Regulation of Fat-Stimulated Neurotensin Secretion in Healthy Subjects

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Context: Cholecystokinin (CCK) and neurotensin are stimulated during meal intake by the presence of fat in the small intestine. The sequence of events suggests that fat hydrolysis is crucial for triggering the release.

Objective: The aim of this study was to investigate whether CCK mediated the effect of intraduodenal (ID) fat on neurotensin secretion via CCK-1 receptors.

Setting: This was a single center study; 34 male volunteers were studied in consecutive, randomized, double-blind, cross-over studies.

Subjects and Methods: CCK and neurotensin release were quantified in: 1) 12 subjects receiving an ID fat infusion with or without 60 mg orlistat, an irreversible inhibitor of gastrointestinal lipases, in comparison to vehicle; 2) 12 subjects receiving ID long chain fatty acids (C18s), ID medium chain fatty acids, or ID vehicle; and 3) 10 subjects receiving ID C18 with and without the CCK-1 receptor antagonist dexloxiglumide or ID vehicle plus iv saline (placebo). Hormone concentrations were measured by specific RIA systems.

Results: ID fat induced a significant increase in CCK and neurotensin concentrations ($P < 0.001$–0.002). Inhibition of fat hydrolysis by orlistat abolished both effects. C18 stimulated CCK and neurotensin release ($P < 0.001$, respectively), whereas medium chain fatty acid was ineffective. Dexloxiglumide administration partially blocked the effect of C18 on neurotensin; the effect was only present in the first phase of neurotensin secretion.

Conclusions: Generation of C18 through hydrolysis of fat is a critical step for fat-induced stimulation of neurotensin in humans; the signal is in part mediated via CCK release and CCK-1 receptors. **(*J Clin Endocrinol Metab* 93: 1964–1970, 2008)**

Neurotensin is a gastrointestinal peptide stored in specific endocrine cells (N cells) of the distal small intestine; the peptide has been implicated in the regulation of gastrointestinal functions (1–3). Fat ingestion induces a dose-related increase in neurotensin plasma concentrations, whereas glucose and amino acids produce only minor or no effect (4–6). Major physiological functions of neurotensin include the stimulation of pancreatic exocrine secretion, inhibition of gastric acid secretion, and inhibition of gastroduodenal motility (7–10). Intracerebroventricular injections of neurotensin induce a dose-dependent inhibition of food intake in rats (11). The effect of peripheral administration of neurotensin on food intake has not been investigated. Therefore, the role of neurotensin in regulating appetite is not clear yet.

Several gut hormones have been implicated in the physiological control of appetite or food intake with consequences for conditions such as obesity or anorexia. A recent review summarized the changes observed in fasting concentrations of gut hormones in obese persons; the following changes were documented: increased levels of cholecystokinin (CCK), insulin, and amylin; and decreased concentrations of ghrelin, pancreatic polypeptide, peptide YY (PYY), and glucagon-like peptide

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Abbreviations: CCK, Cholecystokinin; C18, long chain fatty acid; C8, medium chain fatty acid; DEXLOX, dexloxiglumide; GLP, glucagon-like peptide; ID, intraduodenal; PYY, peptide YY.

TABLE 1. Relative binding of CCK-like peptides to the antibody

Peptide	% Binding
CCK-33 (human)	100
CCK-33 (porcine)	100
CCK-8 (sulfated)	100
CCK-8 (unsulfated)	0.01
CCK-9 (sulfated)	77.3
Cerulein	94.5
Gastrin (sulfated)	0.34
Gastrin (unsulfated)	0.15

Details of the assay have been previously described (19, 20).

(GLP)-1 (12, 13). Interestingly, in obese human subjects, lower neurotensin levels have been observed compared with lean controls (14). More important, after gastric banding and bypass procedures, respectively, neurotensin levels are increased (15, 16). Plasma neurotensin levels show a more pronounced increase after gastric bypass compared with gastric banding (15), suggesting that specific bariatric surgical procedures result in distinct alterations of gastrointestinal hormone metabolism. The fact that weight loss after gastric bypass surgery is associated with increased circulating levels of neurotensin can be interpreted as evidence that increased neurotensin levels are mechanistically linked to reduced appetite and weight after bariatric surgery. Therefore, it is important to understand the physiological factors controlling neurotensin release.

