Role of cholecystokinin in the anorexia produced by duodenal delivery of peptone in rats

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Woltmann, Todd, and Roger Reidelberger. Role of cholecystokinin in the anorexia produced by duodenal delivery of peptone in rats. Am. J. Physiol. 276 (Regulatory Integrative Comp. Physiol. 45): R1701–R1709, 1999.—We used the cholecystokinin receptor antagonist devazepide to assess the importance of CCK in mediating the anorexia produced by 2-h duodenal infusions of peptone, a protein digest, at dark onset in nonfasted rats. Peptone alone (0.14–2.24 g/h) suppressed food intake dose dependently by 18–96%, with an approximate half-maximal dose of 1 g/h. Peptone-induced reductions in caloric ingestion were comparable to the caloric loads infused. Devazepide alone (30–1,000 µg/kg) stimulated food intake dose dependently by 30–73%, with a minimal effective dose of 100 µg/kg. Devazepide appeared to reverse the anorexic response to peptone (1.1 g/h) dose dependently by 29–65%, with a minimal effective dose of 30 µg/kg. The magnitudes of these devazepide-induced effects were similar to, and in some cases were larger than, those produced when the same doses of devazepide were administered alone. Coadministration of devazepide (1,000 µg/kg) and a lower peptone dose (0.8 g/h) produced similar results. These results suggest that an essential CCK mechanism plays a significant role in mediating the satiety response to duodenal delivery of protein.

meal patterns; satiety; protein; cholecystokinin receptor antagonist; devazepide

The physiological mechanisms that produce satiety in response to ingestion of protein are not clearly defined. In sham-feeding rats, duodenal infusions of graded doses of the intact protein bovine serum albumin (10) and single doses of casein hydrolysate (23) and l-phenylalanine (22) have been shown to inhibit food intake, whereas a single dose of casein had no effect (1). In a more recent study in rats (6), duodenal infusion of l-tryptophan, but not l-phenylalanine, l-alanine, l-arginine, or l-leucine, dose dependently inhibited sham feeding, and a combined infusion of l-tryptophan and l-phenylalanine dose dependently inhibited real feeding. Thus it remains to be determined whether the satiety response to duodenal delivery of protein is produced by intact protein, partial hydrolysates of protein, or free amino acids.

A role for CCK in mediating the satiety response to ingested food is supported by evidence that the type 1 CCK receptor (CCK-AR) antagonist devazepide stimulates food intake in a variety of species (2, 7, 11, 12). CCK's role in specifically mediating protein-induced satiety is controversial, however. In rats, intact protein and partial hydrolysates of protein are potent stimuli for CCK release into the bloodstream (3, 13, 22), and as cited above, each has been shown to suppress sham feeding when infused into the duodenum. Protein and protein digestive products in the small intestine are thought to stimulate CCK secretion by reducing trypsin degradation of CCK releasing factor(s) in the intestinal lumen (15). Administration of trypsin inhibitors into the duodenum significantly elevates plasma CCK levels (14), and duodenal administration of trypsin inhibitor has been shown to decrease food intake in young 9- to 12-day-old rats (17), but not in adult sham-feeding rats (14). Furthermore, CCK receptor blockade has been shown to reverse the anorexic response to trypsin inhibitor in young rats (17) but to have no effect on the anorexia produced by duodenal infusion of l-phenylalanine in sham-feeding rats (22). In contrast, CCK receptor blockade has been shown to attenuate the anorexic response to a gastric preload of protein in real-feeding adult rats (16). Our previous work suggests that in real-feeding adult rats an essential CCK mechanism plays a significant role in mediating the satiety response to duodenal delivery of small, but not large, loads of oleic acid and glucose. It remains to be determined whether this is also true for duodenal delivery of protein.

The aims of the present study were first to determine the dose-response effects of 2-h duodenal infusions of peptone, an enzymatic digest of protein, at dark onset in meal patterns in nonfasted rats with free access to food and then to assess the effects of intravenous injection of the CCK-AR antagonist devazepide on anorexic responses to low- and high-dose peptone infusions. If CCK plays an essential role in mediating the anorexia produced by protein, then devazepide should significantly attenuate peptone-induced anorexia. Data from this study were presented previously in abstract form (21).

METHODS

Animals. Male Sprague-Dawley rats (Sasco, Omaha, NE; ~330 g at the start of the study) were provided care according to guidelines established by the Medical Research Service of the Department of Veterans Affairs. The animals were provided ground rat chow (Purina #5001, 3.3 kcal/g) and water ad libitum and were maintained on a 12:12-h light-dark cycle with lights off at 1600.

