

Regulation of Gastric Acid Secretion by Neurotensin in Man

Evidence Against a Hormonal Role

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Abstract

The present study was designed to evaluate neurotensin as a hormonal regulator of gastric acid secretion in man. After a fat-rich meal, the strongest known stimulus of neurotensin release, plasma neurotensin-like immunoreactivity (NTLI) was elevated from 7.6 ± 1.9 to 15.5 ± 2.5 pM. Plasma NTLI was measured with antiserum L170, which requires the biologically active carboxyl-terminal hexapeptide of the neurotensin molecule for recognition and does not crossreact significantly with any known natural catabolite in human plasma. Intravenous infusion of neurotensin at $25 \text{ pmol} \cdot \text{kg}^{-1} \text{ h}^{-1}$ resulted in a plasma level of 14.7 ± 2.1 pM, similar to the maximal physiological level observed after the fat-rich meal. Intravenous infusion of neurotensin at $25 \text{ pmol} \cdot \text{kg}^{-1} \text{ h}^{-1}$ during 2 h, however, failed to significantly inhibit peptone meal-stimulated gastric acid secretion measured by intragastric titration. The 2-h acid output to peptone was 40.8 ± 6.2 and 41.3 ± 6.9 mmol during the vehicle and the neurotensin infusion, respectively. Intravenous infusion of neurotensin at 100 or $400 \text{ pmol} \cdot \text{kg}^{-1} \text{ h}^{-1}$ did not affect acid output, whereas at $1,600 \text{ pmol} \cdot \text{kg}^{-1} \text{ h}^{-1}$, which resulted in a plasma neurotensin concentration of 725 ± 80 pM, significantly reduced peptone meal-stimulated gastric acid secretion. The neurotensin-induced inhibition of acid output was independent of the hormone gastrin. The present results provide evidence against a hormonal role for neurotensin in the regulation of meal-stimulated gastric acid secretion in man.

Introduction

Neurotensin, pGlu-Leu-Tyr-Glu-Asn-Lys-Pro-Arg-Arg-Pro-Tyr-Ile-Leu-OH, a candidate regulator of gastric functions, was originally isolated from bovine hypothalamus (1). Related peptides have been immunologically detected in species throughout evolutionary stages from the primitive organism *Escherichia Coli* (2) to the *Homo sapiens* (3, 4). During evolution, the carboxyl-terminal neurotensin-like immunoreactivity (NTLI)¹ has been conserved (5, 6). The carboxyl-terminal hexapeptide is required for biological activity (7), whereas no action has been positively identified for amino-terminal fragments. Tissue concentrations of NTLI determined by different region specific antisera vary considerably, indicating neuroten-

sin molecular heterogeneity. In mammals, intact neurotensin is found in endocrine-like N cells predominantly in the distal small intestine, whereas it is sparse in the stomach (8). In contrast, high levels of a smaller molecule with carboxyl-terminal NTLI have been found in rat gastric tissues (9). Neurotensin has been suggested to be a hormonal regulator of postprandial gastrointestinal functions since NTLI is elevated in peripheral blood after a meal, and intravenous administration of this peptide affects various gastrointestinal functions (10) and causes inhibition of gastric acid secretion (11). However, critical analysis reveals that a hormonal role for neurotensin cannot be concluded from studies performed so far. In previous studies on neurotensin-induced acid secretory inhibition, neurotensin was measured by radioimmunoassay (RIA) in nonextracted plasma (10, 12, 13) and/or with amino-terminally directed antiserum (10). Such measurements may result in artificially high values and do not necessarily reflect biologically active circulating levels. Chromatographic examinations indicate that most of the elevation of plasma NTLI after a meal corresponds to the amino-terminal catabolites NT 1-8 and NT 1-11, whereas the bioactive parent peptide NT 1-13 only accounts for a minor fraction (14-16). An antiserum specific for the biologically active portion of neurotensin was employed in the present study to determine whether or not the postprandial rise in neurotensin could account for hormonal inhibition of acid secretion, and it is concluded that it does not.

Methods

Physiological studies. Studies were performed on eight healthy male volunteers: mean age, 32 ± 4 (18-41) yr; mean weight, 78 ± 4 (63-92) kg. The study was approved by the Veterans Administration Wadsworth Committee on Human Studies, and informed consent was obtained from each subject. Synthetic neurotensin was administered under IND 23 018 of the Food and Drug Administration.

