The Role of Neurotensin in Human Gallbladder Motility

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Gallbladder contraction in response to a fatty meal is thought to be caused by release of cholecystokinin (CCK). We have previously demonstrated a close correlation between circulating concentrations of CCK and contraction of the gallbladder in normal humans and in gallstone patients. Recent studies in animals, however, have shown that other potentially cholecystokinetic hormonal agents are released by a fatty meal, which suggests that other hormones may be involved in postprandial gallbladder contraction. Neurotensin, a 13-amino acid peptide, is released by fat; we have shown it to cause gallbladder contraction in dogs. In the present study, we measured release of neurotensin in seven normal adult volunteers. We determined the effects of infused neurotensin (4 pmol/kg-min) on gallbladder contractility, measured by ultrasonography in 10 adult volunteers, and we evaluated release of neurotensin in eight patients with gallstones. After ingestion of fat, we found significant release of neurotensin in normal volunteers from a mean basal concentration of 15.9 ± 3.5 pg/ml to a maximum of 34.7 ± 0.2 pg/ml. In the gallstone patients after fat ingestion, neurotensin rose from a basal of 16.8 ± 3.1 pg/ml to a maximum of 53.4 ± 28.1 pg/ml, which was a significantly greater release than in controls. Intravenous infusion of neurotensin produced dilatation of the gallbladder (from a mean basal volume of 13.7 ± 2.3 cc to 20.0 ± 1.8 cc). Neurotensin causes relaxation of the gallbladder in humans and, by contributing to stasis, may be involved in the formation of gallstones.

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NEUROTENSIN IS a tridecapeptide that was initially isolated during attempts to purify fractions of substance "P" from extracts of bovine hypothalamus.[1] Although neurotensin was initially found to produce vasodilatation and hypotension when injected into rats, it was soon found to function as a gastrointestinal hormone as well.[2] Neurotensin has been found throughout the human gastrointestinal tract with concentrations increasing from duodenum to distal ileum.[3] In the ileum, the immunoreactive N-cell is the third most prevalent endocrine cell in the region, after the EC-cell (enterochromaffin) and the L-cell (glucagon).[4] Fat is the single most potent stimulant of neurotensin release,[5] and ingestion of a meal releases neurotensin in man.[6] The pancreatic effects of neurotensin infusion mimic those of oleate perfusion of the duodenum.[7] Actions of neurotensin include stimulation of pancreatic bicarbonate and protein secretion.[8] Sakamoto and colleagues from our laboratory[9] found that neurotensin *potentiated* the stimulatory action of intraduodenal amino acids and HCl, as well as amino acids plus HCl, on pancreatic bicarbonate secretion, whereas it acted in an *additive* manner with these agents to stimulate pancreatic protein secretion.

In contrast to its stimulatory effects on pancreatic secretion, neurotensin inhibits gastric acid secretion,[10] decreases gastric motility,[11] decreases lower esophageal sphincter pressure,[12] alters the motility pattern in the duodenum and proximal jejunum in man from a fasting to a fed type,[13] and increases antiperistaltic activity in the proximal colon.[14] Fujimura and colleagues[15] have shown that neurotensin causes gallbladder contraction in dogs. Otherwise, the effects of neurotensin on gallbladder motility are largely unstudied.

The primary effect of cholecystokinin (CCK) on the pancreas is to stimulate enzyme (protein) output, although CCK will augment the stimulation of bicarbonate output by secretin. Release of CCK in humans is stimulated by ingestion of fat, and we have found that release of endogenous CCK by fat (Lipomul®) is strongly correlated with contraction of the gallbladder in humans.[16] We later showed that this action can be reproduced in man by infusion of pure CCK-33 in doses that achieve circulating levels similar to those found after a fatty meal.[17]

Although other hormones, such as gastrin,[18] have weak cholecystokinetic activity, the chief stimulant of gallbladder contraction is thought to be cholecystokinin.

