Adaptation to high-fat diet reduces inhibition of gastric emptying by CCK and intestinal oleate

MIHAI COVASA AND ROBERT C. RITTER
Department of Veterinary and Comparative Anatomy, Pharmacology, and Physiology, and Program in Neuroscience, Washington State University, Pullman, Washington 99164

Adaptation to high-fat diet reduces inhibition of gastric emptying by CCK and intestinal oleate. Am. J. Physiol. Regulatory Integrative Comp. Physiol. 278: R166–R170, 2000.—Rats maintained on a low-fat (LF) or high-fat (HF) diet were fitted with gastric and duodenal cannulas. Intraperitoneal injection of 0.250–2.0 pg/kg cholecystokinin (CCK) significantly inhibited gastric emptying of a 5-ml NaCl load in LF rats by 26.2%–55.1% compared with emptying after vehicle injection. By contrast, CCK-induced inhibition of gastric emptying was significantly less in HF rats given the same CCK doses (10.0%–31.7% inhibition over the same CCK dose range). A 20-min intraduodenal infusion of oleate (0.03 or 0.06 kcal/ml) also resulted in significant inhibition of gastric emptying in LF rats (24 and 89%, respectively). Oleate-induced inhibition of gastric emptying was significantly attenuated in rats maintained on the HF diet (2 and 56%, respectively). Unlike CCK injections or oleate infusion, intraduodenal maltotriose infusion inhibited gastric emptying to a similar degree in LF and HF rats (77 and 78%, respectively). These results indicate that feeding HF diets diminishes the enterogastric sensitivity to the satiation-producing effects of intraintestinal infusion of CCK and intestinal oleate. In the experiments described here, we tested this hypothesis by measuring gastric emptying of isotonic saline after intraperitoneal CCK or intraintestinal infusion of maltotriose or oleate in rats maintained on an HF or LF diet.

METHODS

Adult male Sprague-Dawley rats (350–450 g) were housed individually in a temperature-controlled room with ad libitum access to water and pelleted laboratory rodent diet, except as indicated. Lights in the animal room were on from 0600 to 1800, and gastric emptying measurements were always performed between 0900 and 1200. After 1 wk of habituation to the housing conditions, rats were placed on an LF diet or an HF diet that was isocaloric with the LF diet. The composition and the source of the ingredients used in formulating the diets have been previously described (5). Briefly, the LF diet (3.86 kcal/g) contained the following nutrients, as percentages by weight: 65% starch, 5% fat, 20% protein, and 3% cellulose. The HF diet, which was isocaloric to the LF diet (3.86 kcal/g), contained no starch, 54% fat, 20% protein, and 39% cellulose. Both diets were balanced and equivalent with regard to mineral and vitamin content. Experiments began after rats had been maintained on these diets for 2 wk.

Gastric and duodenal cannulation. All rats were implanted with stainless steel gastric cannulas. In addition to gastric cannulas, rats that participated in intestinal infusion experiments also were implanted with silicone rubber intestinal catheters, according to the procedure previously described by Yox and Ritter (31). Briefly, the animals were anesthetized with methoxyflurane (Metofane, Pitman-Moore, Mundelein, IL), and the flanged end of a stainless steel gastric cannula (13 mm long, 6 mm ID, 8 mm OD) was inserted through the ventral wall of the nonglandular portion of the stomach near the greater curvature. The cannula was secured with a purse-string suture, a piece of Marlex mesh was placed around it, and the nonflanged end of the cannula was externalized through an incision in the left paramedian abdominal wall. The cannula was kept closed with a stainless steel screw, except during experiments.

