PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 170, 264–272 (1982)

Inhibition of Sham Feeding-Induced Gastric Secretion and Serum Hormonal Responses by Analogs of (pyro)Glu–His–Gly-OH (41429)

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Abstract. The effects of intravenous infusion of analogs of (pyro)Glu–His–Gly-OH on secretion of gastric acid and elevation of serum gastrin and insulin levels induced by sham feeding were evaluated in conscious dogs. Tripeptides (pyro)Glu–3Me–His–Gly-OH, (pyro)Glu–His–D-Ala-OH, D-(pyro)Glu–His–Gly-OH, (pyro)Glu–His–Trp-NH_2, (pyro)Glu–His–Gly-NH_2, and (pyro)Glu–His–Gly-ethylamide, but not control peptides Glu–His–Pro-OH or Leu–Arg–Phe-OH, significantly suppressed the cephalic phase rise in serum insulin and gastrin as compared to saline-infused controls and lowered these hormonal levels below basal values. Analogs (pyro)Glu–3Me–His–Gly-OH, D-(pyro)Glu–His–Gly-OH, and (pyro)Glu–His–D-Ala-OH also significantly inhibited the gastric acid response to sham feeding. The reductions in gastric acid caused by (pyro)Glu–His–Gly-NH_2 or (pyro)Glu–His–Gly-ethylamide were less intense. Statistical analyses of food intake in 86 sham feeding experiments in seven dogs showed that the reduction during the infusion of (pyro)Glu–His–Gly-OH and its analogs was not significant by Duncan's multiple range test, and only that induced in (pyro)Glu–3Me–His–Gly-OH was significant by Student's *t*-test as compared to saline control. Our findings suggest that some analogs of (pyro)Glu–His–Gly-OH are more powerful inhibitors of the hormonal and gastric secretory responses during the cephalic phase stimulation than the original tripeptide.

The regulation of appetite and food intake involves mesencephalic and hypothalamic centers, but the exact mechanisms of this control are unknown (1). The hypothalamus has also been implicated in the excitation and inhibition of gastrointestinal functions (2, 3). Hypothalamic lesions and electrical stimulation of hypothalamic centers can produce alterations in the gastric secretion of rats (2–4). We have previously suggested that the mechanisms regulating feeding may be neurohormonal and that the hypothalamus may elaborate a substance responsible for direct control of appetite (3, 4). Such a substance could act on receptors localized in the CNS centers and involved in the regulation of appetite. Trygstad *et al.* (5) and Reichelt *et al.* (6) reported that a tripeptide, (pyro)Glu–His–Gly-OH, isolated from the urine of patients with "hypothalamic" anorexia nervosa produced a reduction in food consumption and a weight loss in mice, suggesting its anorexigenic properties. Although these claims could not be confirmed by several groups (7–9), our previous collaborative studies (10) established that the administration of (pyro)Glu–His–Gly-OH inhibited sham feeding-induced gastric acid and pepsin secretions, as well as the elevation of serum gastrin and insulin in dogs.

It is well known that hunger, anticipation of a meal, and sham feeding induce insulin secretion (10, 11). Stimulation of gastric secretion and rise in serum gastrin induced by sham feeding or by anticipation of a meal also depend on the desire for food (10, 12). Agents which reduce hunger and appetite decrease "psychic" stimulation of food intake and gastric secretion. The inhibition of gastric and hormonal responses during the cephalic phase by (pyro)Glu–His–Gly-OH observed in our first study (10) might reflect the suppression of appetite. The success in the development of various analogs of luteinizing hormone-releasing hormone

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0037-9727/82/070264-09$01.00/0

(LH-RH), somatostatin, thyrotropin-releasing hormone (TRH), enkephalin, and other peptides with greatly improved activity, led us to synthesize several analogs of (pyro)-Glu–His–Gly-OH. These were then tested for their effect on gastric and hormonal responses and food intake during sham feeding in dogs. Our findings are hereby reported.

Materials and Methods. *Peptide synthesis.* The tripeptides (pyro)Glu–His–Gly-OH, (pyro)Glu–3Me–His–Gly-OH, D-(pyro)-Glu–His–Gly-OH, (pyro)Glu–His–D-Ala-OH, (pyro)Glu–His–Gly-ethylamide, (pyro)-Glu–His–Gly-NH_2, and Leu–Arg–Pro-OH were prepared by solid phase methods. The free acids were cleaved from the Merrifield resin by HF treatment and the ethylamide by treatment with ethylamine. The amide was prepared on a benzhydrylamine resin support and cleaved by HF. Peptides were purified by gel filtration on Sephadex G-15 in 2 *M* acetic acid followed by chromatography on silica gel in a 1-butanol: acetic acid:water:ethyl acetate (1:1:1:1) solvent system. All peptides were homogeneous in four solvent systems and gave the correct amino acid analyses after acid hydrolysis. These tripeptides had very low TRH activity and prolactin-releasing activity, which will be reported in detail elsewhere (T. W. Redding *et al.*, in preparation). Glu–His–Pro-OH (Hoffman–La Roche Co., Basle, Switzerland) and (pyro)-Glu–His–Trp-NH_2 (Ayerst Research Lab) were made by classical methods of synthesis and represented gifts from the respective companies. All the peptides were dissolved in 0.15 *M* NaCl immediately before infusion.

