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INHIBITORY EFFECT OF NEUROTENSIN ON PENTAGASTRIN-STIMULATED GASTRIC ACID SECRETION IN PYLORUS-LIGATED RATS

Robert A. Hammer*+, Betty Wagner and Hilda S. Soyode

Gastroenterology Section, Dept. of Medicine, Northwestern Univ. Medical School, and VA Lakeside Medical Center, Chicago, IL

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Neurotensin is a known inhibitor of gastrin-stimulated acid secretion in dogs and humans. In order to study the dose-related effect of neurotensin, we prepared pentobarbital-anesthetized rats by pyloric ligation and collected gastric secretions one hour after injection of saline (Basal), pentagastrin, 6 µg/Kg subcutaneously (PG Alone), or pentagastrin plus neurotensin by tail vein injection (PG + NT). Acid output was calculated from the volume and pH of the samples, which correlated well with the output determined by titration with 0.02 N NaOH ($r = 0.92$). Basal output was 36 $\pm$ 4 µEq/hr; stimulated output (PG Alone) was 64 $\pm$ 5 µEq/hr, and output after PG + NT, 250 pmol/Kg, was 33 $\pm$ 3 µEq/hr ($p < 0.001$). The effect of neurotensin was dose-related over a range from 125 to 500 pmol/Kg. This technique may be useful in the biological evaluation of neurotensin-related peptides.

Shortly after the isolation of the mammalian tridecapeptide neurotensin (NT) from bovine hypothalamus (1), NT was shown to be capable of inhibiting gastric acid secretion in Pavlov-pouch dogs (2) and in humans with peptic ulcer disease (3). The subsequent isolation of human intestinal NT (4), and the demonstration that ingestion of a meal resulted in an increase in neurotensinlike immunoreactivity (NTLI) in peripheral plasma of volunteers (5, 6, 7), suggested that NT may function as an enterogastrone in the regulation of post-prandial acid secretion (8).

Rosell and his colleagues have shown that infused synthetic NT acts as an enterogastrone (8), decreasing lower esophageal sphincter tone (9), decreasing gastric acid secretion (2) and delaying gastric emptying (10). NT also has effects on small intestinal tone and motility (11). Kihl and his colleagues have suggested that gastric acid output in ulcer patients (and normal individuals) is inversely correlated with the release of NT into the circulation in response to graded doses of intra-duodenal lipid (3).

*Present address: Department of Medicine, Tulane Univ. Medical School, 1430 Tulane Avenue, New Orleans, LA 70112.

+To whom reprint requests should be sent.

Abbreviations: NT, neurotensin; PG, pentagastrin.

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However, the nature of the response of gastric acid secretion to various doses of NT has never been defined, nor has a model been described that can be utilized to test the bio-activity of small amounts of NT or NT-like compounds that might be isolated in the future. The Pavlov-pouch dog model (2) is not useful in screening new substances during purification because of the large amounts of material that must be injected to achieve an active concentration.

Therefore, we modified the pylorus-ligated rat model (12) to provide a sensitive, reproducible bio-assay of the inhibitory effect of NT on acid secretion. This model was originally described by Shay as a method to produce gastric ulceration (12), with the animals maintained for up to 19 hours after ligation. Others have shown that short periods of pyloric ligation can be used to demonstrate the stimulatory effects of gastrin (13) or histamine (14), or the effect of various inhibitors of acid secretion (13, 15).

