Inhibitory effects on intake of cholecystokinin-8 and cholecystokinin-33 in rats with hepatic proper or common hepatic branch vagal innervation

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Abstract

The inhibitory effects of exogenous cholecystokinin (CCK)-8 and CCK-33 for decreasing meal size depend on the route of administration. Inhibitory potencies after intraperitoneal administration (19), systemic intravenous administration (36). The rank order of potencies of CCK-33, a major form of endogenous CCK in the rat small intestine (43), after intraperitoneal, intraportal, and intraperitoneal CCK-8 was intraperitoneal > intraportal > intravenous. Although CCK-33 and CCK-8 had equivalent inhibitory potencies after intraperitoneal administration (19), CCK-33 was significantly more potent than CCK-8 after intraperitoneal administration (36). This was consistent with the demonstration that CCK-33 was much less vulnerable to hepatic uptake, metabolism, and excretion than CCK-8 (10).

These results suggested that intraportal administration of CCK-33 inhibited intake by stimulating CCK₁ receptors on vagal afferent terminals in the liver, because devazepide intraperitoneally blocked the satiating effect of CCK-33 in vagally-intact rats (22) and devazepide or section of the common hepatic branch of the vagus nerve blocked the weak inhibitory effect of intraportal CCK-8 (31).

In this paper, we extend the investigation of the apparent differential potency of exogenous CCK-8 and CCK-33 on hepatic vagal afferent fibers by fixing the route of administration while varying the pattern of innervation provided by the common hepatic branch of the vagus in the absence of all other feeding.

In rats with hepatic proper or common hepatic branch vagal innervation. Am J Physiol Regul Integr Comp Physiol 289: R456–R462, 2005. First published April 14, 2005; doi:10.1152/ajpregu.00062.2005.—The relative potencies of cholecystokinin (CCK)-8 and CCK-33 for decreasing meal size depend on the route of administration. Inhibitory potencies are equal after intraperitoneal administration, but CCK-33 is significantly more potent after intraportal administration. This suggests that CCK-33 is a more effective stimulant of hepatic afferent vagal nerves than is CCK-8. To investigate this possibility, we administered both peptides intraperitoneally in rats with abdominal vagotomies that spared only the hepatic proper vagal nerves (H) and in rats with abdominal vagotomies that spared the common hepatic branch that contains the fibers of the hepatic proper and gastroduodenal nerves (HGD). The vagal afferent innervation in H and HGD rats was verified with a wheat germ agglutinin-horseradish tracer strategy. Intraportal administration of CCK-33 decreased 30-min intake of 10% sucrose in H rats as much as in sham rats, but CCK-8 decreased intake significantly less in H rats than in sham rats. The larger inhibitory effect of CCK-33 than of CCK-8 in H rats is consistent with the hypothesis that CCK-33 is a more effective stimulant of the hepatic proper vagal afferent nerves than CCK-8. In contrast to the results in H rats, the inhibitory potencies of both peptides were significantly and equivalently reduced in HGD rats compared with sham rats. This suggests that there is an inhibitory interaction between the stimulation of the gastroduodenal and hepatic proper afferent fibers by CCK-33.

Intraperitoneal injection of CCK-8 for decreasing meal size. Intraportal injection of CCK-8 is much more potent than intraportal or systemic intravenous injection or infusion or subcutaneous injection (11, 12, 18, 19, 31, 33, 42). The weak inhibitory effect of CCK-8 administered intraperitoneally was mediated by CCK₁ receptors, because devazepide, a specific antagonist of CCK₁ receptors blocked it (31). The receptors were probably on terminals of hepatic vagal afferent fibers, because intraportal CCK-8 had no effect on intake after section of the common hepatic branch of the abdominal vagus nerves (31).

The third of potent effect of exogenous CCK-8 administered intraperitoneally suggested that the mode of the satiating action of endogenous CCK-8 in rats is paracrine rather than endocrine (11, 12, 33). The anatomical relationship between vagal afferent terminals and CCK-immunoreactive cells in the proximal small intestine is consistent with a paracrine mode of action of endogenously released CCK on the afferent terminals (4).

A paracrine effect of exogenous CCK-8 was also supported by Cox’s demonstration (7) that local injection of a small dose of devazepide into the superior pancreaticoduodenal artery that perfuses the proximal duodenum blocked the satiating effect of CCK-8 intraperitoneally. This was a local effect of the antagonist, because when the same dose of antagonist was injected into the jugular vein it did not block the satiating effect of CCK-8 intraperitoneally.