Animals and experimental procedure. Six adult male mongrel dogs and one pure bred beagle were used for studies on:

(a) plasma hormonal responses (insulin and gastrin) to sham feeding;

(b) total gastric acid secretion in response to sham feeding;

(c) food consumption.

The dogs were first surgically equipped under pentobarbital anesthesia with esophageal and gastric fistulae essentially as described by Olbe (13). Three weeks after surgery the animals were used for sham feeding experiments. The dogs were deprived of food, but not water, 48 hr prior to the experiments. Experiments on a given dog were conducted no more than once a week. During the experiments each animal was kept in a metabolic cage while the esophageal fistula was opened and the esophagus distal to the fistula was occluded as for sham feeding. In each experiment the peptides were administered by intravenous infusion at a standard dose of 100 μg/kg-hr for 30 min before, during (30 min), and after sham feeding (105 min), using a peristaltic pump calibrated to deliver 40 ml/hr. The order in which various peptides were tested was completely randomized. In control studies 0.15 *M* NaCl was infused. Venous blood samples were drawn every 15- to 30-min period and placed in prechilled tubes containing EDTA for hormonal determinations. Gastric juice was withdrawn from the stomach with an 18-gauge Foley catheter by syringe suction every 15 min, except for the sham feeding period when it was collected after 30 min in order not to interfere with this procedure.

The animals were fed a semi-liquid food prepared by mixing commercial dog food consisting of meat, soybean, etc. (Kam Dog Food, Strongheart Products, Kansas City) in a blender with 150 ml water per can of dog food. For some dogs, it was necessary to use beef broth or Alpo liver chunks to stimulate eating behavior. In these cases this diet was used in all sham feeding experiments in a given dog. The sham feeding period lasted 30 min. The food which fell from the esophageal fistula into the stainless steel funnel of the metabolic cage was reconsumed in most cases. Because of great variation in food intake between dogs and within the same dogs on different days, several experiments were usually performed in each dog. Since the weight of the dogs ranged from 10 to 20 kg, gastric acid secretion and food consumption were expressed per kilogram of body weight (b.w.).

Determinations. The volume of gastric acid was measured to the nearest 0.2 ml and the total acidity was determined by titration with 0.1 *M* NaOH. Serum insulin was determined by double antibody RIA using a commercial kit (Cambridge Nuclear Co.).

The variation within each assay was 1.4% and the interassay variation was 2.9%. Serum gastrin was also determined by double antibody RIA with a kit (Cambridge Nuclear Co.). The variation within each assay was 1.2% and the interassay variation was 2.4%.

All data were expressed as the mean ± standard error. Statistical evaluation of the levels of insulin and gastrin was made by Duncan's new multiple range test (14). Gastric acid secretion and food consumption were compared both by Duncan's test and Student's *t* test (15), using programs adapted for our computer.

Results. *1. Insulin responses.* After 30 min of iv infusion, none of the nine peptides tested or saline produced changes in the basal levels of insulin (15.7 ± 0.5 μU/ml) as compared with preinfusion values (15.6 ± 0.5 μU/ml) (Fig. 1). In all 21 experiments in 7 dogs, when saline was infused serum insulin increased after sham feeding, highest levels (32.8 ± 0.9 μU/ml) being reached 15 min after sham feeding was stopped (Period 5). Insulin remained significantly elevated for the next 75 min, but returned to basal values (16.4 ± 0.6 μU/ml) after 2 hr (Fig. 1). Infusion of (pyro)Glu–His–Gly-OH and six of its analogs suppressed the sham feeding-induced rise in insulin. The levels of insulin actually fell below basal values and remained significantly suppressed as compared with saline controls until the 10th 15-min period. The greatest depression in insulin levels was obtained with (pyro)Glu–3Me–His–Gly-OH and D-(pyro)Glu–His–Gly-OH (8.0 ± 0.6 and 8.4 ± 0.05 μU/ml, respectively ($P < 0.01$ vs saline control). Control tripeptides Glu–His–Pro-OH and Leu–Arg–Phe-OH did not alter the cephalic phase rise in serum insulin, the peak levels being 32.0 ± 3.6 and 32.3 ± 2.5 μU/ml, respectively (Fig. 1). No visible side ef-