Surgical procedures. Rats were surgically implanted with duodenal and jugular vein cannulas by means of procedures described previously (19). The duodenal cannula was implanted in the aborad direction 1 cm distal to the pyloric
shown to reduce to intermeal interval criterion of 5 min. These criteria have been minimum meal size criterion of 50 mg and a minimum each experiment, individual meals were defined using a interval/meal size), the amount of food ingested each hour, meal sizes, postmeal intervals, and satiety ratios (postmeal CA). Output from each balance was monitored at code-activated switch (CAS-161, Western Telematic, Irvine, CA) were connected to an IBM XT-compatible computer through a (0.01 g sensitivity). The 16 balances in this 16-cage system

Below the hole was a food cup fixed to an Ohaus E400 balance

scribed previously (18). Briefly, each rat was housed individually in a metabolism cage modified to include a stainless steel side compartment with a 3-cm diameter hole in the base. Below the hole was a food cup fixed to an Ohaus E400 balance (0.01 g sensitivity). The 16 balances in this 16-cage system were connected to an IBM XT-compatible computer through a code-activated switch (CAS-161, Western Telematic, Irvine, CA). Output from each balance was monitored at ~20-s intervals, and changes in food bowl weight were recorded and data processed to determine latency to first meal, individual meal sizes, postmeal intervals, and satiety ratios (postmeal interval/meal size), the amount of food ingested each hour, and food intake and number of meals cumulated hourly. For each experiment, individual meals were defined using a minimum meal size criterion of 50 mg and a minimum intermeal interval criterion of 5 min. These criteria have been shown to reduce to <1% the possible influence of intrameal pauses of no eating in the determination of meal size in our experimental model (18).

Excess amounts of fresh food were provided each day at 1200. Animals were adapted to experimental conditions, which usually required 1–2 wk. This included alternating days of intravenous injection and duodenal infusion of vehicle at the times described below for each experiment. Intravenous injections were given manually; duodenal infusions were administered using a syringe infusion pump (model 22, Harvard Apparatus, South Natick, MA). Pumps were turned on and off by a computer program. Experiments were begun when daily food intake and meal patterns had stabilized.

Dose-dependent effects of duodenal infusion of peptone on food intake. In an attempt to assess the importance of dietary protein in producing satiety, feeding patterns were monitored in nonfasted rats receiving, at dark onset, 2-h duodenal infusion of vehicle (distilled water) or an approximate half-maximal dose of peptone (1.1 g/h). Meal patterns were monitored as described above for the first 4 h of the dark period. On any given day, rats received either one of the devazepide doses or vehicle, paired with duodenal infusion of vehicle or peptone in random order at 48-h intervals.

Effects of the CCK receptor antagonist devazepide on suppression of feeding by duodenal infusion of peptone (0.8 g/h). A subsequent experiment of similar design in a separate group of rats (n = 14) examined the effects of intravenous injection of devazepide (1,000 µg/kg) on the anorexic responses to duodenal infusion of a lower dose of peptone (0.8 g/h).

Measurement of meal patterns. Procedures have been described previously (18). Briefly, each rat was housed individually in a metabolism cage modified to include a stainless steel side compartment with a 3-cm diameter hole in the base. Below the hole was a food cup fixed to an Ohaus E400 balance (0.01 g sensitivity). The 16 balances in this 16-cage system were connected to an IBM XT-compatible computer through a code-activated switch (CAS-161, Western Telematic, Irvine, CA). Output from each balance was monitored at ~20-s intervals, and changes in food bowl weight were recorded and data processed to determine latency to first meal, individual meal sizes, postmeal intervals, and satiety ratios (postmeal interval/meal size), the amount of food ingested each hour, and food intake and number of meals cumulated hourly. For each experiment, individual meals were defined using a minimum meal size criterion of 50 mg and a minimum intermeal interval criterion of 5 min. These criteria have been shown to reduce to <1% the possible influence of intrameal pauses of no eating in the determination of meal size in our experimental model (18).