Local treatment with devazepide also decreased the satiating effect of ingested sucrose, because sucrose intake increased in deprived and nondeprived rats (7). This is strong evidence that endogenous CCK released by intraduodenal sucrose acted locally in a paracrine manner to inhibit intake. These effects of devazepide were probably the result of blockade of CCK₁ receptors on vagal afferent terminals in the duodenum because the superior pancreaticoduodenal artery does not perfuse the pyloric sphincter, the site of another population of CCK₁ receptors (41).

That intraportal CCK-8 is significantly less potent than intraperitoneal CCK-8 could be due to hepatic uptake and metabolism of peptides composed of 10 amino acids or less (9, 10, 14). To test that hypothesis, we compared the satiating potencies of CCK-33, a major form of endogenous CCK in the rat small intestine (43), after intraperitoneal, intraportal, and systemic intravenous administration (36). The rank order of potency of CCK-33 was intraperitoneal > intraportal > intravenous. Although CCK-33 and CCK-8 had equivalent inhibitory potencies after intraperitoneal administration (19), CCK-33 was significantly more potent than CCK-8 after intraportal administration (36). This was consistent with the demonstration that CCK-33 was much less vulnerable to hepatic uptake, metabolism, and excretion than CCK-8 (10).

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In this paper, we extend the investigation of the apparent differential potency of exogenous CCK-8 and CCK-33 on hepatic vagal afferent fibers by fixing the route of administration while varying the pattern of innervation provided by the common hepatic branch of the vagus in the absence of all other feeding.

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abdominal vagal innervation. Specifically, we administered both peptides intraperitoneally to two groups of vagotomized rats. One group (HGD rats) had the common hepatic branch (3, 24, 28, 30) intact and all other abdominal vagal branches sectioned. This has been referred to previously as a hepatic-spared vagotomy (16, 38). This surgical group had vagal innervation of the liver through the hepatic branch proper and vagal innervation of the proximal duodenum, distal ileum, and proximal stomach through the fibers of the gastroduodenal branch that traverse the liver and descend to the small intestine. Previous experiments in HGD rats showed that the common hepatic branch of the vaga was sufficient to mediate the inhibition of intake by CCK-8 in real feeding and sham feeding in some (15, 16) but not all (39) conditions. The inhibitory effect of CCK-8 in HGD rats is presumably mediated by afferent fibers of the common hepatic branch. Because CCK1 receptors are on the afferent fibers (6, 21), CCK-8 produces electrophysiological stimulation of the afferent fibers of the common hepatic branch (8, 35) and total, abdominal, afferent vagotomy abolished the inhibitory effect of CCK-8 (20, 40).

To our knowledge, CCK-33 has not been tested in HGD rats. The other group of vagotomized rats (H rats) had vagal innervation of the liver by the fibers of the hepatic branch proper, but had no other abdominal vagal innervation because the gastroduodenal branch of the common hepatic branch and all other abdominal vagal branches were sectioned. Neither CCK-8 nor CCK-33 has been tested in rats with this type of vagal innervation.

On the basis of previous results with intraperitoneal administration of the peptides in vagally intact rats (11, 36), we predicted that CCK-33 would be significantly more potent than CCK-8 in H rats. We also predicted that CCK-33 and CCK-8 would be equipotent in HGD rats as they are after intraperitoneal administration in vagally intact rats (19). The results confirmed the first prediction, but not the second.

MATERIALS AND METHODS

Male Sprague-Dawley rats weighing between 200–225 g (Charles River Laboratories, Wilmington, MA) were housed individually in polycarbonate, solid-bottomed cages (24 × 20 × 29 cm) with cob bedding. Room temperature was ~23°C, and there was a 12:12-h light/dark cycle (lights on 0700). The rats were maintained on ground chow (Purina no. 5001; St. Louis, MO) and tap water ad libitum for 1 wk, following which they were also offered a milk diet consisting of equal parts of sweetened condensed milk (Eagle Brand; Borden, Columbus, OH) and tap water supplemented with Poly-Vi-Sol vitamin drops, (2.0 ml/0.6 liter of diet; Mead Johnson, Evansville, IN). Rats were vagotomized after at least 2 wk of access to the milk diet. All procedures and protocols were approved by the Institutional Animal Care and Use Committees of Weill Medical College of Cornell University and Purdue University.

