, 3.4 or 6.8 μg OF PYRO-GLU-HIS-GLY-OH (TRI-P) ADMINISTERED ACROSS THE 20 DAY INJECTION PERIOD

Groups	(n)	Preinjection	Injection Day 20	Postinjection Day 14
Uninjected Controls	(12)	31.85 ± 0.50	34.75 ± 0.45	36.45 ± 0.45
0.0 μg Tri-P*	(11)†	31.56 ± 0.45	35.28 ± 0.51	37.42 ± 0.35
3.4 μg Tri-P	(12)	31.49 ± 0.48	34.57 ± 0.64	36.19 ± 0.73
6.8 μg Tri-P	(12)	32.35 ± 0.66	34.17 ± 0.75	36.60 ± 0.90

*pyro-Glu-His-Gly-OH.
†Sample size reduced due to death of one animal.

TABLE 2

MEAN TOTAL CUMULATIVE FOOD INTAKE (PER 2 ANIMALS) OF FEMALE MICE AFTER A 20 DAY TREATMENT PERIOD WITH EITHER NOTHING (UNINJECTED CONTROLS) OR ELSE TOTAL DOSES OF 0.0, 3.4 OR 6.8 μg OF PYRO-GLU-HIS-GLY-OH (TRI-P) AND ALSO SUBSEQUENT TOTAL FOOD INTAKE AFTER AN ADDITIONAL TWO WEEKS POST-INJECTION INTERVAL

Groups	(n)	Injection Day 20	Postinjection Day 14
Uninjected Controls	(6)	210.13 ± 5.22	151.60 ± 4.13
0.0 μg Tri-P*	(5)†	223.98 ± 11.37	154.24 ± 4.95
3.4 μg Tri-P	(6)	119.95 ± 7.38	151.78 ± 3.21
6.8 μg Tri-P	(6)	200.48 ± 3.58	157.52 ± 5.06

*pyro-Glu-His-Gly-OH.
†Sample size reduced due to death of one animal.

during the experiment. Thus, much like the results of Experiment 1, the above data offer no support for the results of Trygstad *et al.* [3] with respect to the reported anorexigenic potency of this new tripeptide. In additional experiments of a preliminary nature, we have also tried even higher doses (up to 3.4 μg/day) of the peptide in mice and have yet to observe any effects on food intake or body weight.

GENERAL DISCUSSION

Our data failed to reveal any anorexic effect of pyro-Glu-His-Gly-OH in rats or mice. Although the major discrepancy between our results and those of Trygstad *et al.* [1,3] may eventually be clarified, we must conclude at this time that the anorexigenic activity of this new tripeptide has yet to be firmly established.

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