MATERIALS AND METHODS

Female Sprague-Dawley rats (Holtzman, Inc., Madison, WI) were maintained on a standard chow diet (Purina) in a 14-hour light/10hr dark cycle, and kept NPO except for water for 36-48 hr prior to use. During fasting, they were housed singly in wire-bottom cages to minimize coprophagy. Pentagastrin (PG, Peptavlon, Ayerst Pharmaceuticals) and NT (Peninsula Labs) were diluted for injection in sterile 0.9% NaCl. The concentration of NT in the stock solution was confirmed by quantitative amino acid analysis. Rats were anesthetized with sodium pentobarbital (35-45 mg/Kg i.p.) and additional anesthetic was administered when necessary. Animals' temperature was maintained at 37°C with a Delta-phase isothermal pad (Braintree Scientific). Each animal underwent a 1 cm midline laparotomy starting just below the xiphoid process. The pylorus was identified and a double ligature of 3-0 silk was passed around it with curved forceps and secured. Care was taken not to manipulate the stomach nor to induce bleeding. The time of pyloric ligation was recorded, and the abdominal incision was closed with 2 or 3 full-thickness sutures of 3-0 silk on a curved needle. PG or saline was immediately administered by subcutaneous injection in the thigh, and saline or NT in saline (2 ml/Kg body weight) was injected into the tail vein with a 1 ml syringe and disposable 26 Ga needle. Animals were marked for identification and placed on their sides on a heated surface (40°C). This process was completed within 3 to 5 min after pyloric ligation. Thus, 12 to 15 rats were tested on each occasion; 4-6 received PG alone, 4-6 received PG plus NT, and 2 or 3 served as basal controls (saline injected both subcutaneously and intravenously).

An hour after pyloric ligation, the sutures were removed from each rat's abdomen and the incision extended inferiorly. The gastro-esophageal junction was located along the greater curvature of the stomach (separating the spleen by blunt dissection) and the distal esophagus was clamped. The stomach was cut out above the clamp and below the pyloric ligature, and a small incision was made with scissors into the fundus; the gastric contents were expressed through this cut into a graduated 15 ml conical glass centrifuge tube, and the volume and pH were measured (Orion Research, Cambridge MA). The rats, which were still anesthetized, were killed by exsanguination or by creation of a bilateral pheumothorax. If solid material was present in a sample of gastric juice, it was sedimented by centrifugation at 800 g for 10 min, and the volume of solids was subtracted from the total to determine the volume secreted.

Acid output was calculated in μEq/hr by multiplying the H+ concentration (in μEq/ml), determined from the antilog of -(pH), by the gastric volume secreted (in ml/hr). In one series of animals, titration of gastric contents

to pH 7 with 0.02 N NaOH was also performed. In analyzing the dose related effect of NT, results from the rats that received NT were expressed as percent inhibition of the mean PG-stimulated output minus the basal output under the particular conditions (PG dose, pentobarbital dose, period of fasting, animal weight) of the assay on a given day. Differences between groups were analyzed by Student's t test or analysis of variance and significance was accepted if p was less than 0.05. The correlation of H+ output by titration and by calculation from the volume and pH was determined by linear regression analysis.

RESULTS

Acid output calculated from volume and pH data was highly correlated with that determined by titration (Fig. 1, $r = 0.92$, $p < 0.001$, $n = 67$). Calculated acid output was 15 $\pm$ 11 µEq/hr (mean $\pm$ SD) less than that determined by titration; this difference was uniform throughout the range tested (Fig. 1, slope of regression line = 0.96).

Basal acid output one hour after pyloric ligation (saline only injected) was 35.7 $\pm$ 3.9 µEq/hr (mean $\pm$ SEM, $n = 22$). The acid output stimulated by pentagastrin, 6 µg/Kg, was 64 $\pm$ 4.9 µEq/hr ($n = 56$, $p < 0.01$ compared to saline). The mean level of PG-stimulated acid output after the administration of NT, 250 pmol/Kg, was 33.1 $\pm$ 3.2 µEq/hr ($n = 84$, $p < 0.001$ compared to PG alone). Figure 2 shows the distribution of acid outputs in the three groups of animals. The inhibition of PG-stimulated acid output by NT was dose-related (Figure 3).