Surgery. Rats (250–325 g body wt at surgery) were anesthetized by administration of 3 ml/kg ip of a mixture containing pentobarbital (8.9 mg/ml) and chloral hydrate (42.5 mg/ml). Rats were laparotomized and the right (anterior or ventral) and left (posterior or dorsal) vagal trunks were visualized microscopically (magnification, ×10) as they emerged along the esophagus below the diaphragm. This was facilitated by gentle traction on the ends of a 3-0-vicryl (Ethicon; Johnson & Johnson, Somerville, NJ) suture placed in the middle of the greater curvature of the stomach. The left trunk and the gastric and accessory celiac branches of the right trunk were ligated individually with greater curvature of the stomach. The left trunk and the gastric and accessory celiac branches of the right trunk were ligated individually with three 0-vicryl ties placed about 5 mm apart and then sectioned between the ligatures. This left the common hepatic branch intact. Thus HGD rats had the hepatic branch proper and the gastroduodenal branch intact.

To prepare H rats with only the hepatic branch proper intact, the same procedure was performed as in HGD rats, and then the gastroduodenal branch of the common hepatic branch was sectioned using the technique described by MacIsaac and Geary (17). The hepatic artery was exposed, isolated, and then sectioned between two 4-0-vicryl ligatures placed about 2.5 mm apart. The proximal ligature was distal to the bifurcation of the celiac artery into the hepatic and gastroduodenal arteries, so blood flow in the gastroduodenal artery was not compromised. After the hepatic artery was sectioned, forceps were used to strip the bile duct and portal vein with cotton-tipped applicators. A piece of paraffin cut to size was placed behind the bile duct and portal vein to prevent the underlying and surrounding tissue from the solution. After application of the solution, the area was flushed copiously with 0.15 M saline and the piece of paraffin was removed. Sham rats underwent laparotomy, but the vagal nerves were not touched. At the end of all surgeries, the abdominal musculature was closed with interrupted 3-0-vicryl sutures, and the skin was closed with wound clips (Clay Adams; Becton Dickinson, Sparks, MD).

Test procedure. After recovering from surgery, the rats were slowly weaned from the milk diet and returned to their maintenance diet of ground chow. During this time, the rats were adapted to intraperitoneal injections and the test diet of 10% sucrose.

On test days, rats were food deprived at 0900 and water deprived at 1245. At 1255 rats received an intraperitoneal injection of CCK-8 or CCK-33 in 1 ml of 0.9% bacteriostatic saline (Abbott Laboratories, North Chicago, IL) or 1 ml of 0.9% bacteriostatic saline alone. The doses of CCK-8 (Peninsula Laboratory, San Carlos, CA) were 4, 8, and 16 μg/kg; molar equivalent doses of porcine CCK-33 (Peptide, Osaka, Japan) were 13.2, 26.4, and 52.8 μg/kg. Each test with CCK-8 or CCK-33 was preceded by a control test in which saline alone was injected. At 1300, the rats were given access to a 10% sucrose solution in a graduated burette fitted with a pipper tube. Intake was measured at 1330. Under these conditions, the 30-min intake was the size of a single meal. At the end of a test, rats were returned to their home cages, water was replaced at 1345, and chow was replaced at 1400.

At the end of behavioral testing, rats were shipped to Purdue University for verification of the vagal afferent denervation.

Verification of vagal afferent denervation. After arrival at Purdue University, rats were housed individually at 23°C and had ad libitum access to tap water and pelleted rat chow before verification surgery. Vagotomies were verified with a wheat germ agglutinin-horseradish peroxidase (WGA-HRP) tracer strategy (24, 27). Briefly, each rat was anesthetized with pentobarbital sodium (60 mg/kg ip) and the left nodose ganglion was exposed and pressure injected (PicoSpritzer II; General Valve, NJ) with 4% WGA-HRP (3.0 μl; Vector Laboratories) through a glass micropipette (25 μm ID). The incision was closed and treated with nitrofurazone. Then the rat was returned to its cage.

Three days after receiving WGA-HRP, each rat was deeply anesthetized with pentobarbital (180 mg/kg ip) and injected in the left cardiac ventricle with 0.1 ml heparin (1,000 U/ml; Elkins-Sinn, Cherry Hill, NJ) to prevent coagulation and 0.1 ml propranolol (Ayerst Laboratories, Philadelphia, PA) to produce vasodilatation. The rat was perfused transcardially with 500 ml of 0.9% saline at 40°C followed by 3% paraformaldehyde and 0.5% glutaraldehyde in 0.1 M sodium phosphate buffer, pH 7.4 at 4°C, and finally by 0.1% sucrose in the same buffer at 4°C.