As mentioned before, fat is the most potent stimulus for the release of neurotensin (4, 5); moreover, products of fat digestion have been implicated in stimulating the release of various other peptides, which have been associated with inhibition of food intake (CCK, GLP-1, and PYY) (17–19). In fact, fat hydrolysis is crucial for stimulating CCK and PYY release (19). Therefore, we were interested in investigating whether fat hydrolysis is essential for stimulating neurotensin release; we were also interested in testing whether the effect of hydrolyzed fat on neurotensin secretion is mediated by CCK release via CCK-1 receptors. Therefore, the aim of this study was to investigate whether CCK mediated the effect of intraduodenal (ID) fat on neurotensin secretion via CCK-1 receptors.

Subjects and Methods

Subjects

A total of 34 male subjects, aged 20–30 yr (mean 25.2), participated in the study. The body weight of all subjects was within the normal range for age, sex, and height. Each subject gave written informed consent for the study. The human ethics committee of the University Hospital, Basel, approved the protocol. Before acceptance, each participant was required to complete a medical interview, receive a full physical examination, and participate in an initial laboratory screening. No subject was receiving any medications, or had a history of food allergies or dietary restrictions.

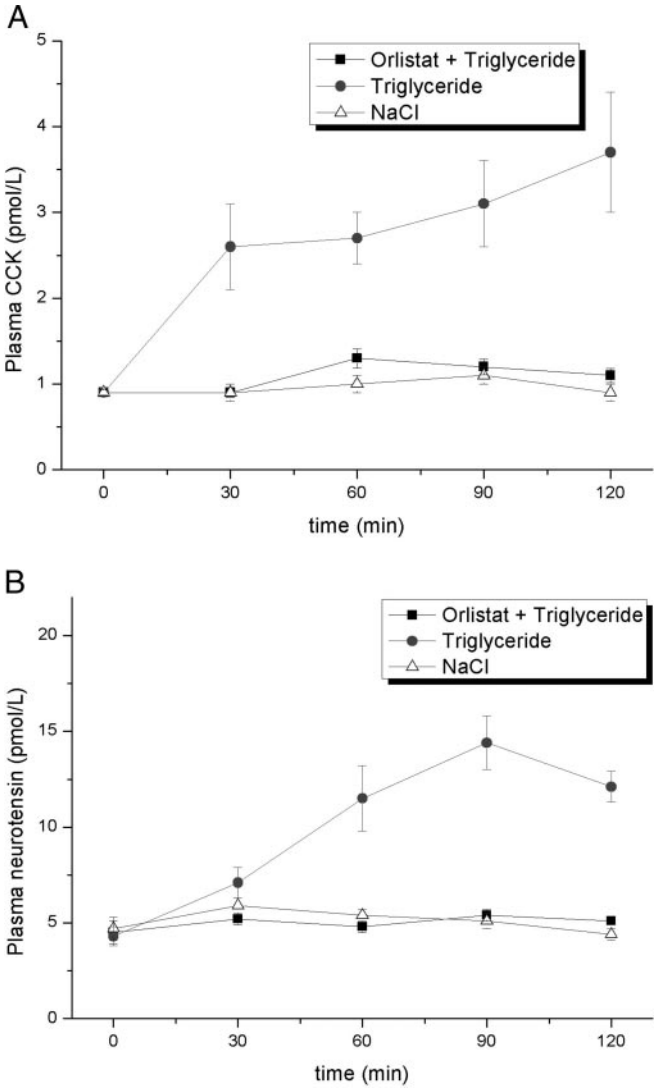

FIG. 1. Plasma concentrations of CCK (A) and neurotensin (B) during ID infusions of triglycerides with or without the lipase inhibitor orlistat (30 mg/h) or infusion of vehicle (control treatment) in 12 healthy male subjects. Triglycerides infused to the duodenum induced a treatment × time interaction for CCK and neurotensin ($P < 0.01$ each). CCK and neurotensin increased progressively during triglyceride infusion. Inhibition of fat hydrolysis completely abolished these effects. Plasma CCK and neurotensin concentrations were not different from control values. Data are mean ± SEM.

Experimental procedures

Part 1: effect of orlistat dissolved in olive oil on neurotensin release

Three treatments, separated by at least 7 d, were performed in 12 subjects in a randomized order. On the evening preceding each treatment, subjects swallowed a radioopaque polyvinyl feeding tube (external diameter 8 French), which had an opening at the tip of the tube. The tube was inserted through the nose because this procedure allowed the tube to be retained overnight and for the duration of the experiment. The position of the tube was located fluoroscopically, and the tip of the tube was positioned in part III of the duodenum (~100-cm distal to the teeth). It was firmly attached to the skin behind the ear to prevent further progression of the tube during the experiment.