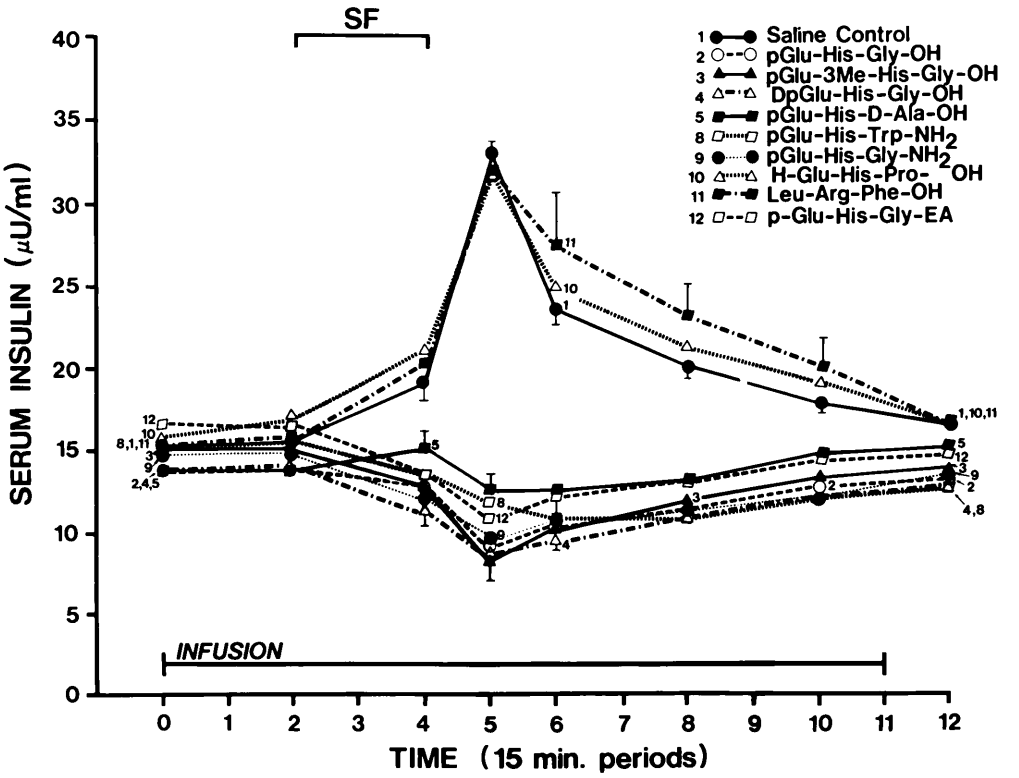

FIG. 1. Effect of intravenous infusion of saline (control), (pyro)Glu–His–Gly-OH and its analogs on serum insulin levels in response to sham feeding (SF) in dogs with esophagostomy.

fects were observed during the infusion of any of the tripeptides.

2. Gastrin responses. Serum gastrin levels (44.5 ± 0.9 pg/ml) were not altered by the initial 30-min infusion of saline (44.7 ± 0.7 pg/ml) or any of the nine tripeptides (Fig. 2). Sham feeding produced a marked rise in gastrin with the peak (73.8 ± 1.8 pg/ml) occurring 15 min after cessation of sham feeding (Fig. 2). (Pyro)Glu–His–Gly-OH and all six of its peptide analogs, but not Glu–His–Pro-OH or Leu–Arg–Phe-OH, inhibited the sham feeding-induced rise in gastrin. Some tripeptides actually depressed gastrin levels below basal values, the most potent being again (pyro)-Glu–3Me–His–Gly-OH and D-(pyro)Glu–His–Gly-OH, which lowered gastrin to 27.1 ± 1.9 and 28.0 ± 0.9 pg/ml, respectively (Fig. 2). The least active appeared to be (pyro)Glu–His–D-Ala-OH and (pyro)Glu–His–Trp-NH_2, which produced levels of 40.0 ± 4.4 and 34.4 ± 2.7 pg/ml, respectively. Two hours after sham feeding serum gastrin returned to essentially control levels.