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We have found that the relationship between circulating CCK concentrations and gallbladder contractility is shifted in patients with gallstones[19] and with duodenal ulcer.[20]

The purpose of the present study was to measure release of neurotensin in normal human volunteers, to assess the physiologic effects in humans of neurotensin infusion on gallbladder contractility measured by ultrasonography, and to determine the pattern of neurotensin release in patients with gallstones. We have also measured concentrations of CCK and pancreatic polypeptide (PP), since both agents are released by fat in man.[21]

Methods

Experimental Plan

In one experiment, Lipomul (corn oil, 1.5 ml/kg) was ingested by seven volunteers (three women and four men ranging in age from 21 to 57 years). Plasma samples were obtained for measurement of neurotensin, CCK, and PP, before and at intervals up to 60 minutes after ingestion of Lipomul. Gallbladder volume was measured by real-time ultrasonography, as originally described by Everson and colleagues[22] and as modified by us.[16,17,19] Gallbladder volume was calculated using the sum of cylinders method.[16]

In a separate experiment, pure neurotensin from Penninsula Laboratories (San Carlos, CA) was infused initially into one 29-year-old male volunteer at doses that ranged from 1 to 16 pmol/kg-min. This was done to establish the dose at which plasma levels corresponded to levels seen after ingestion of fat (Fig. 1). Filter sterilization of the solution was performed using millipore filters because of a previous experience in losing bioactivity of CCK after similar filtration.[23] *In vivo* bioactivity of the sterilized neurotensin solution was determined by measurement of gallbladder pressure after bolus injection of various doses of neurotensin into awake dogs that had been prepared with pressure transducers attached to their gallbladder walls in such a manner as to allow measurement (in grams) of the force of gallbladder contraction (Fig. 2). After gallbladder responses were determined and bioactivity assured, a 1-hour infusion of neurotensin (4 pmol/kg-min) was carried out in ten volunteers. During the infusion, blood samples were obtained at intervals for measurement of neurotensin and pancreatic polypeptide.

Gallbladder contraction was measured using real-time ultrasound after ingestion of oral Lipomul.[16] CCK[24] and neurotensin[9] were measured in plasma by specific radioimmunoassays developed in our laboratory. Pancreatic polypeptide levels were measured by a specific radioimmunoassay employing reagents provided by Dr. R. E. Chance (Lilly Research Laboratories, Eli Lilly Co., Indianapolis, Indiana).[25]

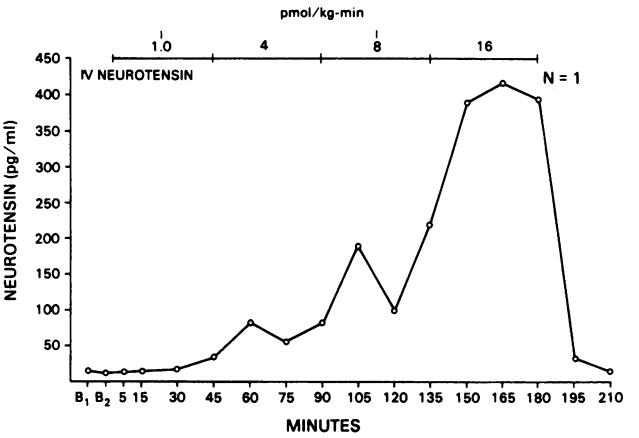

FIG. 1. Plasma concentrations of neurotensin achieved during the infusion of graded doses of neurotensin in one volunteer.

In another experiment, eight asymptomatic patients with gallstones were given oral Lipomul (1.5 ml/kg). Plasma levels of CCK and neurotensin and PP levels were measured before and at intervals after Lipomul.

All studies performed had been previously reviewed and approved by our Instutional Review Board.

Hormone concentrations and gallbladder size are expressed as the mean ± the standard error of the mean (except in Fig. 1, in which the data are recorded from a single subject). Student's t-test for paired observations was used to analyze data for statistical significance of differences between means. Differences with p values less than 0.05 were considered significant.