In rats intended for intestinal infusion experiments, a silicone rubber catheter (0.025 in. ID, 0.047 in. OD) was threaded through a 16-gauge stainless steel cuff (5.0 mm long) that was soldered to the inside wall of the gastric cannula. The catheter was anchored to this cuff with Dow...
Gastric emptying after CCK injection. The rats were deprived of food, but not water, overnight for 17 h, before the start of gastric-emptying measurements. At the start of each gastric-emptying experiment, each rat was removed from its home cage, the gastric cannula was opened, and the stomach was gently washed with warm (37°C) tap water. A drainage tube was attached to the open cannula, and the rat was placed in a Plexiglas gastric-emptying cage, which has been described previously (31). The drainage tube exited through a longitudinal slot in the wire-mesh floor of the cage and rested in a graduated cylinder. After connection of the drainage tube and while the rats rested in the Plexiglas cages, the stomach was flushed twice with warm 0.9% NaCl, via the drainage tube, by means of a syringe attached to the drainage tube. The second saline wash contained phenol red (60 mg/l) to saturate gastric mucosal binding of phenol red and minimize loss of the dye due to adsorption during subsequent emptying measurements (12). Phenol red recovery after this procedure was 95–97% of the infused loads. After the final, dye-containing wash, 30 min were allowed to drain any remaining wash solution from the stomach. Then, 5 min after intraperitoneal injection of CCK (1 ml/kg), 5 ml of warm 0.9% NaCl containing 0.006% phenol red, were instilled into the stomach via the drainage tube, and the tube was clamped. At the end of a 10-min emptying period, the clamp was removed, the volume remaining in the stomach was withdrawn, and the stomach was washed twice with saline and allowed to drain for another 30 min into a collection flask. Collected volume was measured, and the gastric contents were centrifuged at 10,000 rpm for 5 min to remove any particulate matter. A 1-ml sample from the centrifuged gastric contents was buffered with 24 ml of 0.014 M NaPO₄·12 H₂O, and the spectrophotometric absorbance of each buffered sample was compared with that of a 1-ml buffered sample from the originally instilled phenol red solution to determine the volume of the original test load remaining in the stomach at the end of the 10-min emptying period. Gastric emptying was measured after CCK doses of 0.250, 0.500, 1.0, and 2.0 µg/kg, administered in ascending order. All rats were tested for inhibition of gastric emptying by each CCK dose, and the order in which doses were tested was randomized. Each CCK dose was preceded and followed by gastric-emptying measurements after control injections of 0.9% NaCl. A minimum of two tests were conducted for each CCK and infusate dose, and all injections or infusions were separated by ≥48 h. The results represent comparisons between the mean of two injections of CCK at each dose, with the mean of the saline injections occurring immediately before and immediately after the CCK doses.

Gastric emptying after intestinal infusions. Overnight food deprivation and all other preparations for measurement of gastric emptying after intestinal nutrient infusions were similar to those described for measurement of gastric emptying after CCK injection. However, for intestinal infusion experiments, the free end of each intestinal catheter was connected to polyethylene (PE-90) tubing that was passed through the drainage tube to a 10-ml syringe mounted in a syringe pump. Intestinal infusions were delivered at a rate of 0.48 ml/min for 20 min. The 5-ml load of saline was placed in the stomach 5 min after the end of intestinal infusion. At 10 min after instillation of the gastric load, gastric contents were collected for emptying measurements, as described above. Each intestinal infusate was made isotonic (300 mosmol/kg) by addition of NaCl, and the pH was adjusted to 7.4. Tonicity was checked using a vapor pressure osmometer (model 5130A, Wescor). Gastric emptying was measured after saline (0.06 and 0.03 kcal/ml) or maltotriose (0.52 kcal/ml) infusions. Measurements made after nutrient infusions were separated by measurements made after intraintestinal infusions of isotonic saline. A minimum of two tests were conducted for each infusate concentration, and all infusions were separated by ≥48 h. The results represent comparisons between the mean of two nutrient infusions at each caloric concentration, with the mean of the saline infusions occurring immediately before and immediately after the nutrient infusion.

Analysis of results. Results are graphically expressed as percent inhibition of 10-min gastric emptying. Percent inhibition of emptying was calculated for each treatment in each rat according to the following formula: %inhibition = [(1 – (experimental/control)) × 100. The numerator (experimental) is the amount emptied in 10 min after an intraperitoneal CCK-8 or intraintestinal nutrient infusion. The denominator (control) is the amount emptied 10 min after an intraperitoneal saline or intraintestinal infusion of the nutrient vehicle. The mean percent suppressions were compared between LF and HF animals by two-way ANOVA, with repeated measures on dose of CCK or nutrients. Significant differences between individual means were identified using Dunnett’s test.