3. Effects on sham feeding-induced gastric secretion. The amounts of gastric acid collected in periods 0 to 2 (0–30 min) before the start of sham feeding ranged from 0 to 0.02 mmole H^+/kg b.w. (mean = 0.017 ± 0.0069 mmole H^+/kg). During the infusion of saline, sham feeding produced a marked increase in gastric acid output as in our previous studies (10, 16). The highest amount was collected in the 30-min period that began at the start of sham feeding (minutes 30–60 of the experiment), and then gastric secretion gradually returned to basal levels

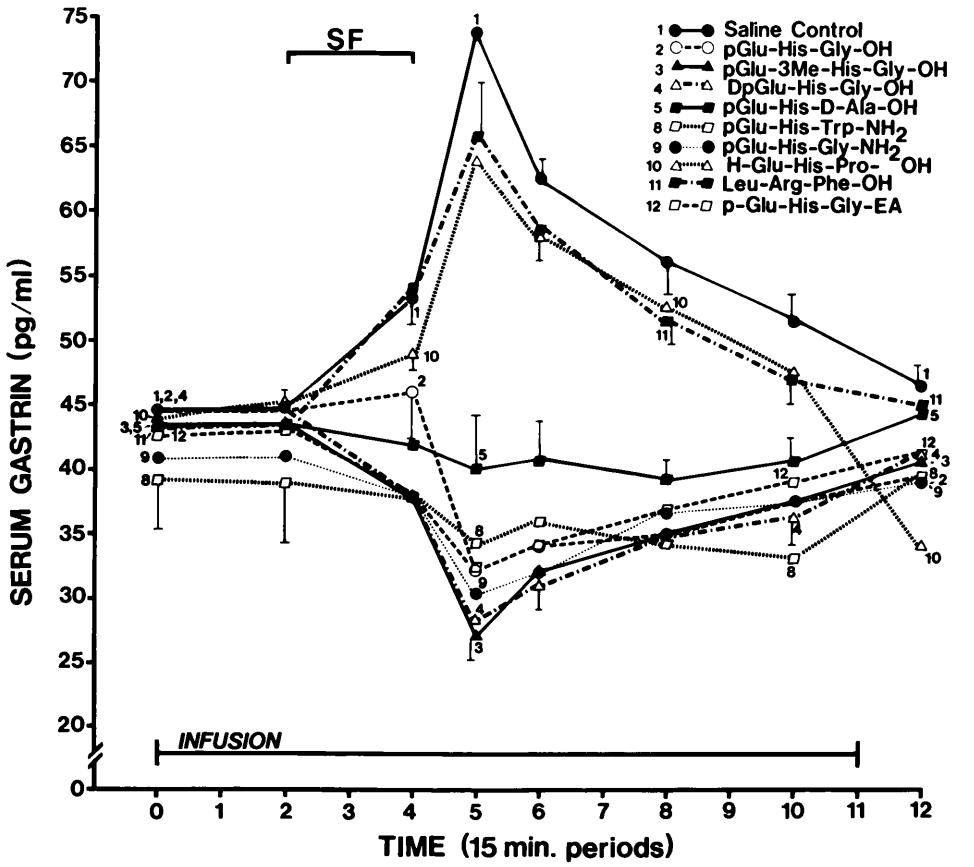

FIG. 2. Effect of intravenous infusion of saline (control), (pyro)Glu–His–Gly-OH and its analogs on serum gastrin levels in response to sham feeding (SF) in dogs with esophagostomy.

in about 1.5 hr (Table I). In 21 experiments in seven dogs, the mean output of gastric acid during the sham feeding period was 0.77 ± 0.09 mmole H^+/kg (Table I). The infusion of (pyro)Glu–His–Gly-OH diminished the secretion to 0.54 ± 0.07 mmole H^+/kg in agreement with our previous results, but this reduction was not significant either by Duncan's test or by Student's *t* test. In contrast, (pyro)Glu–3Me–His–Gly-OH, D-(pyro)Glu–His–Gly-OH and (pyro)Glu–His–D-Ala-OH caused a significant reduction in the gastric acid output as compared with saline both by Duncan's test and Student's *t* test (Table I). (Pyro)Glu–3Me–His–Gly-OH appeared to be the most active and diminished acid secretion by more than twofold. The reductions induced by (pyro)Glu–His–Gly-NH_2 and (pyro)Glu–His–Gly-ethylamide were significant only by Student's *t* test. There was a significant difference between (pyro)-Glu–3Me–His–Gly-OH and the original tripeptide (No. 2) by Student's *t* test. (Pyro)-Glu–His–Trp-NH_2, Glu–His–Pro-OH and Leu–Arg–Phe–OH were inactive (Table I). Infusion of (pyro)Glu–3Me–His–Gly-OH, D-(pyro)Glu–His–Gly-OH and (pyro)-Glu–His–D-Ala-OH also decreased gastric acid output somewhat, as compared with saline during the 45- to 60-min period following sham feeding, but the differences were not significant statistically.