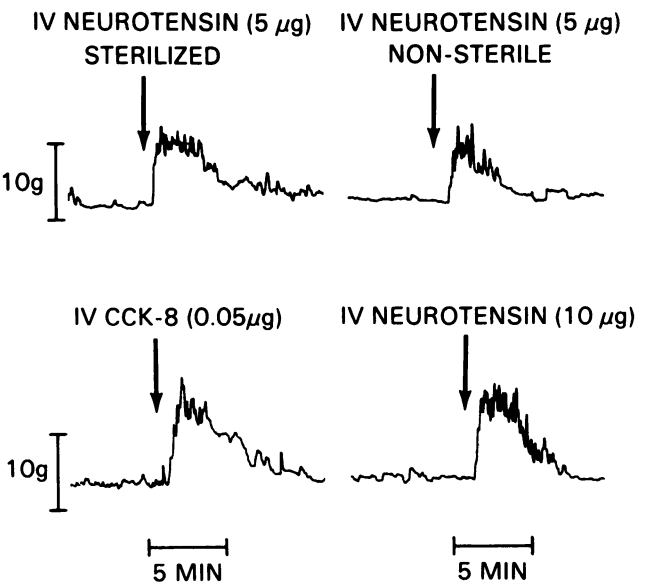

FIG. 2. Gallbladder contractions in a dog measured by force-gate transducer. These findings show that millipore sterilization of a neurotensin solution did not alter bioactivity, measured by gallbladder contraction of a dog. The effect of injection of CCK-8 is shown for comparison. In this canine preparation, the cholecystokinetic action of 0.05 μg CCK-8 is approximately equal to that of 10 μg of neurotensin.

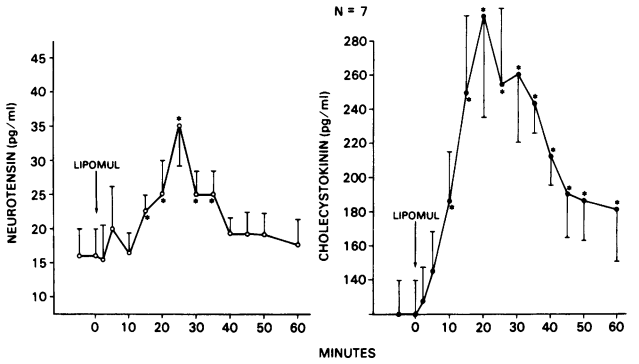

FIG. 3. Plasma concentrations of neurotensin and cholecystokinin-33 in seven normal adult volunteers after ingestion of Lipomul (1.5 ml/kg). Asterisks indicate significant elevation above basal concentrations ($p < 0.05$).

Results

Hormone responses to oral Lipomul were prompt in the seven volunteers, with release of neurotensin from a basal of 15.9 ± 3.5 pg/ml to a peak of 34.7 ± 7.2 pg/ml at 25 min; CCK rose from a basal level of 120 ± 23 pg/ml to a peak of 295 ± 59 pg/ml at 20 min (Fig. 3). Ultrasonography revealed a prompt contraction of the gallbladder as we have previously reported.[16]

During continuous neurotensin infusion (4 pmol/kg-min), plasma levels of between 70 to 80 pg/ml were achieved (Fig. 4). Neurotensin infusion did not release CCK (data not shown) but was a potent releaser of PP (Fig. 4). Although neurotensin infusion caused no initial effect on the gallbladder (Fig. 5), there was a gradual but steady increase in gallbladder volume as the infusion continued (from a basal volume of 13.7 ± 2.3 cc to 20.2 ± 1.8 cc at 90 min), an increase of 46%. Blood pressure and heart rate remained constant during the infusion.

In the eight gallstone patients, CCK release after ingestion of Lipomul was again prompt, but the mag-