After an overnight fast, a double-lumen tube was introduced into the stomach. The position of the tube was checked by a water recovery test. After complete emptying of the stomach by aspiration, 500 ml of a 6% glucose solution was instilled intragastrically and acid secretion was quantified by automated intragastric titration at pH 5.5 (17). This procedure of liquid meal administration was repeated five times using 8% peptone (Bacto Peptone; Difco Laboratories Inc., Detroit, MI).

All subjects were studied on three separate days in random order. On 1 d, synthetic human neurotensin (Peninsula Laboratories, Inc., Belmont, CA), dissolved in 0.15 M NaCl containing 0.25% human serum albumin, was infused into the left antecubital vein during the last three peptone meals at sequentially increasing doses each of 30 min duration: 100, 400, and $1,600 \text{ pmol} \cdot \text{kg}^{-1} \text{ h}^{-1}$. On a 2nd d, only vehicle was infused. On a 3rd d, the subjects were given a fat-rich meal consisting of 88 g fat, two slices bread, 4 oz cream cheese, and 1 qt milk.

Pilot experiments revealed that the plasma NTLI level obtained after a fat-rich meal could be reproduced by intravenous infusion of neurotensin at $25 \text{ pmol} \cdot \text{kg}^{-1} \text{ h}^{-1}$. To evaluate the effect of a physiological plasma NTLI level on gastric acid secretion, six of the eight volunteers were reexamined using similar methods as described above. Basal

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1. Abbreviation used in this paper: NTLI, neurotensin-like immunoreactivity.

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acid output was measured by suction aspiration for 30 min, and peptone meal-stimulated acid output was measured by intragastric titration during 2 h (four 30-min peptone meals). In random order on two different days, the subjects received neurotensin or vehicle intravenously during the 2-h peptone meal stimulation. Plasma samples for NTLI measurements were obtained at 30 min intervals.

RIA. Blood samples were collected into chilled tubes, containing 500 kIU aprotinin (Trasylol; Bayer, FRG) and 0.13 mg EDTA per ml whole blood, from a needle in the right antecubital vein and centrifuged at 4°C for 10 min. The plasma was stored at -20°C until measured by RIA for gastrin with antiserum 1611 (18) and for neurotensin with antiserum L170 (19), as previously described. To concentrate neurotensin and to eliminate nonspecific assay interference, 3 ml of plasma was extracted on Sep Pak C₁₈ cartridges (Waters Associates, Milford, MA), as previously described (19). The recoveries of synthetic neurotensin added to hormone-free plasma to obtain plasma levels of 0, 10, 15, 25, 50, 100, and 500 pM were 0, 105±15, 86±11, 92±6, 84±6, 88±8, and 85±6%, respectively (*n* = 8). The relative immunoreactivity, as determined by the amount of peptide needed to inhibit by 50% the binding of iodinated neurotensin in the RIA, is demonstrated in Table I. Neurotensin antiserum L170 requires the biologically active carboxyl-terminal hexapeptide of the neurotensin molecule for recognition and does not cross-react significantly with any known natural catabolite in human plasma. On gel permeation chromatography (see below) of postprandial human plasma, only one homogenous peak of carboxyl-terminal NTLI was detected, confirming observations by others (14, 15). This material may be identified as neurotensin since equal amounts of amino- and carboxyl-terminal NTLI were measured.

Gel permeation chromatography of postprandial plasma NTLI. 100 ml plasma from one subject was obtained at 30 min after the fat meal. The plasma NTLI was extracted on Sep Pak C₁₈ cartridges and concentrated by rotary evaporation. An analytical amount of 2 ml was characterized on an analytical Sephadex G-50 column (1.5 × 100 cm) equilibrated and eluted with 0.05 M CH₃CO₂NH₄ at pH 5.5. The eluted fractions were assayed for NTLI by RIA with antisera L170 and 7852 (20).

Data analysis. The data are presented as mean and standard errors. The range is given in parentheses after data. Repeated measures analysis of variance was used to evaluate significance of differences between the experimental groups, and *P* < 0.05 was considered as significant.