4. Food consumption. During the infusion of saline, the mean amount of food ingested in 30 min by seven hungry dogs in 20 sham feeding experiments was 299.9 ± 28.6 g/kg b.w. (Table II). The infusion of (pyro)Glu–His–Gly-OH reduced the food intake to 255.6 ± 35.9 g/kg b.w., but this decrease was not significant statistically either by Duncan's or by Student's *t* test vs controls infused with saline. (Pyro)Glu–3Me–His–Gly-OH caused the greatest reduction in food consumption (182.9 ± 35.8 g/kg b.w.), which was significant by Student's *t* test. D-(Pyro)Glu–His–Gly-OH and (pyro)Glu–His–Gly-ethylamide also appeared to inhibit feeding, but the differences were not significant vs the control. Infusion of the other five tripeptides did not influence food intake, as the dogs con-

TABLE I. OUTPUT OF GASTRIC ACID IN DOGS AFTER SHAM FEEDING AND INFUSION OF SALINE OR OF TRIPEPTIDES

Peptide	Peptide No.	Number of experiments	Number of dogs	Time periods (min)					*P* values for period 30–60 min vs control	
				30–60	60–75	75–90	90–105	105–120	Duncan's test	Student's *t* test
Saline control		21	7	0.77 ± 0.09[a]	0.19 ± 0.02	0.16 ± 0.02	0.1 ± 0.01	0.08 ± 0.01		
pGlu–His–Gly-OH	2	10	7	0.54 ± 0.07	0.18 ± 0.04	0.13 ± 0.03	0.1 ± 0.02	0.08 ± 0.02	NS	NS
pGlu–3Me–His–Gly-OH	3	16	7	0.29 ± 0.04	0.13 ± 0.02	0.1 ± 0.01	0.1 ± 0.01	0.08 ± 0.01	<0.05	<0.001
D-pGlu–His–Gly-OH	4	12	7	0.37 ± 0.03	0.14 ± 0.02	0.09 ± 0.02	0.07 ± 0.01	0.09 ± 0.02	<0.05	<0.001
pGlu–His–D-Ala-OH	5	6	7	0.32 ± 0.06	0.16 ± 0.04	0.09 ± 0.03	0.06 ± 0.03	0.04 ± 0.02	<0.05	<0.001
pGlu–His–Trp-NH_2	8	3	3	0.64 ± 0.03	0.25 ± 0.05	0.21 ± 0.04	0.015 ± 0.04	0.09 ± 0.04	NS	NS
pGlu–His–Gly-NH_2	9	5	5	0.43 ± 0.03	0.14 ± 0.02	0.13 ± 0.03	0.06 ± 0.03	0.14 ± 0.05	NS	<0.01
H-Glu–His–Pro-OH	10	3	3	0.5 ± 0.11	0.14 ± 0.0	0.1 ± 0.03	0.09 ± 0.03	0.06 ± 0.03	NS	NS
Leu–Arg–Phe-OH	11	5	5	0.71 ± 0.12	0.17 ± 0.06	0.13 ± 0.05	0.11 ± 0.04	0.06 ± 0.02	NS	NS
pGlu–His–Gly-EA	12	5	5	0.45 ± 0.1	0.21 ± 0.1	0.15 ± 0.07	0.09 ± 0.03	0.09 ± 0.04	NS	<0.025
								3 vs 2	NS	<0.025
								4 vs 2	NS	NS

Note. Saline or the peptides were infused from time 0 for 11 periods of 15 min = 2 hr 45 min. Last blood was taken at period 12 = 3 hr from time 0. Sham feeding for 30 min (two 15-min periods) was begun at 30 min from the start of the infusion.

[a] Results are mmole H^+/kg (mean ± SE).

TABLE II. FOOD CONSUMPTION IN DOGS DURING SHAM FEEDING AND INFUSION OF SALINE OR OF TRIPEPTIDES

Peptide	Peptide No.	Number of experiments	Number of dogs	Food consumed g/kg b.w. (mean ± SE)	P values vs. control	
					Duncan's test	Student's *t* test
Saline control		20	7	299.9 ± 28.6	—	—
pGlu–His–Gly-OH	2	12	7	225.6 ± 35.9	NS	NS
pGlu–3Me–His–Gly-OH	3	14	7	182.9 ± 35.8	NS	0.025
D-pGlu–His–Gly-OH	4	12	7	241.1 ± 36.7	NS	NS
pGlu–His–D-Ala-OH	5	7	7	320.7 ± 56.8	NS	NS
pGlu–His–Trp-NH_2	8	3	3	338.4 ± 100.1	NS	NS
pGlu–His–Gly-NH_2	9	4	4	305.0 ± 68.2	NS	NS
H-Glu–His–Pro-OH	10	3	3	319.0 ± 106.5	NS	NS
Leu–Arg–Phe-OH	11	6	5	288.2 ± 82.5	NS	NS
pGlu–His–Gly-EA	12	3	3	198.8 ± 87.8	NS	NS

Note. Saline or the peptides were infused from time 0 for 11 periods of 15 min = 2 hr 45 min. Last blood was taken at period 12 = 3 hr from time 0. Sham feeding for 30 min (two 15-min periods) was begun at 30 min from the start of the infusion.

sumed about 300 g/kg b.w. in each case, which was similar to the value obtained for controls.