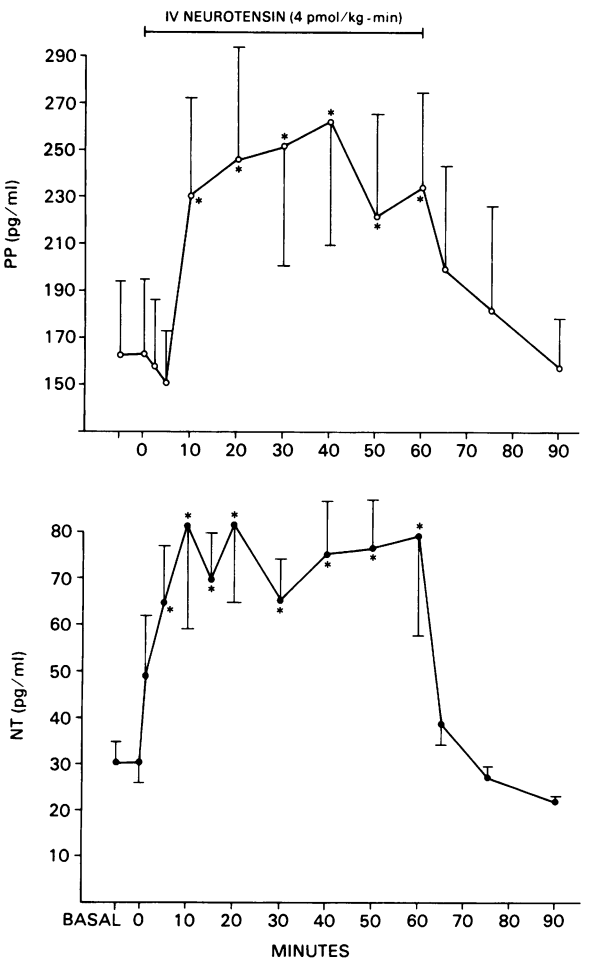

FIG. 4. Plasma concentrations of pancreatic polypeptide (PP) and neurotensin (NT) in 10 normal adult volunteers during an infusion of neurotensin (4 pmol/kg-min). Asterisks indicate significant elevation above basal concentrations ($p < 0.05$).

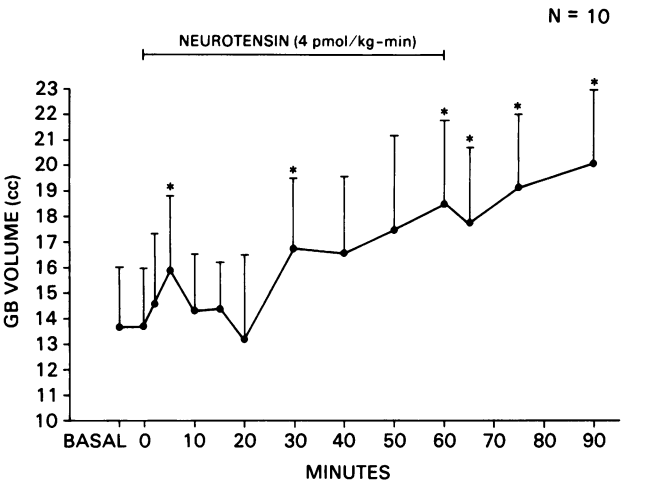

FIG. 5. Gallbladder volume measured by ultrasonography in 10 normal adult volunteers before, during, and after infusion of neurotensin (4 pmol/kg-hr). Asterisks indicate significant increase in gallbladder volume above basal.

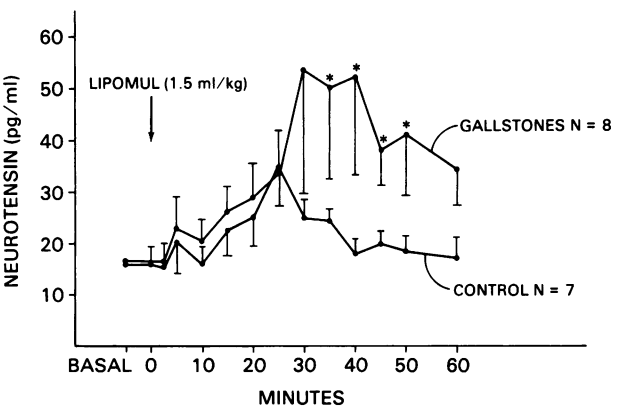

FIG. 6. Neurotensin concentrations in plasma in seven normal adult volunteers (control) and eight gallstone patients before and after oral ingestion of Lipomul (1.5 ml/kg). Asterisks indicate significant elevation above basal concentrations in the gallstone patients.

nitude was less (but not significant) than in volunteers without gallstones (data not shown). The fat-stimulated release of neurotensin in the gallstone patients was significantly greater (from a basal of 16.8 ± 3.1 to a peak of 53.4 ± 28.1 pg/ml) than that of normal controls (Fig. 6). Contraction of the gallbladder was not significantly different from controls.