The treatments were identical in design except for the ID infusions. One treatment consisted of ID saline infusion for the duration of the experiment (120 min). In the second and third experiments, ID fat (olive

TABLE 2. Plasma pharmacokinetics of CCK and neurotensin during ID infusions of fat or vehicle (control treatment) with and without orlistat in 12 healthy male subjects

	Treatments		
	ID vehicle (control)	**ID fat alone**	**ID fat plus Orlistat**
CCK			
AUC (0–120 min) (pmol/min·liter)	115.4 ± 4.18 (NA)	319.5 ± 46.63 ($P < 0.001$)	131.4 ± 5.6 (NS)
Cmax (pmol/liter)	1.3 ± 0.07 (NA)	3.9 ± 0.63 ($P < 0.001$)	1.4 ± 0.11 (NS)
Tmax (min)	67.5 ± 11.75 (NA)	105.0 ± 7.83 ($P < 0.013$)	67.5 ± 7.5 (NS)
	ID vehicle plus iv saline (control)	**ID TG plus iv saline**	**ID TG plus iv Orlistat**
Neurotensin			
AUC (0–120 min) (pmol/min·liter)	628.3 ± 30.0 (NA)	1234.6 ± 102.4 ($P < 0.001$)	605.2 ± 25.6 (NS)
Cmax (pmol/liter)	6.6 ± 0.3 (NA)	16.1 ± 1.6 ($P < 0.001$)	6.2 ± 0.2 (NS)
Tmax (min)	25.0 ± 6.2 (NA)	85.0 ± 6.3 ($P < 0.001$)	65.0 ± 15.6 ($P < 0.02$)

Data are mean ± SEM (n = 12 per group). *P* values represent statistically significant differences *vs.* control. AUC, Area under the curve; NA, not applicable; NS, nonsignificant; Cmax, maximum plasma concentration; Tmax, time at which plasma concentration is maximum.

oil) with or without 60 mg orlistat was used instead of saline throughout the experiments. An infusion rate of 0.5 ml/min oil was chosen for the duration of the experiment; this rate was taken from previous experiments (19). The ID fat infusion solution was indistinguishable in appearance from the control solution (saline). The treatments were given in a double-blind manner. During the experiments, samples of blood (7.5 ml each) were drawn at regular intervals into ice-chilled EDTA coated tubes containing aprotinin (1000 kallikrein inhibiting unit/ml blood) for plasma CCK and neurotensin determinations. Samples were immediately centrifuged at 4 C and deep frozen until analysis.

Part 2: effect of free fatty acids on neurotensin release

The design of the second series was similar to part 1: 12 healthy male subjects swallowed a feeding tube, which was positioned in part III of the duodenum under fluoroscopic control. The study was conducted in a randomized, double-blind, three-period crossover fashion. All treatments were given in the morning after an overnight fast. One treatment consisted of ID infusion of medium chain fatty acids (C8s) in the form of sodium caprylate. Sodium caprylate was perfused at a concentration of 0.049 g/ml at a rate of 0.5 ml/min, resulting in a load of 8 mmol/h. In the second experiment, long chain fatty acids (C18s) in the form of sodium oleate were infused at a concentration of 0.086 g/ml at a rate of 0.5 ml/min, resulting in a load of 8 mmol/h. On the third experimental day, volunteers received ID vehicle (control) instead of free fatty acids. The C8 and C18 loads were chosen from previous experiments (19). During each treatment, samples of blood were drawn (7.5 ml each) at regular intervals into EDTA coated tubes containing aprotinin (1000 kallikrein inhibiting unit/ml blood) for plasma CCK and neurotensin determinations.

Part 3: effect of C18 with and without iv dexloxiglumide (DEXLOX) on neurotensin secretion

The design of the third series was similar to part 2: healthy male subjects swallowed a feeding tube, which was positioned in part III of the duodenum under fluoroscopic control. Three treatments, separated by at least 7 d, were performed after fasting overnight in 10 subjects in a randomized order. Subjects received on 2 experimental days continuous ID infusion of C18 (sodium oleate, 8 mmol/h, for a total of 120 min) together with either an iv infusion of isotonic saline (control) or an infusion of the CCK-1 receptor antagonist DEXLOX (5 mg/kg·h) for the duration of the study. The dose of DEXLOX was chosen from previous experiments. Intravenous infusions were started 30 min before ID infusion (19). On the third experimental day, subjects received ID saline and iv saline during the study. Ambulatory infusion pumps through a Teflon catheter (DuPont Co., Wilmington, DE) inserted into a forearm vein delivered infusions. Blood was taken (7.5 ml) at regular intervals for hormone determinations.

