Effect of medium- and long-chain triglycerides on lower esophageal sphincter pressure: role of CCK

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Ledeboer, M., A. A. M. Masclee, I. Biemond, and C. B. H. W. Lamers. Effect of medium- and long-chain triglycerides on lower esophageal sphincter pressure: role of CCK. Am. J. Physiol. 274 (Gastrointest. Liver Physiol. 37): G1160–G1165, 1998.—Fat meals are known to decrease lower esophageal sphincter (LES) pressure, possibly through postprandial CCK release. Dietary fat consists mainly of long-chain triglycerides (LCT), which potentiate CCK release. This effect contrasts with that of medium-chain triglycerides (MCT), which do not induce CCK secretion. This effect contrasts with that of medium-chain triglycerides (MCT), which do not induce CCK secretion. The effect of MCT on LES pressure is mediated by CCK. The effect of MCT is not dependent on CCK, since MCT does not release CCK and loxiglumide does not prevent the MCT-induced reduction in LES pressure.

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INGESTION OF FATTY MEALS results in a reduction in lower esophageal sphincter (LES) pressure (3, 32). Fat is a potent stimulus of CCK release, and CCK is thought to be responsible for fat-induced reduction of LES pressure (3, 17, 27, 32). Results of studies on the effect of CCK on LES pressure have been controversial. Infusion of CCK in humans reduced LES pressure in some studies (12, 33, 35), but in the study of Brazer et al. (7) a reduction in LES pressure was demonstrated only at supraphysiological doses of CCK-8. We addressed this question in a previous study and concluded that intravenous infusion of CCK-33 to physiological postprandial plasma levels significantly reduced LES pressure (26). However, infusion of exogenous CCK in the interdigestive state may not represent the digestive or postprandial state, and therefore studies with endogenously released CCK are needed to solve this issue. By using specific CCK-A receptor antagonists, the role of endogenous CCK as a physiological regulator of LES pressure can be further clarified. Loxiglumide (CR-1505) is a potent and specific antagonist of peripheral CCK-A receptors, which antagonizes the effects of CCK by competitive binding (34). No effects other than those mediated through inhibition of the CCK-A receptor have been observed after administration of loxiglumide in vitro or in vivo (2, 34). Loxiglumide has been used in the past to study the role of endogenous CCK in the regulation of gallbladder motility and pancreatic enzyme secretion in humans (10, 14, 23, 30). Infusion of loxiglumide does not influence interdigestive basal LES pressure (29), but Katschinski et al. (25) recently reported that loxiglumide prevented the decrease in LES pressure that occurs after ingestion of a mixed meal.

Dietary fat mainly consists of long-chain triglycerides (LCT), which are known to potently release CCK. In this study we have investigated the effect of intraduodenal administration of LCT on CCK release and on LES pressure. The studies were performed with and without the infusion of loxiglumide. In addition, gallbladder volumes were measured, because gallbladder contraction is mainly regulated by CCK. In contrast to LCT, medium-chain triglycerides (MCT) do not induce CCK release or gallbladder contraction (18). It is, however, not known whether MCT affects LES pressure. Therefore, the effect of an equimolar amount of intraduodenal MCT on LES pressure, CCK release, and gallbladder motility was studied without and with concomitant infusion of loxiglumide.

METHODS

Subjects. Six healthy volunteers (4 male, 2 female; mean age 26 yr, range 19–39 yr) participated in the study. The subjects were studied after an overnight fast of at least 10 h. None of the subjects had a history of gastrointestinal disease, gallstones, or surgery, and none of the subjects were taking any medication. The presence of gallstones was excluded by ultrasonography. Informed consent was obtained from each individual, and the protocol was approved by the ethics committee of the Leiden University Medical Center.