Discussion

Neurotensin is a potent gastrointestinal regulatory peptide whose role in pancreatic secretion has been carefully studied.[7,8] The purpose of the present investigation was to provide evidence relating to the question of whether neurotensin has a physiologic role in gallbladder contraction in man, as well as to demonstrate patterns of release of neurotensin in normal volunteers and in patients with gallstones. Before the study, we assumed that neurotensin would cause contraction of the gallbladder, in a manner similar to CCK, as we have shown it to do in dogs.[15] To our surprise, neurotensin caused gradual dilatation of the human gallbladder; we found no contraction in any volunteer with infusion of neurotensin. The dilating effect of neurotensin infusion on the gallbladder may be direct or may be mediated by another peptide hormone. Pancreatic polypeptide might merit consideration, since it was released by NT infusion (Fig. 4). The possibility seems unlikely, since PP does not affect isolated gallbladder muscle strips or CCK-stimulated gallbladder muscle contraction *in vitro*,[26] although it has been shown to decrease bilirubin output in humans.[27] Another possibility, though doubtful, is that the dilatation was caused by the release of a peptide hormone which we did not measure (such as vasoactive intestinal polypeptide, which decreases gallbladder tension[28,29]).

In the dose infused (4 pmol/kg-min), there was a prompt rise in plasma neurotensin levels to a plateau of 70 to 80 pg/ml. The infusion was well-tolerated with no side-effects in human volunteers, although after the infusion was over, several individuals noted a need to defecate, a phenomenon previously reported.[12,13,30] In the test infusion, during which neurotensin was infused at levels up to 16 pmol/kg-min in a single individual, explosive diarrhea developed upon cessation of the infusion. This was brief and self-limiting. The diarrhea was probably due to an increased rate of net fluid secretion and a diminished small bowel transit time, which have been reported in man[31] and dogs.[32]

The gallstone patients had significantly greater fat-stimulated release of neurotensin than the normal volunteers. This may somehow be a result of gallbladder disease or, conversely, may indicate that neurotensin, by causing dilatation of the gallbladder, may play a role in the pathogenesis of gallstone formation. The increased release of neurotensin in gallstone patients is in contrast to the decreased release of CCK in these patients.[19] CCK and neurotensin may play opposite roles in regulation of gallbladder mortility, and the shifting of response to one that favors stasis may favor gallstone formation.

This study serves as a reminder that multiple events are triggered by ingestion of food (for example, fat causes release of CCK, PP, and neurotensin), and that we cannot assume any action for any agent in any species until it has actually been demonstrated in that species.