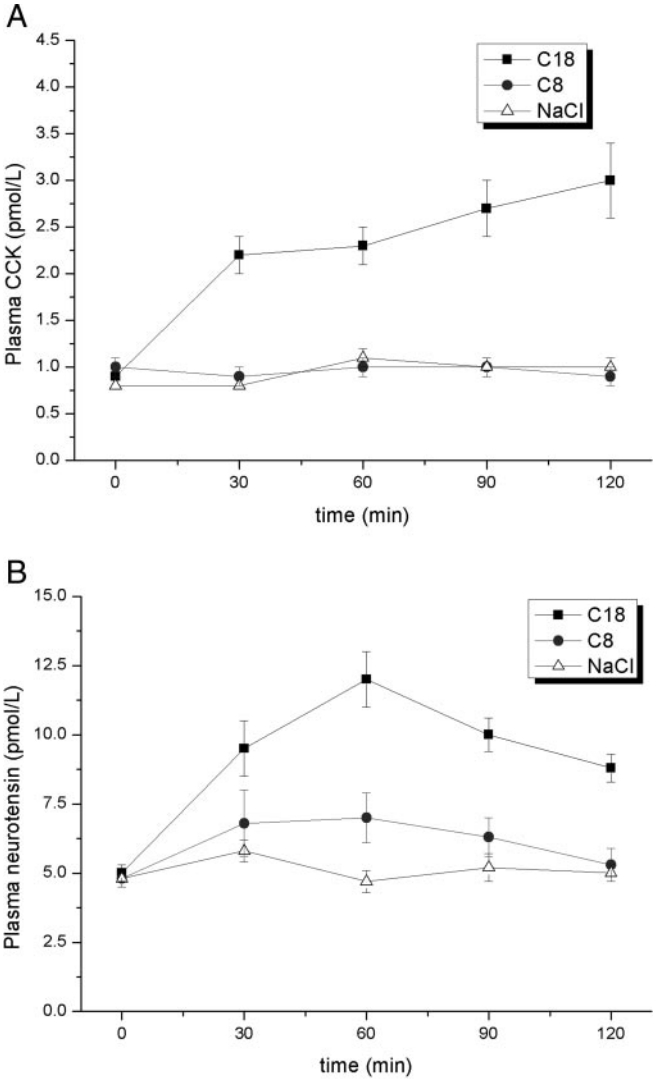

FIG. 2. Plasma concentrations of CCK (A) and neurotensin (B) during ID infusions of C18 (sodium oleate, 8 mmol/h), C8 (sodium caprylate, 8 mmol/h), or infusion of vehicle (control treatment) in 12 healthy male subjects. C18 increased CCK and neurotensin levels progressively ($P < 0.01$ each) compared with controls. C8 did not affect hormone levels. Plasma CCK and neurotensin concentrations were not different from control values. Data are mean ± SEM.

TABLE 3. Plasma pharmacokinetics of CCK and neurotensin during ID infusions of C18s, C8s, or infusion of vehicle (control treatment) in 12 healthy male subjects

	Treatments		
	ID saline (control)	**ID C8**	**ID C18**
CCK			
AUC (0–120 min) (pmol/min·liter)	114.2 ± 5.2 (NA)	113.1 ± 3.8 (NS)	272.3 ± 23.1 ($P < 0.001$)
Cmax (pmol/liter)	1.1 ± 0.1 (NA)	1.2 ± 0.1 (NS)	3.3 ± 0.3 ($P < 0.001$)
Tmax (min)	48.0 ± 11.1 (NA)	63.0 ± 13.7 (NS)	93.0 ± 11.4 ($P < 0.02$)
Neurotensin			
AUC (0–120 min) (pmol/min·liter)	616.9 ± 40.3 (NA)	754.9 ± 93.5 ($P = 0.001$)	1149.8 ± 59.4 ($P < 0.001$)
Cmax (pmol/liter)	6.1 ± 0.4 (NA)	7.6 ± 1.0 ($P = 0.0019$)	12.7 ± 1.1 ($P < 0.001$)
Tmax (min)	45.0 ± 12.0 (NA)	51.0 ± 6.4 (NS)	66.0 ± 6.0 (NS)

Data are mean ± SEM (n = 12 per group). *P* values represent statistically significant differences *vs.* control. AUC, Area under the curve; NA, not applicable; NS, nonsignificant; Cmax, maximum plasma concentration; Tmax, time at which plasma concentration is maximum.