Discussion. Both neural and hormonal pathways have been implicated in the complex mechanisms of regulation of gastric secretion and control of appetite (1–4). The involvement of the central nervous system in these processes has been demonstrated by several independent lines of investigation. The cephalic phase, induced by sham feeding or by anticipation of a meal, involves the release of gastrin and insulin and stimulation of gastric and pancreatic secretions (10, 12, 16). Alterations in appetite during sham feeding by various peptides might in turn alter vagally mediated gastric secretions. Based on the changes in eating behavior following stimulation or destruction of the hypothalamic centers, a neural control of appetite and satiety has been proposed (17). It has also been suggested that the hypothalamus may elaborate a substance responsible for the central control of appetite (3, 4). Direct evidence for hypothalamic, diencephalic, or mesencephalic neurotransmitters involved in the control of appetite is still lacking, although the presence of a lipid mobilizing factor which could be implicated in the neural control of obesity has been shown in extracts of ventral hypothalami of sheep, cattle, pigs, and human beings (18, 19). Demonstration of a peptide in the urine of patients with "hypothalamic" anorexia nervosa (5, 6), which was characterized as (pyro)Glu–His–Gly-OH and which was reported to reduce food consumption in mice, prompted new investigations. Several groups, including ours, subsequently found that this tripeptide will not influence food intake or body weights in mice and rats (7–9). Nevertheless, since hunger is associated with changes in gastrointestinal functions, we examined the effects of (pyro)Glu–His–Gly-OH in dogs and found that it decreases gastric secretion and insulin and gastrin responses to sham feeding (10).

In view of ample evidence from various laboratories, including our own, that the biological activity of a variety of peptides can be increased to a very high degree by

discreet structural modifications, we decided to synthesize some analogs of (pyro)-Glu–His–Gly-OH and test their effects in dogs and rats. Our findings indicate that infusion of several analogs of (pyro)Glu–His–Gly-OH can significantly suppress the elevation of insulin and gastrin in dogs in response to sham feeding. In fact, the levels of both gastrin and insulin fell below control values after 1 hr of infusion with a distinct nadir between 60 and 90 min. (Pyro)Glu–3Me–His–Gly-OH and D-(pyro)Glu–His–Gly-OH appeared to cause the greatest depression in serum insulin and gastrin levels. Control tripeptides, H-Glu–His–Pro-OH (corresponding to the amino acid sequence of uncyclized TRH) and Leu–Arg–Phe-OH, were inactive. The cephalic phase of insulin and gastrin secretion has been established in rats (20, 21), dogs (22, 23, 10), and human beings (11, 25). Since (pyro)Glu–His–Gly-OH and its analogs did not influence basal or postprandial levels of insulin and gastrin in dogs ((10) and unpublished results), the suppression of hormonal responses during the cephalic phase might reflect the inhibition of appetite. The infusion of (pyro)Glu–3Me–His–Gly-OH, D-(pyro)Glu–His–Gly-OH, and (pyro)Glu–His–D-Ala-OH also significantly suppressed gastric acid response to sham feeding. The suppression of gastric acid produced by (pyro)Glu–His–Gly-NH_2 and (pyro)Glu–His–Gly–ethylamide was significant only by Student's *t* test. Although (pyro)Glu–His–Gly-OH inhibited gastric acid release in accord with our previous work (10), this inhibition was not significant statistically in the present study.

Our recent work indicates that TRH significantly suppressed gastric acid secretion induced by sham feeding or pentagastrin (16). However, in contrast to our analogs, TRH did not inhibit the rise in gastrin following sham feeding (16). TRH is a powerful releaser of prolactin in addition to thyrotropin and differs from our tripeptides in having C-terminal proline. (Pyro)Glu–His–Gly-OH and its tripeptide analogs reported here have less than 0.1% TRH activity and 0.3% of prolactin-releasing (PRH) activity of TRH. TRH is found in the highest concentrations in the hypothalamus, but it is distributed throughout the CNS and even in the G.I. tract (26). The administration of TRH is associated with brief side effects which might be due in part to a variety of actions on the CNS and in part to action on the G.I. tract (26). (Pyro)Glu–His–Gly-OH and its analogs reported in this study were devoid of any visible side effects in dogs when infused in doses of 100 μg/kg for 2 hr 45 min. An autopsy on a dog which received more than 10 infusions of analogs and died from an unrelated infection, showed no gross abnormalities in major organs. (Pyro)Glu–His–Gly-OH given to mice as a rapid iv injection produced no visible side effects in doses as high as 20–50 mg/kg and no evidence of toxicity was found after chronic administration of 1 mg/kg/day for 20 days.