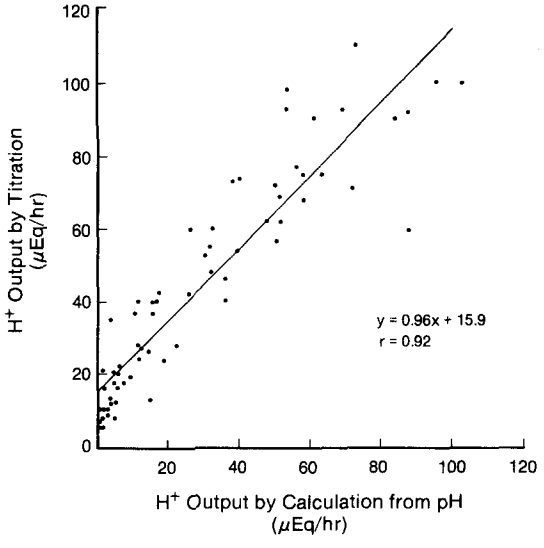
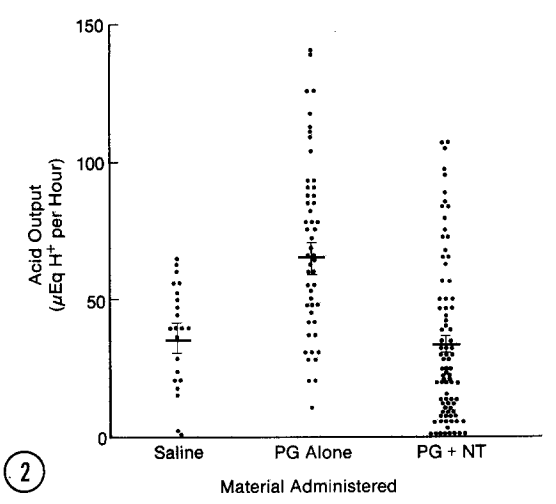


Fig. 1. Determination of acid output by titration to pH 7 (using 0.02 N NaOH, y axis) and by calculation as described in Methods (x axis) in 50 consecutive samples of gastric contents. Solid sediment was pelleted prior to pH determination and resuspended during titration. Linear regression analysis was used to determine the equation of the line shown. The coefficient of correlation is significant ($p < 0.001$).



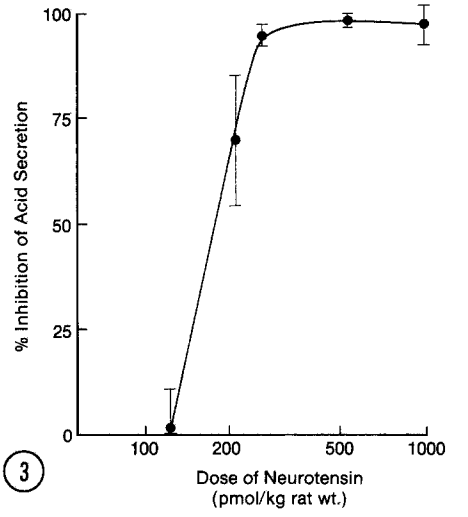


Fig. 2. Gastric acid output in rats injected with saline alone (Saline, n = 22), pentagastrin 6 μg/Kg subcutaneously and saline 2 ml/Kg intravenously (PG Alone, n = 57), and pentagastrin and NT 250 pmol/Kg in saline (PG + NT, n = 84). Difference in distribution of acid output between PG Alone and each of the other groups is significant ($p < 0.01$ vs. Saline; $p < 0.001$ vs. PG + NT). If Basal values on a given day were high, there was a tendency for the response to PG alone and to PG + NT to be very high as well (but $0.05 < p < 0.1$ by analysis of variance); data from all bio-assays are combined in this Figure.

Fig. 3. Dose-related effect of intravenous NT on acid output in response to pentagastrin, 6 μg/Kg subcutaneously. The inhibitory effect is expressed as described in Methods. Each point represents the mean of 5 to 7 rats, and bars indicate SEM.

DISCUSSION

Neurotensin was a potent inhibitor of pentagastrin-stimulated gastric acid secretion in rats anesthetized with pentobarbital and surgically prepared with a ligated pylorus. The full inhibitory effect of NT occurred at a dose that was only one half of the minimum hypotensive dose (500 pmol NT per Kg body weight, Ref. 1). The half-life of intravenous NT in rats is only 30 sec (16), so it is unlikely that the effect of NT on acid output could have been due to hypotension.

The acid output determined by calculation from the volume and pH measurements correlated well with the output determined by titration, and the calculation method was used throughout the study. The consistently higher value determined by titration was probably due to the buffering effect of the gastric juice. Since this effect seemed to be constant over the full range of acid outputs, the calculation method was used throughout the remainder of the study.

The acid output in response to PG + NT (Fig. 2) was not normally distributed; this may have been due to an underestimation of some acid outputs by the calculation method (i.e., those values below 16 μEq/hr by titration, Fig.