Plasma hormone determinations

Plasma CCK concentrations were measured using a sensitive RIA based on an antiserum that recognizes the sulfated tyrosine residue of CCK8 but has minor cross-reactivity with sulfated gastrin (<1%) and does not cross-react with unrelated gastrointestinal peptides (Table 1). Synthetic, labeled CCK-8 was bought from Eurodiagnostica (Malmoe, Sweden) and used as a tracer, and synthetic CCK-8 was used as a standard. Separation of free from antibody bound hormone was performed by adsorption of the free peptide to charcoal [addition of 0.5 ml plasma-assay buffer suspension, 1:4 (vol/vol), containing 16 mg charcoal]. The sensitivity of the assay was 0.6 pmol/liter plasma.

Plasma samples were extracted with 96% (vol/vol) ethanol (1:2, vol/vol). The recovery of CCK-33 added to charcoal-treated plasma before the extraction procedure was more than 75%.

Neurotensin was measured with a commercially available kit (Phoenix Europe GmbH, Karlsruhe, Germany). ^{125}I-neurotensin was used as a label; the labeled peptide was purified by HPLC (specific activity 302 μCi/μg). The lowest level of neurotensin that could be detected by this assay was 10 pg/ml when using a 100 μl plasma sample. Intraassay and interassay variabilities were less than 11% and less than 13%, respectively. Plasma samples were extracted with C18-Sep-Pak (Millipore Corp., Billerica, MA).

In a recent study, it was suggested that a reliable measurement of mature neurotensin is difficult due to its instability and its rapid clearance from the circulation with a half-life of 2–6 min, often leading to an underestimation of the actual neurotensin levels (14, 21). The identification of the more stable precursor peptide pro-NT/NMN, which is produced in stoichiometric amounts to mature neurotensin, allowed us to compare directly the two molecular forms in plasma. Therefore, some samples were also assayed for pro-NT/NMN. Pro-NT/NMN was measured by A. Ernst (SphingoTec GmbH, Borgsdorf, Germany) using a new sandwich immunoassay as previously described (21). Briefly, pro-NT/NMN was measured in a sandwich immunoassay using polyclonal rabbit antibodies against the pro-NT/NMN-peptides 44–62 and 98–117. Antibodies were purified from rabbit antisera by affinity chromatography. Antibodies to amino acids 44–62 were coated on polystyrene tubes (2 μg/tube) as solid phase, and antibodies to amino acids 98–117 were labeled with acridinium ester-N-hydroxy-succinimide for chemiluminescence detection. The assay was calibrated using a human reference serum pool. The analytical assay sensitivity as determined with normal horse serum was 9.95 pmol/liter. Serum samples with pro-NT/NMN concentrations above the highest calibrator were pre-diluted in normal horse serum, measured again, and the concentration was subsequently extrapolated.

Statistical analysis

Hormone parameters were compared among treatment groups by the general linear model analysis using repeated measurement option. If this analysis revealed significant differences between the treatments, treatment groups were compared with the control group by simple linear contrasts. The level of significance was $P = 0.05$. All statistical comparisons were performed using SPSS for Windows software (version 14.0; SPSS, Inc., Chicago, IL).

Results

Part 1

Plasma neurotensin concentrations were significantly increased with fat infusion ($P < 0.001$) compared with the control treatment (Fig. 1 and Table 2). Orlistat (60 mg, infused together with fat) reversed the effects of ID fat. The stimulation of neurotensin release was virtually blocked by the lipase inhibitor; as a consequence, neurotensin concentrations were similar to controls. These data indicate that fat induced increase in circulating neurotensin can be abolished by inhibition of fat hydrolysis. CCK measurements served as positive controls. As expected, orlistat infusion markedly attenuated the fat induced increase in circulating CCK (Fig. 1 and Table 2), thereby confirming previous results (19).

Part 2

Fasting hormone concentrations were comparable in the different treatments. During the control treatment and during infusion of C8, plasma neurotensin and CCK levels remained largely unchanged. ID administration of C18 caused a significant increase in both plasma CCK and neurotensin levels ($P < 0.001$ and $P < 0.001$, respectively) compared with controls (Fig. 2 and Table 3).

Part 3

ID infusion of C18 with iv infusion of saline caused a significant ($P < 0.001$) increase in plasma neurotensin compared with controls (Fig. 3 and Table 4). In contrast, ID C18 infusion together with iv DEXLOX resulted in a blunted neurotensin response in the first 60 min compared with ID C18 plus iv saline ($P < 0.003$; Fig. 3 and Table 4). After 60 min, neurotensin levels markedly increased in response to C18 infusion together with iv DEXLOX; the area under the curve for the time period 60–120 min was higher for C18 infusion together with iv DEXLOX ($P = 0.06$) compared with ID C18 infusion plus iv saline (Table 4).