RESULTS

Ten-minute gastric emptying of a 5-ml NaCl load after intraperitoneal saline injection was 4.1 ± 0.2 ml (n = 6) in LF rats, which was not significantly different from emptying in HF rats (4.0 ± 0.3 ml, n = 6, P > 0.5). CCK inhibited gastric emptying at all doses tested. However, it produced significantly greater inhibition of emptying in LF than in HF rats (Fig. 1). CCK (2 µg/kg) inhibited gastric emptying by 55.1 ± 6.2% in LF rats, whereas the same CCK dose inhibited emptying significantly less (P < 0.01), i.e., 30.3 ± 4.5%, in HF rats. The lowest CCK dose (250 ng/kg) inhibited gastric emptying by 26.2 ± 3.6% in LF rats, which was significantly greater than the inhibition of emptying produced by this dose in HF rats (10 ± 2.9%, P < 0.01).

Intraintestinal saline infusions did not reduce gastric emptying in LF or HF rats (P > 0.5). On the other hand, oleate infusion (0.03 and 0.06 kcal/ml) caused a significant inhibition of gastric emptying in LF rats (24.2 and 89.5%, respectively). However, the effect of oleate was significantly attenuated in HF rats (12.2 and 56.3%, respectively, P < 0.01; Fig. 2). Maltotriose infusion (0.52 kcal/ml) caused a significant suppression of gastric emptying in LF (n = 6) and HF (n = 6) rats, with no significant difference between the groups (76.8 and 78.5% in LF and HF, respectively, P > 0.5; Fig. 3).
DISCUSSION

Exogenous CCK inhibited gastric emptying less in rats maintained on an HF diet than in those maintained on an LF diet. Intestinal infusion of oleate also inhibited gastric emptying less in rats fed an HF diet than in those fed an LF diet, whereas inhibition of gastric emptying by maltotriose did not differ between rats fed an HF diet and those fed an LF diet.

Inhibition of gastric emptying after injection of exogenous CCK is well documented in a variety of mammals, including rats, monkeys, and humans (14, 17, 19). The fact that exogenous CCK inhibits gastric emptying at doses resulting in plasma CCK concentrations comparable to those produced by eating (2) has led to the hypothesis that CCK mediates intestinal control of gastric emptying by some components of the meal, including fat (11, 23, 24, 32). In support of this hypothesis, experimental results indicate that ingestion or intestinal infusion of fat triggers CCK secretion (3, 10, 13), and HF meals generally empty more slowly than LF meals (17). In addition, administration of CCK-A receptor antagonists increases the rate of emptying of HF meals in rats, humans, and monkeys (11, 16). Furthermore, CCK-A receptor antagonist administration attenuates or abolishes inhibition of gastric emptying by intraintestinal infusion of triglyceride or fatty acids (11). Thus the evidence for CCK-mediated, physiological control of gastric emptying by intestinal fat is convincing.