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RESULTS

Dose-dependent effects of duodenal infusion of peptone on food intake. Duodenal infusion of the vehicle (deionized water) for 2 h beginning 15 min before onset of the dark period had no effect on 3-h cumulative food intake when compared with that occurring with no infusion (data not shown). In contrast, Fig. 1 shows that duodenal infusion of peptone under the same conditions dose dependently inhibited food intake during the first 4 h by 18–96%. ANOVA demonstrated a significant effect of peptone dose on cumulative intake at 1 h [F (5,70) = 9.1, P < 0.00001], 2 h [F (5,70) = 30.8, P < 0.00001], 3 h [F (5,70) = 35.2, P < 0.00001], and 4 h [F (5,70) = 33.7, P < 0.00001]. The minimally effective dose was 1.12 g/h (0.15 kcal/min) at 1 h and 0.28 g/h (0.037 kcal/min) at 2, 3, and 4 h. The half-maximal dose was ~1 g/h.

Table 1 shows the dose-dependent effects of the peptone infusions on first meal parameters and on mean meal parameters for all meals consumed during the first 3 h of the test period. ANOVA demonstrated statistically significant effects only at the two highest doses of peptone administered. The 1.12 g/h dose increased first postmeal interval by 138% and, during the 3-h period, decreased the number of meals and mean meal size by 24 and 21%, respectively. The 2.24 g/h dose increased first meal latency and postmeal interval by 280 and 120%, respectively, and decreased first meal size by 45%. During the 3-h period this dose...
Effects of duodenal infusion of peptone on meal parameters

Table 1. Effects of duodenal infusion of peptone on meal parameters

<table>
<thead>
<tr>
<th>Peptone, g/h</th>
<th>First Meal</th>
<th>Average for All Meals During First 3 h</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Latency, min</td>
<td>MS, g</td>
</tr>
<tr>
<td>0</td>
<td>45 ± 6</td>
<td>2.9 ± 0.4</td>
</tr>
<tr>
<td>0.14</td>
<td>39 ± 6</td>
<td>2.7 ± 0.3</td>
</tr>
<tr>
<td>0.28</td>
<td>39 ± 12</td>
<td>1.9 ± 0.2</td>
</tr>
<tr>
<td>0.56</td>
<td>39 ± 6</td>
<td>2.3 ± 0.4</td>
</tr>
<tr>
<td>1.12</td>
<td>69 ± 18</td>
<td>1.8 ± 0.2</td>
</tr>
<tr>
<td>2.24</td>
<td>171 ± 24*</td>
<td>1.6 ± 0.2*</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 15. Peptone was infused into the duodenum for 2 h beginning 15 min before dark onset. MS, meal size; PMI, postmeal interval; SR, satiety ratio (PMI/MS). *P < 0.05.
epide in the second, third, and fourth hour after onset of infusion (Fig. 3). The minimal effective dose of devazepide was 30 µg/kg (Fig. 3A). The 30, 100, and 300 µg/kg doses appeared to produce comparable effects (29–47% reversals by 2 and 3 h), whereas the 1,000 µg/kg dose produced the largest effects (54 and 65% at 2 and 3 h). The magnitudes of these devazepide-induced effects were similar to, and in a few cases were larger than, those produced when the same devazepide doses were administered alone (Fig. 3). No dose of devazepide completely reversed peptone-induced anorexia. Cumulative intakes in response to the combined treatments of devazepide and peptone were always significantly less than those occurring in response to treatment alone with the same dose of devazepide.

Table 2 shows that the peptone-induced reduction in first meal size was not significantly altered by prior treatment with the different doses of devazepide. In contrast, devazepide did appear to reverse the peptone-induced increase in first meal latency (83 and 94% reversal by 100 and 1,000 µg/kg doses) and the peptone-induced decrease in mean meal size during the 3-h period (45 and 71% reversal by 300 and 1,000 µg/kg doses).

Effects of the CCK receptor antagonist devazepide on suppression of feeding by duodenal infusion of peptone (0.8 g/h). Figure 4 and Table 3 show the individual and combined effects of intravenous injection of devazepide (1,000 µg/kg) and duodenal infusion of a lower dose of peptone (0.8 g/h) on food intake and meal patterns. ANOVA demonstrated a significant main effect of devazepide on cumulative intake at 1 h [F(1,39) = 5.2, P < 0.05], 2 h [F(1,39) = 11.9, P < 0.01], 3 h [F(1,39) = 4.4, P < 0.05], and 4 h [F(1,39) = 6.8, P < 0.05]; a significant main effect of peptone on cumulative intake at 1 h [F(1,39) = 8.0, P < 0.01], 2 h [F(1,39) = 9.4, P < 0.01], 3 h [F(1,39) = 14.4, P < 0.0001], and 4 h [F(1,39) = 11.7, P < 0.01]; and a significant interaction between devazepide and peptone on cumulative intake at 2 h [F(1,39) = 3.3, P < 0.05], but not at 1 h [F(1,39) = 0.4, P > 0.05], 3 h [F(1,39) = 1.1, P > 0.05], or 4 h [F(1,39) = 0.0, P > 0.05]. The significant interaction at 2 h indicates that devazepide was more effective in stimulating feeding when administered with peptone than when administered alone.