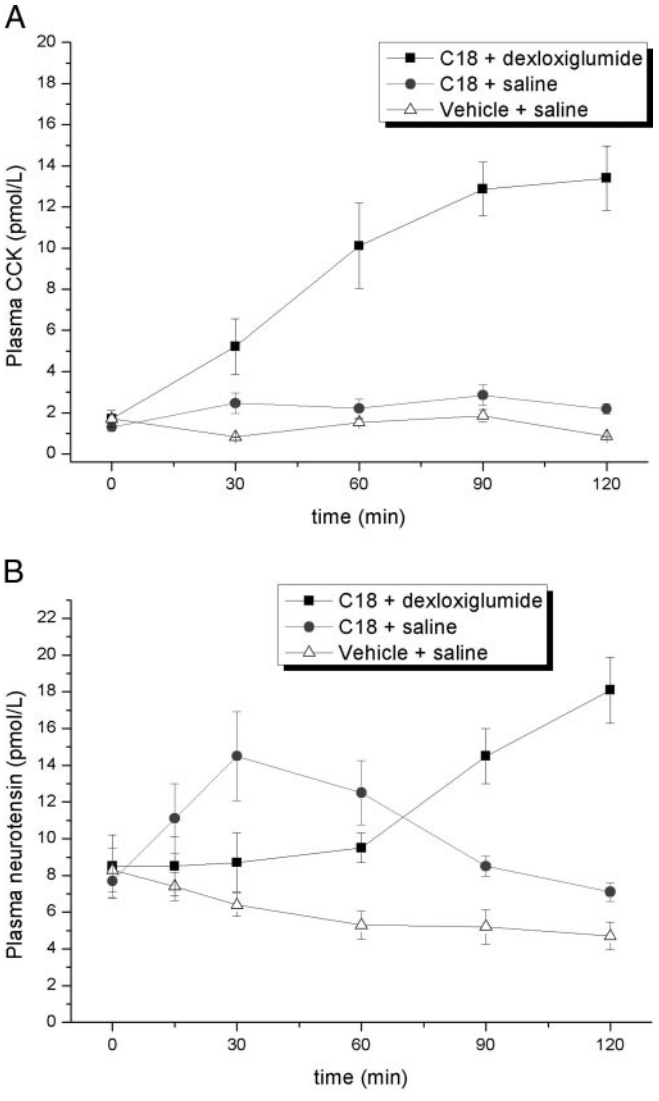

FIG. 3. Effect of DEXLOX, a specific CCK-1 receptor antagonist, on plasma concentrations of CCK (A) and neurotensin (B) during ID infusions of C18s or infusion of vehicle (control treatment) in 10 healthy male subjects. C18 increased CCK and neurotensin concentrations progressively ($P < 0.01$ each). Intravenous administration of DEXLOX (5 mg/kg·h) partially antagonized the effects induced by intraluminal fat. Plasma neurotensin concentrations were not different from control values in the first 60 min but increased progressively thereafter. Data are mean ± SEM.

These data indicate that the increase in plasma neurotensin concentrations induced by ID C18 administration can only be blocked in part by the CCK-1 receptor antagonist DEXLOX. The results suggest that circulating CCK via CCK-1 receptors mediates the first phase of C18-stimulated neurotensin release.

As depicted in Fig. 3 and Table 4, ID infusion of C18 with iv infusion of saline caused a significant increase in plasma CCK compared with controls (ID saline plus iv saline). ID C18 infusion together with iv DEXLOX resulted in a significantly augmented CCK response (Fig. 3 and Table 4) in comparison to ID C18 plus iv saline.

Pro-NT/NMN measurement

Figure 4 depicts the time course of pro-NT/NMN in response to ID infusion of C18 with iv infusion of saline or ID C18 infusion together with iv DEXLOX. The pattern of pro-NT/NMN is very similar to the data obtained with mature neurotensin (Fig. 3), suggesting that both assays were able to quantify reliably the respective molecules.

Discussion

Neurotensin is a regulatory signal from the gastrointestinal tract that can modulate digestive functions (8, 9, 11). Recently, it has also been implicated in the regulation of appetite and food intake (11, 14–16). Further understanding of the physiological mechanisms by which this hormone is stimulated by nutrients is required to appreciate the regulatory circuits controlling appetite and food intake. Different macronutrients exert specific effects on satiety signals. Fat, as an example, is one macronutrient that has been shown before to stimulate CCK, GLP-1, PYY, and neurotensin release (5, 17–19, 22–26). Here, we investigated the importance of different digestion products of fat in regulating neurotensin secretion.