Results

Acid secretion (Fig. 1). The gastric acid secretory responses to the glucose meal were 4.6±1.0 (0–8.5) and 5.1±1.7 (0–11.2) mmol·30 min⁻¹ in the control and test experiment, respectively. The peptone meal significantly stimulated acid output to 9.1±2.5 (2.8–22.7) and 9.4±2.1 (1.8–17.7) mmol·30 min⁻¹

at 90 min, respectively. In the control group, acid output remained at this rate throughout the experiment. The 1,600-pmol·kg⁻¹ h⁻¹ dose of neurotensin significantly reduced the acid output to 5.8±2.2 (0–19.3) mmol·30 min⁻¹. The 100- and 400-pmol·kg⁻¹ h⁻¹ doses of neurotensin did not significantly affect acid output.

No significant effect of neurotensin was observed in the study with infusion of the “physiological dose” (25 pmol·kg⁻¹ h⁻¹) during 2 h of peptone meal stimulation. Basal acid outputs were 3.2±0.6 (1.1–5.1) and 3.2±0.7 (2.0–6.1) mmol·30 min⁻¹, and the 2-h acid outputs in response to peptone were 40.8±6.2 (22.3–62.4) and 41.3±6.9 (22.1–72.1) mmol during the vehicle and neurotensin infusion, respectively.

Gastrin and neurotensin-like immunoreactivity in plasma during acid secretory test. The administration of peptone stimulated gastrin release from basal levels near 30 pM to a plateau of 60–80 pM. Intravenous infusion of neurotensin at sequentially increasing doses, 100–1,600 pmol·kg⁻¹ h⁻¹, did not affect plasma gastrin.

Intravenous infusion of neurotensin at 100, 400, and 1,600 pmol·kg⁻¹ h⁻¹ significantly elevated plasma NTLI from a basal of 10±2 (6–19) pM to 63±13 (22–110), 164±28 (102–336), and 725±80 (414–840) pM, respectively, after 30 min of infusion (Fig. 2).

Intravenous infusion of neurotensin at 25 pmol·kg⁻¹ during 2 h significantly elevated plasma NTLI from a basal of 8.5±2.0 pM to a mean concentration of 14.7±2.1 (7–19) pM, whereas the mean plasma NTLI during the corresponding saline infusion period was 7.6±1.1 (5–12) pM. The steady state level was reached within 30 min of infusion.

Endogenous NTLI release (Fig. 3). Basal plasma NTLI was 7.6±1.9 (2–17) pM. After ingestion of a fat-rich meal, plasma NTLI was significantly elevated to a mean peak maximum of 15.5±2.5 (4–24) pM at 40 min (Fig. 3). Plasma NTLI was back to basal at 100 min.

Gel permeation chromatography of postprandial plasma NTLI (Fig. 4). The Sephadex G-50 chromatogram (Fig. 4) of plasma that was obtained 30 min after a meal revealed two peaks of NTLI when eluates were measured with an amino-terminal specific antiserum. The smaller NTLI peak may be identified as neurotensin since it eluted in the same region as did synthetic neurotensin, and equal amounts of amino- and carboxyl-terminal NTLI were measured. The major NTLI peak, only detectable with antiserum 7852, represents amino-terminal fragments, presumably smaller neurotensin catabolites.

Table I. Specificity of Antisera L170 and 7852

Peptide	Cross-reactivity	
	Ab L170	AB 7852
	%	%
Neurotensin 1-13	100	100
Neurotensin 4-13	100	<1
Neurotensin 8-13	29	<1
Neurotensin 9-13	<2	<1
Neurotensin 10-13	<1	<1
Neurotensin 1-12	<1	ND
Neurotensin 1-11	<1	79
Neurotensin 1-8	<1	39
Other peptides*	<1	<1

* Gastrin, somatostatin, bombesin, enkephalin, CCK, GIP, VIP, PP, secretin, motilin, and neuromedin N.