1). If one were to assume that all the calculated acid outputs less than 16 μEq/hr actually were 16 or more, the mean (± SEM) acid output of the PG + NT group would increase to 37.2 ± 2.8 μEq/hr, a value still significantly less than that of the PG alone group ($p < 0.001$) and no different from that of the saline control group.

Ligation of the pylorus alone has been shown to stimulate basal acid secretion as measured 7 to 19 hours later (12). This high basal acid secretion was not simply due to distention, since acid output from vagally intact rats was unaffected by placement of a gastric cannula at the time of surgery (17). During the first hour after ligation, however, basal acid output did not increase markely (18). Thus, Szabo and colleagues were able to demonstrate that the effects of stimulants of acid secretion could be measured 30 min to 1 hour after pyloric ligation (19).

We have confirmed that, after a one hour period, measurement of gastric acid output in the pylorus-ligated rat could be used to demonstrate both stimulation by a secretagogue (13, 14), and inhibition of that stimulation (15). Despite the inherent variability of this model, the technique described here permits the testing of a potential physiological effect of NT in the regulation of the GI tract and requires relatively small quantities of material. It may be useful in the biological evaluation of new substances, including NT-related peptides, isolated from the GI tract or from portal plasma.

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REFERENCES

1. Carraway, R.E., and Leeman, S.E. (1973) J. Biol. Chem. 248,6854-61.
2. Andersson, S., Chang, D., Folkers, K., and Rosell, S. (1976) Life Sciences 19,367-370.
3. Kihl, B., Rokaeus, A., Rosell, S., and Olbe, L. (1981) Scnad. J. Gastroenterol. 16,513-526.
4. Hammer, R.A., Leeman, S.E., Carraway, R.E., and Williams, R.H. (1980) J. Biol. Chem. 255,2476-2480.
5. Mashford, M.L., Nilsson, G., Rokaeus, A., and Rosell, S. (1978) Acta Physiol. Scand. 104,244-246.
6. Blackburn, A.M., and Bloom S.R. (1979) J. Endocrinol. 83,175-181.
7. Hammer, R.A., Carraway, R.E., and Leeman, S.E. (1982) J. Clin, Invest. 70,74-81.
8. Rosell, S., Rokaeus, A., Mashford, M.L., Thor, K., Chang, D., and Folkers, K. (1980) In Neuropeptides and Neural Transmission. C.A. Marsan, and W.Z. Traczyk, eds., pp. 181-189. Raven Press, New York, New York.
9. Rosell, S., Thor, K., Rokaeus, A., Nyguist, O., Lewenhaupt, A., Kager, L., and Folkers, K. (1980) Acta Physiol. Scand. 109,369-375.

10. Andersson, A., Rosell, S., Hjelmguist, U., Chang, D., and Folkers, K. (1979) Acta Physiol. Scand. 100,231-235.
11. Schemann, M., Siegle, M.L., Sahyoun, H., and Ehrlein, H-J. (1986) Z. Gastroenterologie 24,262-268.
12. Shay, H., Komarov, S.A., Fels, S.S., Merenze, D., Gruenstein, M., and Siplet, H. (1945) Gastroenterology 5,43-61.
13. Semb, L.S. (1966) Acta Physiol. Scand. 66,374-382.
14. Baume, P.E., and Law, D.H. (1965) Proc. Soc. Exp. Biol. Med. 119,461-465.
15. Niada, R., Prino, G., Lugaro, G., Carrea, G., Pasta, P., and Casellato, M.M. (1979) Eur. J. Pharmacol. 56,217-233.
16. Aronin, N., Carraway, R.E., Ferris, C.F., Hammer, R.A., and Leeman, S.E. (1982) Peptides 3,637-642.
17. Hakanson, R., Hedenbro, J., Liedberg, G., Sundler, F., and Vallgran, S. (1980) J. Physiol. 305,139-149.
18. Brodie, D.A. (1966) Am. J. Dig. Dis. NS11,231-241.
19. Szabo, S., Reynolds, E.S., Lichtenberger, L.M., Haith, L.R., Jr., and Dzau, V.J. (1977) Res. Comm. Chem. Path. & Pharm. 16,311-323.