Three different approaches were used to establish that fat hydrolysis is a critical step in the control of neurotensin release. First, we used the lipase inhibitor orlistat as a tool to assess the importance of fat hydrolysis in initiating the release of neurotensin. Stimulation of neurotensin release in response to intestinal fat infusion was completely abolished by orlistat administration. We infer from these data that fat-stimulated neurotensin release is dependent on adequate fat hydrolysis. The present results extend the list of digestive functions, which are dependent on adequate fat hydrolysis.

In a second step, we analyzed the importance of the chain length of fatty acids in triggering neurotensin release. In this part of the study, C8s in the form of sodium caprylate containing eight carbons (denominated C8) or C18s in the form of sodium oleate with 18 carbons (denominated C18) were infused into the small intestine. C18 infusion resulted in a marked increase in neurotensin and CCK concentrations. C8 infusion did not change neurotensin concentrations. These observations suggest that in addition to fat hydrolysis, the chain length of free fatty acids is crucial for initiating neurotensin secretion. Jonkers *et al.* (25) had previously compared long chain triglycerides to very long chain triglycerides and saline perfusion, and concluded that CCK release and gallbladder contraction was chain length dependent, whereas neurotensin release and small bowel transit were chain length independent. These data were in part confirmed and extended by the present results.

In the third part, we used the specific CCK-1 receptor antagonist DEXLOX to block the actions of CCK. The C18 stimulated neurotensin response was partially blocked with CCK-1 receptor blockade, suggesting that the first phase of neurotensin secretion stimulated by intestinal fat was mediated by CCK via its CCK-1 receptor. The stimulation of this first phase of neurotensin secretion is mediated either hormonally through circulating CCK or neurally via CCK-containing nerves because it is not possible that luminal fat has reached the distal small intestine to directly stimulate neurotensin release at this stage. In a second phase, a direct stimulation of neurotensin secreting cells through luminal

TABLE 4. Plasma pharmacokinetics of CCK and neurotensin during ID infusions of C18 or infusion of vehicle (control treatment) with and without iv DEXLOX in 10 healthy male subjects

	Treatments		
	ID vehicle plus iv saline (control) (A)	ID C18 plus iv saline (B)	ID C18 plus iv DEXLOX (C)
CCK			
AUC (0–120 min) (pmol/min·liter)	164.0 ± 12.5(NA)	277.4 ± 31.5 (NS)	1073.2 ± 138.8 [$P < 0.001$ *vs.* (A) $P < 0.001$ *vs.* (B)]
Cmax (pmol/liter)	2.1 ± 0.3 (NA)	3.6 ± 0.5 (NS)	14.7 ± 1.5 [$P < 0.001$ *vs.* (A) $P < 0.001$ *vs.* (B)]
Tmax (min)	54.0 ± 14.0(NA)	81.0 ± 11.9 (NS)	96.0 ± 6.0 ($P = 0.033$)
Neurotensin			
AUC (0–120 min) (pmol/min·liter)	419.0 ± 52.8(NA)	768.8 ± 83.4 ($P < 0.001$)	823.6 ± 54.9 ($P = 0.002$)
AUC (0–60 min) (pmol/min·liter)	236.9 ± 25.3(NA)	440.8 ± 64.4 [$P = 0.015$, *vs.* control (A)]	316.1 ± 45.6 (NS) *vs.* control (A)
AUC (60–120 min) (pmol/min·liter)	182.1 ± 29.8(NA)	328.0 ± 22.2 [$P = 0.003$, *vs.* control (A)]	507.5 ± 31.4 [$P < 0.001$, *vs.* control (A)] [$P < 0.001$, *vs.* (B)]
Cmax (pmol/liter)	5.1 ± 0.7 (NA)	9.2 ± 1.3 [$P = 0.001$, *vs.* control (A)]	11.7 ± 0.9 [$P = 0.002$, *vs.* control (A)]
Tmax (min)	12.0 ± 6.2 (NA)	52.5 ± 10.3 [$P = 0.01$, *vs.* control (A)]	90.0 ± 13.4 [$P = 0.001$, *vs.* control (A)]

P values represent statistically significant differences *vs.* the respective treatment (ANOVA, Scheffe's multicomparison test). Data are mean ± SEM (n = 10 per group). AUC, Area under the curve; NA, not applicable; NS, nonsignificant; Cmax, maximum plasma concentration; Tmax, time at which plasma concentration is maximum.