The hilus of the liver (including the portal vein, arteries, and adventitia) and first 8 cm of the duodenum were prepared as whole mounts (29). The slides were then processed with tetramethyl benzidine (5), mounted, air dried, cleared in xylene, and coverslipped with 1,3-Diethyl-8-phenylxanthine (DPX; Aldrich, Milwaukee, WI).

Innervation patterns were assessed using darkfield microscopy in conjunction with a reconstruction strategy standardized for the com-
CCK-33 ACTS ON HEPATIC VAGAL NERVES

Vagal afferent innervation. Seven of eight HGD rats had complete HGD vagotomies and eight of ten H rats had complete H vagotomies. Only these rats were included in the statistical analysis. In both HGD and H rats, the hilus of the liver and the surrounding arterial supply were densely innervated by afferent bundles originating from the hepatic branch proper of the common hepatic branch (Fig. 1). The two groups, however, differed in the pattern of innervation of the first 8 cm of the duodenum by the gastroduodenal branch of the common hepatic branch. The visible bundles of the celiac branch of the vagus) and the pylorus (innervated by the gastric branch) appeared denervated. An H preparation was considered successful if 1) the innervation of the liver by the hepatic branch proper and of the common hepatic branch was comparable to the patterns previously reported (24), and 2) the more distal duodenum (innervated by the celiac branch of the vagus) and the pylorus (innervated by the gastric branch) appeared denervated. An H preparation was considered successful if 1) the innervation of the liver by the hepatic branch proper of the common hepatic branch appeared intact, and 2) the proximal duodenum as well as the more distal duodenum and the pylorus were denervated. A vagotomy was considered incomplete or nonselective if either of the celiac or gastric branches were completely or partially spared (27).

Statistical analysis. The intakes after CCK-8 and CCK-33 were expressed as percent inhibitions of the intakes in the preceding control tests. First, the effects of CCK-8 and CCK-33 were analyzed separately by two-way ANOVAs with surgical condition (sham, HGD, and H) as a between-subjects factor and dose as a within-subjects factor. Then, to compare the potencies of the molar equivalent doses of the peptides, separate two-way ANOVAs were done with peptide (CCK-8 and CCK-33) as a between-subjects factor and dose as a within-subjects factor. Tukey's honestly significant difference test was used to test pairwise differences after significant ANOVAs. Differences were considered significant when \( P < 0.05 \).

RESULTS

Vaginal afferent innervation. Seven of eight HGD rats had complete HGD vagotomies and eight of ten H rats had complete H vagotomies. Only these rats were included in the statistical analysis. In both HGD and H rats, the hilus of the liver and the surrounding arterial supply were densely innervated by afferent bundles originating from the hepatic branch proper of the common hepatic branch (Fig. 1). The two groups, however, differed in the pattern of innervation of the first 8 cm of the duodenum. In HGD rats, the initial segment of the duodenum was richly innervated by vagal afferent axons and endings (Fig. 2, A and C), whereas the same region in H rats was completely denervated (Fig. 2B).

CCK-33. Surgical condition affected the potency of CCK-33 significantly \([F (2, 34) = 7.10, P = 0.006, \text{Fig. } 3]\). CCK-33 produced equivalent percent inhibitions in H and sham rats \((P > 0.05)\), but significantly less inhibition in HGD rats than in sham rats or H rats \((P < 0.05)\).

There was a significant effect of dose of CCK-33 \([F (2, 34) = 8.01, P = 0.001]\). The largest dose of CCK-33 \((52.8 \mu g/kg)\) produced a significantly larger inhibition of intake than \(13.2 \mu g/kg (P < 0.05)\), but not a larger inhibition than was produced by \(26.4 \mu g/kg (P > 0.05)\). There was no significant interaction between dose and surgical condition \([F (4, 34) = 1.26, P = 0.30]\).

CCK-8. Surgical condition also affected the potency of CCK-8 significantly \([F (2, 34) = 11.63, P = 0.001, \text{Fig. } 4]\). In contrast to the effects of CCK-33, however, CCK-8 decreased intake significantly less in H and HGD rats than in sham rats \((P < 0.05)\). There was no significant difference in the weak inhibitory effects of CCK-8 in H and HGD rats \((P > 0.05)\).