Study protocol. The subjects participated in five experiments performed on separate occasions in random order with an interval of at least 7 days. At 8:00 AM the subjects were intubated with a single-lumen polyurethane feeding tube (Flocare Ch 10; Nutricia, Zoetermeer, The Netherlands) that was positioned under fluoroscopic control in the horizontal part of the duodenum. The manometry catheter to record LES pressure was positioned and secured as described below. Two intravenous cannulas (one for blood sampling, the other for infusion of loxiglumide or saline) were inserted into the antecubital veins of both arms. Basal gallbladder images and blood samples for determination of plasma CCK levels were obtained 30, 15, and 0 min before the start of the experiment (time 0). LES pressure was recorded continuously for a 30-min basal period from −30 to 0 min. At time (t) = −15 min, intravenous infusion of saline or loxiglumide (CR-1505; Rotta Research Laboratories, Monza, Italy; loading dose: 30 mg·kg⁻¹·h⁻¹ for 10 min (=5 mg/kg), followed by continuous infusion of 10 mg·kg⁻¹·h⁻¹) was started. LES pressure was
recorded continuously, and gallbladder images and blood samples were obtained at 15-min intervals during continuous intraduodenal administration of either LCT (corn oil, fatty acid composition 93%, C16–C18), MCT (Ceres-MCT dietary oil; Van den Bergh en Jurgens, Rotterdam, The Netherlands; fatty acid composition 98%, C8–C10), or saline (control, 20 ml/h) at an infusion rate of 20 mmol/h (MCT 15 g/90 min; LCT 30 g/90 min) with concomitant intravenous infusion of either saline (intraduodenal LCT or MCT) or loxiglumide (intraduodenal LCT or MCT) for 90 min.

Manometric recording technique and analysis. Esophageal pressure recordings were obtained using a polyvinyl assembly of 5-mm outer diameter, incorporating a 6-cm-long sleeve sensor and seven side holes, one located at the distal margin to monitor intragastric pressure and six located 0, 3, 6, 9, 15, 24, or 30 cm proximal to the upper margin of the sleeve sensor (Dent Sleeve) (9). The manometric assembly was passed transnasally into the esophagus, and the sleeve sensor was positioned so that it permitted continuous monitoring of LES pressure. Simultaneously, pressures were recorded from the gastric fundus, LES (sleeve), esophageal body (side holes 3, 9, and 15 cm above the upper margin of the sleeve sensor), and pharynx (24 or 30 cm above the sleeve sensor). The side holes and sleeve sensor were connected to a pressure transducer (Medex, Hilliard, OH) and perfused with gas-free distilled water by a low-compliance pneumohydraulic infusion pump (Arndorfer Medical Supplies, Greendale, WI) at a rate of 0.5 ml/min. The side hole located in the pharynx, used to monitor swallowing, was filled with water but not perfused to avoid excessive swallowing. The outputs from pressure transducers were processed by an eight-channel polygraph (Synectics Medical, Stockholm, Sweden) displayed on a monitor and stored on a personal computer.

The pressure tracings were analyzed visually to calculate LES pressure. Each manometric tracing was coded. Analysis of the tracings was performed in a blinded fashion by investigators who were unaware of the code. Mean end-expiratory LES pressure was determined at 10-min intervals and referenced to end-expiratory intragastric pressure, defined as zero.

Measurement of gallbladder volume. Gallbladder volumes measured with real-time ultrasonography (Technicare 3; 5-MHz transducer) were calculated by the sum-of-cylinders method, using a computerized system (11, 16). In this method the longitudinal image of the gallbladder is divided into sections of equal length, with diameters perpendicular to the longitudinal axis of the gallbladder image. The uncorrected volume is the sum of the volumes of these separate cylinders. To correct for the displacement of the longitudinal image of the gallbladder from the central axis, a correction factor was calculated from the longitudinal and transverse scans of the gallbladder. Gallbladder volume is calculated by multiplying the uncorrected volume with the square of the correction factor. The mean of the two measurements was used for further analysis. The assumptions and the mathematical formula used to calculate gallbladder volume have been described and validated previously (11, 16).

Assay of CCK. Blood samples were collected in tubes containing EDTA and kept on ice during the experiment. Plasma CCK was measured by a sensitive and specific radioimmunoassay, using antibody T204 (21, 22). This antibody binds to all carboxy-terminal CCK peptides containing the sulfated tyrosyl region. The detection limit of the assay was 0.3 pmol/l plasma. The intra-assay variation ranged from 4.6% to 11.5%, and the interassay variation ranged from 11.3% to 26.1% (22).

Statistical analysis. Results are expressed as means ± SE. LES pressures are expressed in millimeters of mercury, gallbladder volumes are expressed in cubic centimeters, and plasma CCK levels are expressed in picomoles per liter. Differences in LES pressures, gallbladder volumes, and CCK levels within or between the experiments were analyzed for statistical significance by multiple ANOVA. When this indicated a probability of <0.05 for the null hypothesis, Student-Newman-Keuls analyses were performed to determine which values differed significantly between or within the experiments. The significance level was set at P < 0.05.