TRH infused intravenously inhibits pentagastrin-stimulated gastric secretion in men (27) and in dogs (16), although after intraventricular administration it was reported to increase vagus-dependent gastric secretion in rats (28, 29). TRH given intraventricularly or parenterally was also reported to reduce food intake in rats (30, 31). On the basis of these results it was suggested that TRH may play a role in the control of food intake as a peptidergic mediator of satiety (30, 31).

Other peptides, among them cholecystokinin (32–34), somatostatin (35), and bombesin (36), have been shown to decrease food intake and/or elicit behavioral sequences of satiety in rats, sheep, and men and have been suggested as neuroregulators involved in the control of appetite. Cholecystokinin and somatostatin are distributed throughout the CNS (37, 38). However, recent studies by Smith *et al.* (39) suggest that parenterally administered cholecystokinin might act in the abdomen through gastric vagal fibers and not directly on the brain to produce satiety in the rat.

The presence of (pyro)Glu–His–Gly-OH in hypothalamic extracts has not been confirmed, and its physiological role in the control of gastric secretion and food intake remains speculative. It is interesting, never-

theless, that some of its analogs, particularly (pyro)Glu–3Me–His–Gly-OH, can inhibit the hormonal and gastric secretory events during cephalic phase. The high activity of the 3Me–His analog in the systems tests employed, including a possible effect on food intake, is in agreement with greater TRH, PRH, and CNS potency of (3Me-His^2) TRH as compared to TRH (28, 40). The design and synthesis of additional analogs of (pyro)Glu–His–Gly-OH may possibly lead to a new class of inhibitors of gastric secretion and anorexigenic agents.

This work was supported by a grant from Biomeasure, Inc., U.S. Public Health Service Grant AM 07467 (to A.V.S.) and the Medical Research Service of the Veterans Administration. Two of us, R.M.S. and P.A.B., were recipients of fellowships from Instituto Mexicano Del Seguro Social. We are grateful to Mr. Bill Donovan for preparing computer programs for statistical analyses and for help in calculations.

1. Brobeck JR. Identity of hypothalamic feeding mechanisms. J Neuro-Visc Rel Suppl. 10:277–281, 1971.
2. Smith GP, Brooks FP. Hypothalamic control of gastric secretion. Gastroenterology 52:727–729, 1967.
3. Schally AV, Arimura A, Bowers CY, Kastin AJ, Sawano S, Redding TW. In: Astwood EB, ed. Recent Progress in Hormone Research. New York, Academic Press, Vol 24:pp497–588, 1968.
4. Schally AV, Bowers CY, Locke W. Neurohumoral functions of the hypothalamus. Amer J Med Sci 248:79–101, 1964.
5. Trygstad O, Foss I, Edminson PD, Johanson JH, Reichelt KL. Humoral control of appetite by a urinary anorexigenic peptide. Chromatographic patterns of urinary peptides in anorexia nervosa. Acta Endocrinol (Copenhagen) 89:196–208, 1978.
6. Reichelt KL, Foss I, Trygstad O, Edminson PD, Johansen JH, Boler JB. Humoral control of appetite-II. Puricication and characterization of an anorexigenic peptide from human urine. Neuroscience 3:2107–2111, 1978.
7. Nance DM, Coy DH, Kastin AJ. Experiments with a proposed anorexigenic tripeptide: (Pyro)Glu–His–Gly-OH. Pharmacol Biochem Behav 11:733–735, 1979.
8. Knoll J. Satietin: A highly potent anorexigenic substance in human serum. Physiol Behav 23:497–502, 1979.
9. Redding TW, Comaru-Schally AM, Coy DH, Schally AV. A lack of effect of (pyro)Glu–His–Gly-OH on the control of appetite and body weight in male mice. Fed Proc 39:782, 1980.
10. Coy DH, Jaworek J, Konturek SJ, Kwiecien N, Radecki T, Schally AV, Tasler J. Effects of anorexigenic peptide on gastric and pancreatic secretion. J Physiol 314:225–235, 1981.
11. Parra-Covarrubias A, Rivera-Rodriguez I, Almaraz-Ugalde A. Cephalic phase of insulin secretion in obese adolescents. Diabetes 20:800–802, 1971.
12. Moore JG, Motoki D. Gastric secretory and humoral responses to anticipated feeding in five men. Gastroenterology 76:71–74, 1979.
13. Olbe L. Esophageal cannula dog, a simple mode of preparation for sham feeding experiments. Gastroenterology 37:460–462, 1959.
14. Steel RGD, Torrie HJ. Principles and Procedures of Statistics. New York, McGraw–Hill, 1960.
15. Snedecor GW, Cochran WF. Statistical Methods. Ames, Iowa State Univ Press, pp98–145, 1967.
16. Konturek SJ, Jaworek J, Cieszkowski M, Schally AV. Effect of thyrotropin-releasing hormone on gastro-intestinal secretions in dogs. Life Sci 22:2289–2298, 1981.
17. Anand BK, Brobeck JR. Hypothalamic control of food intake in rats and cats. Yale J Biol Med 24:123–140, 1952.
18. Redding TW, Schally AV. A lipid factor from the hypothalamus. Metabolism 19:641–651, 1970.
19. Redding TW, Schally AV. Effect of hypothalamic preparations on human omental adipose tissue *in vitro*. Metabolism 21:499–506, 1972.
20. Louis-Sylvestre J. Preabsorptive insulin release and hypoglycemia in rats. Amer J Physiol 230:56–60, 1976.
21. Steffens AB. Influence of the oral cavity on insulin release in the rat. Amer J Physiol 230:1411–1415, 1976.
22. Preshaw RM. Gastric acid output after sham feeding and during release or infusion of gastrin. Amer J Physiol 219:1409–1416, 1970.
23. Nilsson G, Simon J, Yalow RS, Berson SA. Plasma gastrin and gastric acid responses to sham feeding and feeding in dogs. Gastroenterology 63:51–59, 1972.
24. Feldman M, Richardson CT, Taylor IL, Walsh JH. Effect of atropine on vagal release of gastrin and pancreatic polypeptide. J Clin Invest 63:294–298, 1979.
25. Goldfine ID, Abraira C, Gruenewald D, Goldstein MS. Plasma insulin levels during imaginary food ingestion under hypnosis. Proc Soc Exp Biol Med 133:274–276, 1970.
26. Morley JE. Extrahypothalamic thyrotropin releasing hormone (TRH)—Its distribution and its functions. Life Sci 25:1539–1550, 1979.
27. Dolva LO, Hanssen KF, Berstad A. Actions of thyrotropin-releasing hormone on the gastrointestinal function in man. II. Inhibition of