There was a significant effect of dose of CCK-8 \([F (2, 34) = 4.59, P = 0.017]\). The largest dose of CCK-8 \((16 \mu g/kg)\) inhibited intake significantly more than did \(4 \mu g/kg (P < 0.05)\) but not more than \(8 \mu g/kg (P > 0.05)\). There was no significant interaction between surgical condition and dose \([F (4, 34) = 0.37]\).

Comparison of potencies of CCK-33 and CCK-8. The potencies of the molar equivalent doses of CCK-33 and CCK-8 were not significantly different in sham rats \((P > 0.05)\) nor in HGD rats \((P > 0.05)\); compare Figs. 3 and 4). In H rats, however, 13.2 and 26.4 \(\mu g/kg\) of CCK-33 produced significantly larger percent inhibitions of intake than the equimolar doses of CCK-8 \((4 \text{ and } 8 \mu g/kg, \text{respectively}; P < 0.05)\).

DISCUSSION

CCK-33. The major result of these experiments is that CCK-33 (intraperitoneal) inhibited 30-min intake significantly more than CCK-8 in H rats in which the presence of the afferent innervation of the hepatic branch proper and the absence of the gastroduodenal, gastric, and celiac afferent innervation of the proximal duodenum were anatomically verified. This confirms our prediction and extends previous results obtained with intraperitoneal administration of CCK-33 and CCK-8 in rats in which all of the abdominal vagal innervation was intact (12, 36). Taken together, the results are consistent with the hypothesis that exogenous CCK-33 is a more effective stimulant of vagal afferent terminals in the liver than CCK-8.

It is probable that CCK1 receptors on hepatic vagal afferent terminals mediated the effect of CCK-33 in H rats, because devazepide abolished the inhibitory effect of CCK-33 on intake in rats with intact abdominal vagal innervation (22). These results with devazepide were obtained under different experimental conditions. Devazepide needs to be investigated in H rats in the conditions used in these experiments.

The larger effect of CCK-33 than CCK-8 in H rats after intraperitoneal administration was related to innervation and not to other conditions of these experiments, because CCK-33 and CCK-8 had equivalent potencies in sham rats and equivalently reduced potencies in HGD rats (compare Figs. 3 and 4).
The larger effect of CCK-33 in H rats was unexpected, given the presence of hepatic proper nerves in H and HGD rats. What explains this differential potency? We suggest the following possible answers to that question. First, when CCK-33 is administered intraperitoneally, it stimulates gastroduodenal vagal afferent terminals first, and then after it gains access to the portal blood, it stimulates hepatic vagal afferent terminals. This sequential stimulation could inhibit the effect of CCK-33 on hepatic vagal afferent terminals peripherally via an axon reflex mechanism, if H and HGD are branches of the same fibers of the common hepatic branch, an anatomical relationship that has not been investigated. Alternatively, the inhibitory interaction could occur centrally in the nucleus tractus solitarius or in other regions of the brain involved in processing the vagal afferent stimulation.

In either case, the larger response in H rats than in HGD rats would result from neural disinhibition of the response of afferent H fibers to CCK-33 by the removal of gastroduodenal afferent fibers. Note that intraportal administration of CCK-33 in vagally-intact rats also bypasses the proposed inhibitory interaction produced by sequential stimulation of gastroduodenal and hepatic afferent fibers.

Second, section of the gastroduodenal fibers probably provoked a regenerative response in the hepatic fibers (25, 26). This could increase the response to CCK-33 in the liver by increasing the number of CCK-1 receptors on hepatic terminals or by increasing the efficiency of their transduction mechanisms.

Third, the increased response to CCK-33 in H rats could have been due to a change in the intrahepatic blood flow produced by the surgical procedure used to section the gastroduodenal branch. The procedure eliminated blood flow from the hepatic artery and removed most, if not all, of the postganglionic sympathetic fibers to the liver (1). This could change the distribution of absorbed CCK-33 within the liver so that it had greater access to hepatic vagal terminals.

Fourth, if the postganglionic sympathetic terminals exerted inhibitory control of the vagal afferent terminals’ response to CCK-33, their removal would also disinhibit the vagal terminals.
All of these suggestions are possible, but none of them is supported by experimental evidence. The problem deserves further investigation because inhibitory interactions among afferent terminal fields of the abdominal vagus have not been described or considered to contribute to the integration of afferent vagal stimulation.

CCK-8. There were two important results of the experiments with CCK-8. First, CCK-8 was significantly less potent in HGD rats than in sham rats. Second, the inhibitory potency of CCK-8 in H rats was not significantly different from its potency in HGD rats.