RESULTS

LES pressure. Basal LES pressures were not significantly different among the five experiments (Figs. 1 and 2). During intravenous infusion of saline, LES pressure 0 min before intraduodenal administration of LCT, MCT, and saline was 29 ± 3, 30 ± 2, and 27 ± 3 mmHg, respectively. During intravenous infusion of loxiglumide LES pressure was 27 ± 3 (LCT) and 27 ± 2 mmHg (MCT) at 0 min. During intraduodenal administration of both LCT and MCT with concomitant intravenous saline, a rapid and significant (P < 0.05) reduction in LES pressure was observed, starting at t = 10 min (to 20 ± 3 and 19 ± 3 mmHg, respectively; Fig. 1). LES pressure remained significantly reduced during all 90 min of intraduodenal administration of both LCT and MCT. The reduction in LES pressure between intraduodenal LCT and MCT was not significantly different. Intravenous infusion of loxiglumide prevented the reduction in LES pressure during intraduodenal administration of LCT (Fig. 2A). However, intravenous infusion of loxiglumide did not affect the reduction in LES pressure during intraduodenal administration of MCT (19 ± 2 mmHg at t = 10 min; Fig. 2B). No significant alterations in LES pressure were observed during intraduodenal administration of saline (Fig. 1).

Plasma CCK concentrations. Basal plasma CCK concentrations (t = −15 min) were not significantly differ-
ent among the five experiments: 2.6 ± 0.3 (LCT), 2.4 ± 0.3 (MCT), and 2.5 ± 0.3 pmol/l (saline) before intravenous infusion of saline (Fig. 3A), and 2.6 ± 0.2 (LCT) and 2.5 ± 0.2 pmol/l (MCT) before intravenous infusion of loxiglumide (Fig. 3B). No significant changes in plasma CCK levels were observed during intravenous infusion of either saline or loxiglumide from t = −15 to t = 0 min. Intraduodenal administration of LCT during concomitant intravenous saline resulted in a significant (P < 0.05) increase in plasma CCK over basal levels starting from 15 min to a maximum of 4.3 ± 0.6 pmol/l at 75 min (Fig. 3A). Administration of LCT also induced significant (P < 0.05) increases in plasma CCK levels from 15 min until 90 min compared with control (saline). No significant alteration in plasma CCK levels over basal was observed during MCT administration.

Infusion of loxiglumide did not affect the plasma CCK increase in response to intraduodenal administration of LCT. The significant increase in plasma CCK started at 60 min and reached a peak value of 4.7 ± 0.5 pmol/l at 90 min (Fig. 3B). During intraduodenal administration of MCT and loxiglumide no significant alterations in plasma CCK levels were observed.

Gallbladder volumes. Fasting gallbladder volumes at t = −15 min were not significantly different between the five experiments: 28.3 ± 4.5 cm³ before LCT, 28.8 ± 3.5 cm³ before MCT, 27.7 ± 3.8 cm³ before saline (Fig. 4A), 28.1 ± 1.7 cm³ before LCT and loxiglumide, and 29.4 ± 3.4 cm³ before MCT and loxiglumide (Fig. 4B). No significant changes in gallbladder volumes were observed during intravenous infusion of saline or loxiglumide from t = −15 to t = 0 min. During intraduodenal administration of LCT with concomitant intravenous infusion of saline a significant (P < 0.05) reduction in gallbladder volume was observed, starting at t = 15 min and reaching a residual volume of 10.8 ± 3.4 cm³ (61 ± 12% contraction) at t = 75 min (Fig. 4A). Intraduodenal administration of MCT with concomitant intravenous infusion of saline did not result in

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**Fig. 2.** LESP (means ± SE; mmHg) after fasting and during intravenous infusion of loxiglumide (10 mg·kg⁻¹·h⁻¹) with simultaneous intraduodenal administration of LCT (A) or MCT (B) in a dose of 20 mmol/h for 90 min. *Significant (P < 0.05) difference compared with basal.

**Fig. 3.** A: plasma CCK concentrations (means ± SE; pmol/l) after fasting and during intravenous infusion of either saline (A) or loxiglumide (10 mg·kg⁻¹·h⁻¹; B), with simultaneous intraduodenal administration of LCT (●), MCT (□), or saline (▲) in a dose of 20 mmol/h for 90 min. *Significant (P < 0.05) difference compared with basal levels and with saline (control).
gallbladder contraction but, on the contrary, resulted in a small but significant (P < 0.05) increase in gallbladder volume, reaching a maximum of 37.8 ± 4.1 cm³ (135 ± 14%) at t = 90 min. No significant alterations in gallbladder volume were observed during intraduodenal administration of saline (control).