C18 is, however, possible. From these observations the following concept can be developed. Adequate fat hydrolysis is required for stimulating neurotensin release; in the first phase, the products of fat digestion stimulate CCK release, which in turn regulates neurotensin secretion via CCK-1 receptors. At this stage it cannot be determined whether circulating CCK is responsible for inducing these effects; it is also conceivable that direct activation of CCK-1 receptors on afferent vagal fibers through C18 stimulation is a potential pathway. After about 60 min, the CCK-1 receptor antagonist was unable to suppress neurotensin release, suggesting that a direct stimulation of the neurotensin cell has occurred through C18s. The receptor and the molecular mechanisms mediating this effect need to be elaborated. Schmidt *et al.* (24) have previously reported on the effects of loxiglumide on neurotensin secretion stimulated with a test meal; no effect on neurotensin release was observed under these conditions. There are several possibilities for these discrepant findings: 1) experimental design (oral test meal administration *vs.* duodenal perfusion of fat), 2) confounding effects of oral meal intake (CCK-1 receptor blockade can accelerate gastric emptying), 3) potency of CCK-1 receptor antagonist (DEXLOX is more potent than loxiglumide on a molar basis), and 4) type of stimulant (C18 *vs.* meal). The exact mechanism needs to be established.

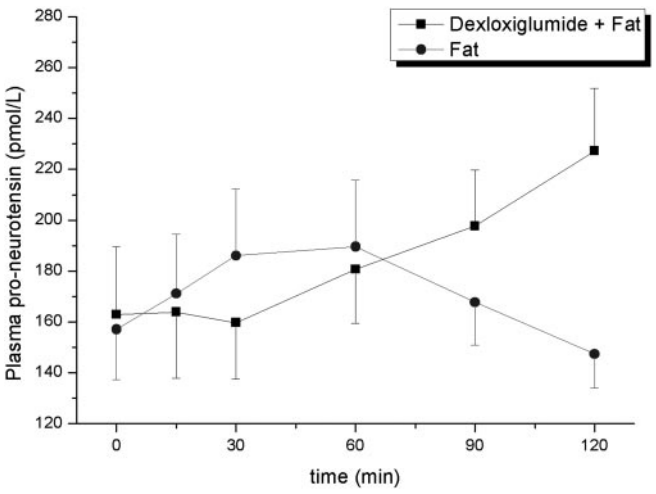

FIG. 4. Effect of iv DEXLOX or saline on plasma concentrations of pro-NT/NMN during ID infusions of C18s in 10 healthy male subjects. C18 induced a progressive increase in pro-NT/NMN concentrations ($P < 0.01$). Intravenous administration of DEXLOX (5 mg/kg·h) partially antagonized the effects induced by intraluminal fat. Plasma pro-NT/NMN concentrations were suppressed in the first 60 min but increased steadily thereafter. Data are mean ± SEM.

The clinical implications of these findings relate to patients who have undergone gastric bypass surgery for obesity. Changes in gastrointestinal peptide concentrations have been reported for GLP-1, PYY, and neurotensin after bariatric surgery (14, 15). The increased levels of these three peptides after bypass surgery have been used to explain the decreased appetite and energy intake after the surgical procedures despite weight loss, which under normal conditions would induce compensatory hyperphagia. However, a role for neurotensin in regulating appetite has not been established and requires further investigation.

The measurement of mature neurotensin has been difficult; the reasons that have been listed include its instability and its rapid clearance from the circulation with a short half-life, sometimes leading to an underestimation of the actual neurotensin levels (21). Measuring the more stable precursor pro-NT/NMN provides an alternative tool to quantify neurotensin levels in clinical practice. The present data reveal that mature neurotensin concentrations can be adequately measured if standard precautions are used, such as blood drawing into ice-chilled EDTA tubes containing aprotinin and immediate centrifugation at 4 C. The pattern of neurotensin release was similar to the pattern of pro-NT/NMN, and no significant differences were documented. The advantage of measuring pro-NT/NMN is probably outside the research setting in daily clinical practice, where the standard precautions stated previously are difficult to follow.

In summary, three pertinent conclusions can be drawn from the present study: 1) adequate fat digestion is crucial for stimulating plasma neurotensin secretion, 2) C18s are necessary to initiate the release of neurotensin, and 3) CCK-1 receptor blockade attenuates the first phase of neurotensin secretion but does not affect the second phase of neurotensin release. The data support the following conclusions: fat hydrolysis in the proximal small intestine is required to start the process; the specific products of fat digestion, C18s, then stimulate the release of CCK (neural or hormonal); and CCK in turn acts on CCK-1 receptors, which then initiates the release of neurotensin secretion. If hy-

drolyzed fatty acids reach the distal small intestine, a direct stimulation of neurotensin occurs through C18s.

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