The decreased potency of CCK-8 in HGD rats compared with sham rats demonstrates that the vagal afferent innervation of the small intestine by the gastroduodenal branch is not sufficient to mediate the normal inhibitory effect of CCK-8 on food intake under these conditions. This confirms our previous result (39) but is different from that of Le Sauter and Geary (16). The reason for the different results is not clear.

The weak potency of CCK-8 in HGD rats suggests that the celiac or gastric afferent innervation of the small intestine that is not present in HGD rats is necessary for the normal potency of CCK-8, at least under these conditions. The celiac afferent innervation may be more important than the gastric because the celiac nerves innervate much more of the small intestine than does the gastric branch (2). Furthermore, celiac afferent vagotomy decreased or abolished the inhibitory effect on food intake of nutrients infused into the small intestine that release endogenous CCK (44). The relative importance of gastric and celiac innervation could be decided by measuring the potency of CCK-8 in HGD rats in which the celiac or gastric vagal innervation was preserved. This experiment has not been reported.

The second important result was that CCK-8 was not more potent in H rats than in HGD rats. Thus when the same intraperitoneal route of administration was used, CCK-8 was still significantly less potent than CCK-33 on hepatic vagal afferent terminals. Our working hypothesis is that this difference in potency occurs because CCK-8 is more vulnerable than CCK-33 to intrahepatic uptake and metabolism. This hypothesis needs more testing.

Vagal afferent innervation. The differential duodenal vagal afferent innervation in H and HGD rats (Figs. 1 and 2) demonstrates that surgical section of the gastroduodenal branch produces a novel preparation for the investigation of the anatomy and function of the fibers of the common hepatic branch of the abdominal vagus. Bellinger and Williams (1) provided evidence that their surgical method of sectioning the gastroduodenal branch eliminated visible nerve fibers to the liver along the bile duct and portal vein. We extend that observation by demonstrating that the surgical method also eliminates the afferent innervation of the proximal duodenum by these fibers.

Fig. 3. Data are means ± SE % inhibition of 30-min intake of 10% sucrose produced by 3 doses of CCK-33 in HGD (n = 7), H (n = 8), and sham (n = 5) rats. The means ± SE of the percent inhibition produced by the largest dose of CCK-33 in sham rats was 0.6; it was too small to be displayed. CCK-33 decreased intake significantly less in HGD rats than in H rats or sham rats (P = 0.006). Inhibition in HGD rats was significantly smaller than in H or sham rats (*P < 0.05). Inhibitions in sham and H rats were not significantly different.

Fig. 4. Data are means ± SE % inhibition of 30-min intake of 10% sucrose produced by 3 doses of CCK-8 in HGD (n = 7), H (n = 8), and sham (n = 5) vagotomized rats. Inhibition in HGD and H rats was significantly smaller than in sham rats (*P < 0.05). Inhibitions in HGD and H rats were not significantly different.
Combining the H or HGD procedures with section of the gastric, accessory celiac, and celiac vagal branches optimizes the analysis of the peripheral and central effects of the hepatic proper and gastroduodenal branch fibers because these fibers are the only abdominal vagal innervation in such rats. A limitation of the technique, however, is that it permits the analysis of the functions of the hepatic proper fibers in the absence of the gastroduodenal fibers, but not the reverse.

Although the anatomical verification demonstrated that the procedure for cutting the gastroduodenal branch was effective, it did not evaluate the loss of other neural fibers.

The procedure lesioned vagal efferent fibers traveling in the gastroduodenal branch and nonvagal fibers, such as spinal visceral afferent fibers and postganglionic sympathetic efferent fibers that ascend into the liver along the hepatic artery and portal vein as previously shown by Bellinger and Williams (1). The extent of this nonvagal damage needs to be assessed in future experiments, because it may be involved in the increased potency of CCK-33 observed in H rats compared with its potency in HGD rats.

In summary, we validated a new surgical procedure to determine the response of hepatic proper and gastroduodenal fibers of the common hepatic branch of the abdominal vagus nerve to intraperitoneal administration of CCK-8 and CCK-33. When the hepatic and gastroduodenal vagal fibers were the only abdominal vagal fibers intact, CCK-8 and CCK-33 decreased intake significantly less than in rats that had all of the abdominal vagal innervation intact. In contrast, when only the hepatic vagal fibers were intact, CCK-33 was as potent as it did not evaluate the loss of other neural fibers.

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