During intravenous administration of both LCT and MCT a significant (P < 0.05) increase in gallbladder volume was observed, starting at 30 min and reaching peak values of 50.9 ± 3.4 cm³ (181 ± 12%) and 52.8 ± 3.0 cm³ (180 ± 10%) at t = 90 min, respectively (Fig. 4B). No significant differences in gallbladder volume were observed between the two experiments.

DISCUSSION

In this study we have shown that intraduodenal administration of LCT or MCT at a dose of 20 mmol/h significantly decreases LES pressure. Loxiglumide, a potent and specific receptor antagonist of the CCK-A receptor, prevented the reduction in LES pressure induced by intraduodenal administration of LCT, but not during administration of MCT. By measuring CCK release during fat administration and the effect of CCK receptor blockade, we were able to determine the role of CCK in the LES pressure reduction induced by LCT and MCT. It appears that the effect of LCT on LES pressure is dependent on endogenous CCK release, whereas the effect of MCT is not mediated by CCK.

The effect of CCK on LES pressure has been questioned in previous studies (7, 12, 26, 33, 35). Infusion of CCK in humans decreases LES pressure, but this effect may occur only at supraphysiological doses, as suggested by Brazer et al. (7) and by Katschinski et al. (25). In a recent study we demonstrated that infusion of CCK in a dose of 1.0 IDU·kg⁻¹·h⁻¹, leading to plasma CCK levels similar to those induced by a fatty meal, decreases LES pressure significantly, by ~40% (26). Infusion of CCK in a lower dose of 0.5 IDU·kg⁻¹·h⁻¹ increased plasma CCK levels to 6.1 ± 0.4 pmol/l but did not affect LES pressure. In the present study, although plasma CCK levels reached during intraduodenal infusion of LCT were lower (at maximum 4.3 ± 0.6 pmol/l), LES pressure decreased significantly. The effect of LCT on LES pressure was completely antagonized during loxiglumide infusion. These results emphasize the role of endogenous CCK in fat-induced reduction of LES pressure. Furthermore, our findings are in agreement with those of Katschinski et al. (25), who demonstrated that duodenal perfusion of a mixed liquid meal significantly reduced LES pressure at relatively low plasma CCK levels, whereas exogenous CCK reduced LES pressure only at higher plasma CCK levels. In the study of Katschinski et al. (25), loxiglumide antagonized the reduction in LES pressure during intraduodenal administration of the mixed meal. In the present study we have demonstrated that intraduodenal LCT decreases LES pressure and that this effect is prevented by blockade of CCK receptors.

It is remarkable that LCT fat induces a decrease in LES pressure at plasma CCK levels well beneath those at which exogenous CCK results in a significant reduction in LES pressure. Several factors may account for the observed discrepancy in the effect between exogenous and endogenous CCK on LES pressure. First, different molecular forms of CCK exist, which may vary in biological activity (6, 22, 27). However, the radioimmunoassay used is highly specific for bioactive CCK peptides. Second, the LES itself or its neurons may differ in sensitivity to various molecular forms of CCK. In our study on the effect of exogenous CCK on LES pressure, we used CCK-33 (26). This molecular form of CCK did not affect LES pressure at physiological plasma levels comparable to those seen after ingestion of a regular meal. Similarly, in studies with CCK-8 a reduction was not observed in LES pressure at plasma CCK levels in the postprandial range (7, 25). Third, intraduodenal LCT may influence the effect of endogenous CCK by acting through vagal afferent pathways.
as has been reported previously for the effect of duodenal distension by nutrients on gastric motility (15). Fourth, it has been suggested that CCK decreases LES pressure through a nonadrenergic, noncholinergic neural mechanism, using peptides such as vasoactive intestinal polypeptide or peptide histidine-isoleucine as neurotransmitters (4, 5). It is not known whether these neurotransmitters are more sensitive to endogenous than to exogenous CCK.