Our results indicate that inhibition of gastric emptying was attenuated in HF rats at all doses of CCK tested. The highest CCK dose tested, 2 µg/kg, reduced gastric emptying by 55% in LF rats, but only by 30% in HF rats. Our higher dose of oleate (0.06 kcal/ml) produced a greater inhibition of gastric emptying (56.3%) in HF rats, whereas inhibition of emptying in LF rats (89.5%) was significantly more profound. Reduced potency of intestinal oleate for inhibition of gastric emptying in HF rats also was apparent with our lower oleate dose (0.03 kcal/ml), suggesting a rightward shift in the dose-response function for oleate-induced inhibition of gastric emptying. Although our higher dose of oleate caused a nearly complete inhibition of emptying in LF rats, we do not know how high an oleate dose would be required to produce maximal inhibition of emptying in HF rats. Nonetheless, our results clearly indicate that HF feeding produces a clear reduction of oleate's potency for inhibition of gastric emptying.
Our results also suggest that the effect of HF feeding on control of gastric emptying is selective, since HF feeding attenuated inhibition of gastric emptying by CCK and intestinal oleate, but not by maltotriose. It may be argued that HF feeding did not attenuate the effect of maltotriose, because it was infused at a higher caloric concentration than oleate. Because we do not have dose-response data for intestinal maltotriose infusion, we cannot rule out this possibility. However, this interpretation seems unlikely, because inhibition of gastric emptying by maltotriose in LF rats (76.8%) actually was slightly less than that produced by oleate (89.5%). Yet, HF feeding resulted in significant attenuation of the response to oleate (56.3% inhibition in HF rats) but not to maltotriose (78.5% inhibition in HF rats). In other words, HF feeding failed to attenuate maltotriose effects, even though the dose used was less effective for inhibiting emptying than oleate. Furthermore, gastric emptying in HF- and LF-adapted rats did not differ after intestinal saline infusion or when no intestinal infusion was made. Our results are in agreement with a report by Cunningham et al. (8), who found that gastric emptying of a test meal that contained 60 g of butter is faster in humans adapted to an HF diet than in those adapted to an LF diet.

The mechanism by which an HF diet reduces responsiveness to intraintestinal fat infusion is not understood. It is possible that HF diets result in a change in the intestinal receptive field for fat. Lin et al. (15) demonstrated that the efficacy of intestinal oleate to inhibit gastric emptying depends on the total length of intestine exposed to this nutrient. It is possible that increased digestion and absorption capacity for fat, which occur during HF adaptation, result in a reduction of the length of intestine coming in contact with fat or fat digestion products. Alternatively, it is possible that attenuation of oleate-induced inhibition of gastric emptying is mediated by reduced responsiveness to CCK, which we also observed in HF-adapted rats.

Vagotomy abolishes inhibition of gastric emptying by exogenous CCK (18, 26, 27) and by intragastric fat (26). In addition, capsaicin treatment, which destroys small unmyelinated sensory neurons, including vagal sensory neurons, attenuates inhibition of gastric emptying by CCK and oleate (11, 22). Inasmuch as vagal sensory neurons express CCK-A receptors (21), it seems plausible that HF adaptation results in reduced vagal CCK-A receptor sensitivity or reduced intracellular signaling after CCK-A receptor activation. In support of this hypothesis, we recently reported that c-fos expression, after intraperitoneal CCK or intestinal oleate, is nearly abolished in the primary vagal sensory nucleus, the nucleus of the solitary tract, of HF-adapted rats. On the other hand, LF-adapted rats express abundant c-fos in the nucleus of the solitary tract after intestinal oleate infusion, and HF- and LF-adapted rats express c-fos in response to maltotriose infusion (6). These results provide strong evidence in support of the hypothesis that altered vagal CCK sensitivity may account for reduced inhibition of gastric emptying in HF-adapted rats.

In conclusion, our results indicate that exposure to high levels of dietary fat results in reduced potency of fat and CCK in inhibition of gastric emptying. This reduction of potency could result in increased passage of fat from the stomach to the small intestine. Considering the fact that capacity for digestion and absorption of fat also is increased in animals maintained on HF diets (25, 28, 29) and that HF feeding diminishes satiation by CCK (5) and intestinal oleate (7), these results suggest that chronic exposure to HF diets may favor increased digestive processing of fat, whereas it desensitizes mechanisms that limit the presentation of fat for digestion and absorption. These changes favor overeating and development of obesity, which often are attendant to ingestion of HF diets.

The authors appreciate the generous donation of CCK-8 by S. J. Lucania (E. R. Squibb, Princeton, NJ). This work was supported by National Institute of Neurological and Communicative Disorders and Stroke Grant NS-20561 to R. C. Ritter.

Address for reprint requests and other correspondence: M. Covasa, Dept. of Veterinary and Comparative Anatomy, Pharmacology, and Physiology, Washington State University, Pullman, WA 99164-6520 (E-mail: mcovasa@vtemed.wsu.edu).

Received 8 February 1999; accepted in final form 5 August 1999.

REFERENCES