Acknowledgments

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References

1. Carraway RE, Leeman SE. The isolation of a new hypotensive peptide, neurotensin, from bovine hypothalami. J Biol Chem 1973; 248:6854–6861.
2. Miller R. Neurotensin as a gastrointestinal hormone. Med Biol 1981; 59:65–68.
3. Hammer RA, Leeman SE, Carraway R, Williams RH. Isolation of human intestinal neurotensin. J Biol Chem 1980; 255:2476–2480.
4. Helmstaedter V, Feurle GE, Forssmann WG. Ultrastructural identification of a new cell type—the N-cell as the source of neurotensin in the gut mucosa. Cell Tiss Res 1977; 184:445–452.
5. Go VLW, Demol P. Role of nutrients in the gastrointestinal release of immunoreactive neurotensin. Peptides 1981; 2:267–269.
6. Hammer RA, Carraway RE, Leeman SE. Elevation of plasma neurotensin-like immunoreactivity after a meal. Characterization of the elevated components. J Clin Invest 1982; 70:74–81.
7. Konturek SJ, Jaworek J, Cieszkowski M, et al. Comparison of effects of neurotensin and fat on pancreatic stimulation in dogs. Am J Physiol 1983; 244:G590–598.
8. Baca I, Feurle GE, Schwab A, et al. Effect of neurotensin on exocrine pancreatic secretion in dogs. Digestion 1982; 23:174–183.
9. Sakamoto T, Newman J, Fujimura M, et al. Role of neurotensin in pancreatic secretion. Surgery 1984; 96:146–153.
10. Skov Olsen P, Pedersen JH, Kirkegaard P, et al. Neurotensin inhibits meal-stimulated gastric acid secretion in man. Scand J Gastroenterol 1983; 18:1073–1076.
11. Blackburn AM, Bloom SR, Long RG, et al. Effect of neurotensin on gastric function in man. Lancet 1980; 1:987–989.
12. Rosell S, Thor K, Rokaeus A, et al. Plasma concentration of neurotensin-like immunoreactivity (NTLI) and lower esophageal spincter (LES) pressure in man following infusion of (Gln^4)-neurotensin. Acta Physiol Scand 1980; 109:369–375.
13. Thor K, Rosell S, Rakaeus A, Kager L. (Gln^4)-neurotensin changes the motility pattern of the duodenum and proximal jejunum from a fasting-type to a fed-type. Gastroenterology 1982; 83: 569–574.
14. Hellstrom PM, Rosell S. Effects of neurotensin, substance P and methionine-enkephalin on colonic motility. Acta Physiol Scand 1981; 113:147–154.
15. Fujimura M, Sakamoto T, Khalil T, et al. Physiological role of neurotensin in gallbladder contraction in the dog. Surg Forum 1984; 35:192–194.

16. Wiener I, Inoue K, Fagan CJ, et al. Release of cholecystokinin in man: Correlation of blood levels with gallbladder contraction. Ann Surg 1981; 194:321–327.
17. Lilja P, Fagan CJ, Wiener I, et al. Infusion of pure cholecystokinin in humans. Correlation between plasma concentrations of cholecystokinin and gallbladder size. Gastroenterology 1982; 83:356–361.
18. Vagne M, Grossman MI. Cholecystokinin potency of gastrointestinal hormones and related peptides. Am J Physiol 1968; 215:881–884.
19. Thompson JC, Fried GM, Ogden WD, et al. Correlation between release of cholecystokinin and contraction of the gallbladder in patients with gallstones. Ann Surg 1982; 195:670–776.
20. Fried GM, Ogden WD, Fagan CJ, et al. Plasma concentrations of cholecystokinin in patients with duodenal ulcer disease. Surgery 1984; 95:27–33.
21. Thompson JC, Marx M. Gastrointestinal hormones. Curr Probl Surg 1984; 21:1–80.
22. Everson GT, Braverman DZ, Johnson ML, Kern F Jr. A critical evaluation of real-time ultrasonography for the study of gallbladder volume and contraction. Gastroenterology 1980; 79: 40–46.
23. Lonovics J, Suddith RL, Guzman S, et al. Loss of biologic and radioimmunologic activity of cholecystokinin during sterilization by intracellulose filtration. N Engl J Med 1979; 201:1066 (letter).
24. Fried GM, Ogden WD, Swierczek J, et al. Release of cholecystokinin in conscious dogs: Correlation with simultaneous measurements of gallbladder pressure and pancreatic protein secretion. Gastroenterology 1983; 85:1113–1119.
25. Chance RE, Moon NE, Johnson MG. Human pancreatic polypeptide (HPP) and bovine pancreatic polypeptide (BPP). *In* Jaffe BM, Behrman HR, eds. Methods of hormone radioimmunoassay. New York: Academic Press, 1979: 657–672.
26. Pomeranz IS, Davison JS, Shaffer EA. *In vitro* effects of pancreatic polypeptide and motilin on contractility of human gallbladder. Dig Dis Sci 1983; 28:539–544.
27. Greenberg GR, Adrian TE, Baron JH, et al. Inhibition of pancreas and gallbladder by pancreatic polypeptide. Lancet 1978; 2: 1280–1282.
28. Vagne M, Troitskaja V. Effect of secretin, glucagon and VIP on gallbladder contraction. Digestion 1976; 14:62–67.
29. Ryan J, Cohen S. Effect of vasoactive intestinal polypeptide on basal and cholecystokinin-induced gallbladder pressure. Gastroenterology 1977; 73:870–872.
30. Calam J, Unwin R, Peart WS. Neurotensin stimulates defecation. Lancet 1983; 1:737–738.
31. Wiklund B, Liljeqvist L, Rokaeus A. Neurotensin increases net fluid secretion and transit rate in the small intestine of man. Regul Pept 1984; 8:33–39.
32. Reasbeck PG, Barbezat GO, Shulkes A, Leader J. Secretion of neurotensin and its effects on the jejunum in the dog. Gastroenterology 1984; 86:1552–1556.