A direct effect of circulating long-chain fatty acids (LCFA) on LES pressure, after absorption from the intestinal lumen, cannot be excluded. Recent studies have shown that circulating fat (LCFA) influences gastric emptying and small intestinal motility (8, 13). LCT are absorbed as LCFA after dispersion by bile salts and hydrolysis by pancreatic lipase, which are both secreted in response to CCK release. CCK receptor blockade with loxiglumide inhibits pancreatic enzyme secretion and gallbladder contraction in response to food stimulation by 60–100% (14). Basal pancreatic enzyme secretion and biliary output are nearly abolished during loxiglumide infusion (23). Therefore, during CCK receptor blockade, digestion and absorption of LCFA may be impaired, and a presumed direct effect of circulating LCFA on LES pressure may be lacking. A direct effect of circulating medium-chain fatty acids (MCFA) may account for the observed reduction in LES pressure after MCT. Hydrolysis of MCT is very rapid and is caused by nonpancreatic lipases (especially gastric lipase), and dispersion occurs in the absence of bile salts (1, 31). Small amounts of MCT are able to enter the intestinal cell without prior hydrolysis. Thus absorption of MCT or MCFA from the intestinal lumen is largely independent of pancreatic enzyme or biliary secretion. Infusion of loxiglumide will not inhibit digestion and absorption of MCT. Therefore, a direct effect of circulating MCT or MCFA on LES pressure may be possible. A direct effect of MCT or MCFA on LES is supported by the finding that loxiglumide does not prevent the MCT-induced reduction in LES pressure. The effects of fatty acids in the postabsorptive or circulatory phase of digestion on LES pressure have not previously been studied in detail. In one study no significant change in LES pressure after intravenous infusion of a lipid emulsion was found. However, LES pressures in that study were not measured with a sleeve catheter, and a possible effect could have been masked by the long interval between fat administration and the onset of LES recording (8).

Other mechanisms involved in the MCT-induced reduction in LES pressure remain speculative. An effect of MCT on LES pressure mediated by CCK is highly unlikely. During intraduodenal administration of MCT no release of CCK was detected, and infusion of loxiglumide did not antagonize the MCT-induced reduction in LES pressure. Other intestinal hormones could be responsible for the observed effect of MCT on LES pressure. However, peptides such as pancreatic polypeptide and gastric inhibitory peptide are not released during MCT ingestion, whereas others such as gastrin and motilin do not reduce LES pressure (19, 24).

During intraduodenal administration of MCT, a significant increase in gallbladder volume to 135% of fasting volume was observed. This increase in gallbladder volume after ingestion of MCT has been reported previously, but its mechanism is unclear (19). MCT accelerates intestinal transit and induces diarrhea (36). Although MCT does not stimulate secretion of proximal gut hormones, recent studies suggest that MCT may activate the ileal brake and peptide YY secretion (28, 36). The relaxation of the gallbladder during loxiglumide infusion and concomitant intraduodenal administration of LCT or MCT reflects the role of CCK in maintaining basal gallbladder tone and mediating postprandial gallbladder contraction, as reported earlier (10, 30). Our results on gallbladder motility confirm the effect of loxiglumide as a specific CCK receptor blocker in vivo.

Postprandial levels of endogenous CCK have been reported to be higher during loxiglumide infusion (14, 20, 25). The augmented CCK response is explained by a negative feedback between biliary acid concentrations in the intestinal lumen and CCK-secreting cells. Decreased intraluminal bile acid concentrations increase CCK secretion. In our study, however, an increase in plasma CCK levels during loxiglumide was observed only after 60 min of LCT administration. The reason for this delayed increase in plasma CCK is probably related to the administration of undigested LCT. Intraduodenally administered LCT has to be dispersed by bile salts and hydrolyzed by pancreatic lipase into monoglycerides and LCFA to release CCK. These split products of lipolysis are potent stimuli of CCK release. During infusion of loxiglumide in the dose we used, both biliary output and pancreatic enzyme secretion are markedly reduced. This reduced pancreatic-cobiliary secretion impairs digestion of LCT and results in a delayed increase in plasma CCK.

In conclusion, LCT and MCT, administered in equimolar amounts intraduodenally, both significantly reduce LES pressure. The effect of LCT on LES pressure is suggested to be mediated by endogenous CCK because 1) intraduodenal LCT increases plasma CCK levels and 2) CCK receptor blockade with loxiglumide prevents the reduction in LES pressure induced by intraduodenal LCT. The effect of MCT on LES pressure is not likely to be dependent on endogenous CCK because 1) intraduodenal MCT does not release CCK and 2) loxiglumide does not prevent the MCT-induced reduction in LES pressure.

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