pentagastrin-stimulated acid secretion. Scand J Gastroenterol **14**:33–34, 1979.
28. Tache Y, Vale W, Brown M. Thyrotropin-releasing hormone—CNS action to stimulate gastric acid secretion. Nature (London) **287**:149–151, 1980.
29. Morley JE, Levine AS, Silvis SE. Endogenous opiates inhibit gastric acid secretion induced by central administration of thyrotropin-releasing hormone (TRH). Life Sci **29**:293–297, 1981.
30. Vijayan E, McCann SM. Suppression of feeding and drinking activity in rats following intraventricular injection of thyrotropin-releasing hormone (TRH). Endocrinology **100**:1727–1730, 1977.
31. Morley JE, Levine AS. Thyrotropin releasing hormone (TRH) suppresses stress induced eating. Life Sci **27**:269–274, 1980.
32. Gibbs J, Young RC, Smith GP. Cholecystokinin elicits satiety in rats with open gastric fistulas. Nature (London) **245**:323–325, 1973.
33. Lorenz DN, Kreielsheimer G, Smith GP. Effect of cholecystokinin, gastrin, secretin and GIP on sham feeding in the rat. Physiol Behav **23**:1065–1072, 1979.
34. Morley JE. The neuroendocrine control of appetite: The role of the endogenous opiates, cholecystokinin, TRH, Gamma-amino-butyric-acid and the diazepam receptor. Life Sci **27**:355–368, 1980.
35. Lotter EC, Krinsky R, McKay JM, Treneer CM, Porte D, Jr, Woods SC. Somatostatin decreases in food intake of rats and baboons. J Comp Physiol Psychol **95**:278–287, 1981.
36. Martin CF, Gibbs J. Bombesin elicits satiety in sham feeding rats. Peptides **1**:131–134, 1980.
37. Straus E, Yalow RS. Brain cholecystokinin in fasted and fed mice. Life Sci **26**:969–970, 1980.
38. Brownstein MJ. Distribution of hypothalamic peptides. In: Beers, RF, Bassett EG, eds. Polypeptide Hormones. New York, Raven Press, pp193–202, 1980.
39. Smith GP, Jerome C, Cushin BJ, Eterno R, Simansky KJ. Abdominal vagotomy blocks the satiety effect of cholecystokinin in the rat. Science **213**:1036–1037, 1981.
40. Rivier J, Vale W, Monahan M, Ling N, Burgus R. Synthetic thyrotropin-releasing factor analogs 3. J Med Chem **15**:479–482, 1972.

Received November 23, 1981. P.S.E.B.M. 1982, Vol. 170.