Discussion

DR. HENRY A. PITT (Los Angeles, California): Dr. Thompson's group has been instrumental in the last few years in demonstrating that a number of hormones are involved with both the contraction and relaxation of the gallbladder. However, data on the effects of various hormones on biliary physiology are conflicting. For example, Dr. Thompson's group presented a study at the Surgical Forum a few weeks ago that showed that neurotensin contracts the canine gallbladder. Are the different findings in these two studies purely species differences, or are there any methodologic differences that might have resulted in opposite conclusions?

In Dr. Thompson's presentation, he brought out the fact that neurotensin in humans stimulates the release of pancreatic polypeptide. We have recently shown in the prairie dog that pancreatic polypeptide relaxes the gallbladder. I wonder, therefore, whether the gallbladder dilatation that you observed in humans was due to neurotensin or was really the effect of pancreatic polypeptide? Moreover, did you measure pancreatic polypeptide levels in the canine study?

DR. FRANK G. MOODY (Houston, Texas): This is a very fine piece of work, and I only rise to question one conclusion; that is, that neurotensin causes relaxation of the gallbladder. Just because the gallbladder gets bigger during an infusion does not mean that it is relaxing. It is possible that neurotensin might be stimulating, for example, secretion of bile, with the bile then filling the gallbladder to give this appearance of relaxation.

Species differences are often implicated in things, but when you get down to basic mechanisms, usually they are the same. For example, when you feed the prairie dog cholesterol, we can find within 3 to 5 days that the smooth muscle of the gallbladder becomes relatively insensitive to cholecystokinin.

I think that Dr. Thompson's group might benefit by taking their observations in humans now into a model where they can actually observe the sequence of events during the formation of gallstones.

DR. SAMUEL A. WELLS, JR. (St. Louis, Missouri): I very much enjoyed Dr. Thompson's paper, another excellent clinical research study from this laboratory, which on an ongoing basis is evaluating the action and interaction of several polypeptide hormones and biogenic amines from the endocrine gut.

I would like to ask Dr. Thompson three questions, two related to the methodology and one, an interesting effect of the drug which I would not call a side effect, but one that was not addressed in the presentation, though it was addressed in this excellent manuscript.

As mentioned by Dr. Thompson, when the infusion of neurotensin was taking place, the dose of 4 picomoles per kilogram per minute was selected as the infusion dose, to mimic the dose achieved with the Lipomul® meal. Actually, I think that the dose is almost twice as high–roughly, 60 or 80 picograms/ml. I wonder, perhaps, if the gallbladder stasis could not be explained on this doubling of dose, seemingly a much higher level than one would achieve physiologically with a Lipomul meal.

The second question I would like to ask regards the effect of cholecystokinin: Is there known to be any effect of the first significant peak above the basal dose with the Lipomul meal? The peak occurred at approximately 10 minutes, and the neurotensin peak at about 20 minutes. Have you looked at the effect of cholecystokin administration on neurotensin secretion?

The last question regards an effect, mentioned in the manuscript, of a fairly potent diarrheal effect of the drug when administered to a patient who was given the first dose of neurotensin, in a range of 1 to 16 picomoles per kilogram per minute. There are several endocrine tumors—the one that I have most experience with is medullary thyroid carcinoma—which may be associated with intractable diarrhea. I wondered if anybody has looked at neurotensin levels in such patients. This polypeptide hormone is derived from a neural crest cell in the endocrine gut, and it is a hypothesis that one might consider.

DR. JAMES C. THOMPSON (Closing discussion): I want to thank the discussants.