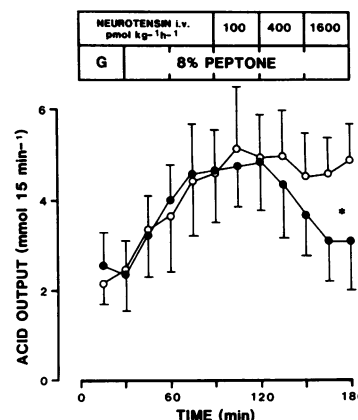


Figure 1. Glucose- and peptone-stimulated gastric acid secretion quantified by intragastric titration in human volunteers. Effect of intravenous neurotensin infusion (●) as compared with control (saline) (○). Neurotensin at 1,600 pmol·kg⁻¹ h⁻¹ i.v. significantly inhibited acid output. $\bar{x} \pm SE$, *n* = 8, *P* < 0.05.

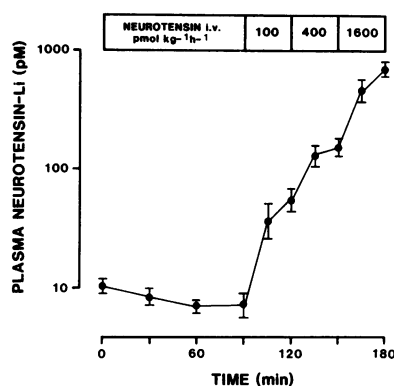


Figure 2. Plasma NTLI during intravenous infusion of synthetic neurotensin, 100, 400, and 1,600 pmol \cdot kg⁻¹ h⁻¹ into healthy volunteers. All doses significantly elevated plasma NTLI, measured with antiserum L170 in Sep Pak-extracted plasma. $\bar{x} \pm$ SE, $n = 8$, $P < 0.05$.

Discussion

Gastric acid secretion. In contrast to previous studies (10, 12, 13) concluding that neurotensin is a hormonal inhibitor of gastric acid secretion, the results in the present study do not support a hormonal role for neurotensin. In fact, the plasma levels of neurotensin obtained after a fat-rich meal, the strongest known physiological stimulus of neurotensin release, were nowhere near the levels required to inhibit acid output during intravenous infusion of synthetic neurotensin. No effect of neurotensin was observed when the physiological plasma NTLI level was mimicked during 2 h of peptone meal stimulation. Blackburn and co-workers (12) reported acid inhibition by a neurotensin dose of ~ 150 pmol \cdot kg⁻¹ h⁻¹ that resulted in a plasma increment of 90 pM, whereas they observed a 27-pM increment after a mixed meal. Their results, therefore, do not support a hormonal role for neurotensin. The inhibition of gastric acid secretion by neurotensin at 300 pmol \cdot kg⁻¹ h⁻¹ observed by Olsen and co-workers (13) was similar to the reduction of acid secretion observed in the present study during infusion of neurotensin at 400 pmol \cdot kg⁻¹ h⁻¹. The inhibition did not reach significance in the present study over 30 min, but significance may have been reached if the neurotensin infusion had continued for 150 min as in the study of Olsen and co-workers. This dose, however, is of limited interest since it resulted in a super-physiological plasma neurotensin level. The potency of the neurotensin batch used in the present study was independently evaluated in a colonic smooth muscle contraction preparation and shown to be fully active (Snape, W., Jr., personal communication). The discrepancy with previous studies may in part be attributed to different RIA methodology, as further discussed below. In support of the present study, similar results were obtained in the dog. Fat administration elevated plasma NTLI (Ab L170) to 35 pM, whereas inhibition of acid secretion during intravenous neurotensin

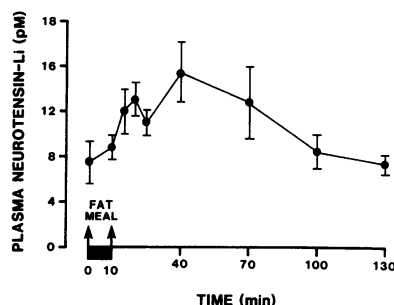


Figure 3. Meal response. Carboxyl-terminal NTLI in healthy volunteers after a fat-rich meal, measured with antiserum L170 in Sep Pak-extracted plasma. $\bar{x} \pm$ SE, $n = 8$.

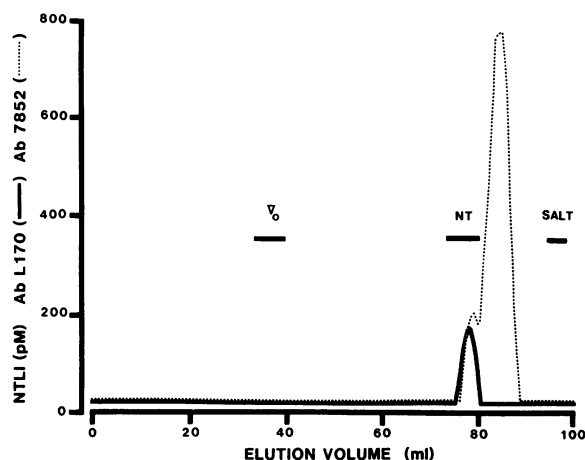


Figure 4. Sephadex G-50 gel permeation chromatogram. Postprandial human plasma, extracted on Sep Pak cartridges and concentrated by rotary evaporation, was characterized on an analytical column (1.5 \times 100 cm). The smaller NTLI peak, equally measured with carboxyl- (L170) and amino- (7852) terminally directed antisera may be identified as neurotensin, whereas the major NTLI peak, later eluting and only detectable with antiserum 7852, represents smaller amino-terminal fragments. Vertical bars, V_0 , NT 1-13, and ¹²⁵I-, indicate the elution positions of blue dextran, neurotensin, and ¹²⁵I-, respectively.

infusion required a supra-physiological plasma NTLI level of 130 pM (21).

Gastrin release was unaffected by exogenous administration of neurotensin, indicating that neurotensin-induced inhibition of gastric acid secretion in man is independent of the hormone gastrin, as previously reported by Olsen and co-workers (13). A direct effect of neurotensin on the parietal cell is unlikely since attempts to demonstrate a neurotensin receptor were negative (22), neurotensin does not inhibit acid secretion stimulated by histamine (11), and neurotensin-induced inhibition is abolished by vagotomy (23).

Neurotensin-like immunoreactivity in plasma. The neurotensin RIA methodology used in the present study has been validated (19) and differs from methods used in previous studies on acid secretion in man with regard to specificity of antiserum and/or extraction procedure. In one previous study (10), an amino-terminally directed antiserum was used. Such antiserum may cross-react with neurotensin catabolites, the main components of plasma NTLI (14-16) that are cleared at slower rates than is neurotensin itself (24) and for which no biological activity on gastric functions has been positively identified (23). In other previous studies that employed carboxyl-terminally directed antisera (12, 13), measurements were performed on plasma without extraction, resulting in artificially high values due to nonspecific interference by plasma components (25).

The chromatographic characterization of Sep Pak-extracted postprandial plasma NTLI in the present study, Fig. 4, suggests that a minor fraction may be neurotensin whereas the main components are smaller amino-terminal fragments. Similar chromatographic data were obtained by other investigators characterizing nonextracted postprandial human plasma (14, 15), but, also, NTLI was measured in the void-volume region. The significance of this NTLI, eliminated by Sep Pak extraction in the present study, is open to question and may reflect

an artifact. It is not likely to be a noncovalent complex of neurotensin to a carrier protein since treatment with 8M urea did not generate small molecular forms of NTLI (14). It is likely that the void-volume NTLI is the result of nonspecific interference in the RIA by plasma proteins or possibly by plasma enzymes eluting in this region. The importance of void-volume NTLI in hormonal regulation of postprandial gastrointestinal functions is minor, if any, since no change in such NTLI occurred after a meal (14, 15). Characterization by high performance liquid chromatography of human plasma, obtained before and after a meal, failed to demonstrate an increase in carboxyl-terminal NTLI, whereas amino-terminal NTLI was significantly elevated (16). On the other hand, the present and other studies (14, 15) in which plasma was taken at earlier time points have demonstrated carboxyl-terminal immunoreactivity in human plasma that appears to be intact neurotensin. Very high levels of carboxyl-terminal NTLI in acid-acetone-extracted human plasma have been reported (26), but not found by other investigators, a fact which may be attributed to the extreme carboxyl-terminal specificity of the antiserum employed, PGL4, and/or generation of NTLI during the extraction. The chromatographic profile of postprandial plasma in the present study is comparable with that of plasma obtained during infusion of synthetic neurotensin (27).

In summary, neurotensin inhibited parietal cell function by a mechanism that did not involve inhibition of gastrin release, but is not likely to function as a physiologically significant hormonal regulator of gastric acid secretion in man since no effect was observed at physiological plasma levels.

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