Figure 4 shows that devazepide treatment alone significantly increased cumulative food intake at 1 and 4 h by 52 and 17%, respectively. Table 3 shows that this effect was associated with an increase in the number of meals (44%) and a decrease in mean postmeal interval (28%) during the 3-h period.

Figure 4 shows that duodenal peptone infusion alone at 0.8 g/h produced a significant decrease in cumulative food intake (~40%) by the second hour of infusion, an effect that was still significant but reduced by the third and fourth hours after onset of infusion. Table 3 shows that this peptone-induced reduction in food intake was associated with an increase in first meal satiety ratio (335%) and a decrease in mean meal size (25%) and an increase in mean postmeal interval (21%) and mean satiety ratio (117%) during the 3-h period.

Figure 4 shows that the inhibitory effects of duodenal peptone infusion on 2-, 3-, and 4-h cumulative intakes appeared to be significantly reversed by prior devazepide treatment. The reversal at 2 h appeared to be complete because cumulative intakes in response to the combined treatments of devazepide and peptone were not statistically different from those produced by devazepide treatment alone.

Table 3 shows that the peptone-induced effects on meal parameters (first satiety ratio, mean meal size, mean satiety ratio) were not significantly altered by prior treatment with devazepide. However, compared with peptone infusion alone, coadministration of devazepide and peptone produced an increase in the number of meals (32%) during the 3-h period.
Fig. 3. Individual and combined effects of graded doses of devazepide and duodenal infusion of peptone (1.1 g/h) on cumulative food intake in 15 rats with free access to ground chow. Devazepide (in µg/kg; A: 30; B: 100; C: 300; and D: 1,000) or vehicle was injected intravenously 15 min before a 2-h infusion of peptone (1.1 g/h) or vehicle, which began 15 min before dark onset. Data are presented as group means ± SE. P, 0.05 as follows: a vehicle-peptone vs. vehicle-vehicle; b vehicle-peptone vs. devazepide-vehicle; c devazepide-peptone vs. vehicle-peptone; d devazepide-vehicle vs. vehicle-vehicle; e devazepide-vehicle vs. vehicle-peptone; f (devazepide-vehicle)-(vehicle-vehicle) vs. (devazepide-peptone)-(vehicle-peptone).

Table 2. Individual and combined effects of devazepide and duodenal infusion of peptone on meal parameters

<table>
<thead>
<tr>
<th>Dev, µg/kg</th>
<th>Peptone, g/h</th>
<th>First Meal</th>
<th>Average for All Meals During First 3 h</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Latency, min</td>
<td>MS, g</td>
<td>PMI, min</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>38.4 ± 6.6</td>
<td>3.2 ± 0.4</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>30.0 ± 6.0</td>
<td>2.4 ± 0.3</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>27.0 ± 7.2</td>
<td>2.6 ± 0.3</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>24.6 ± 4.2</td>
<td>3.1 ± 0.3</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>25.8 ± 4.8</td>
<td>2.6 ± 0.5</td>
</tr>
<tr>
<td>0</td>
<td>1.1</td>
<td>55.8 ± 16.2*</td>
<td>1.6 ± 0.2*</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>40.8 ± 10.2</td>
<td>1.8 ± 0.2</td>
</tr>
<tr>
<td>0</td>
<td>1.1</td>
<td>31.8 ± 7.8†</td>
<td>1.5 ± 0.2†</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>46.8 ± 10.8†</td>
<td>1.8 ± 0.3†</td>
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<tr>
<td>0</td>
<td>1.1</td>
<td>27.6 ± 7.8†</td>
<td>1.4 ± 0.2†</td>
</tr>
<tr>
<td>0</td>
<td>1.1</td>
<td>55.8 ± 16.2*</td>
<td>1.6 ± 0.2*</td>
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</tbody>
</table>

Values are means ± SE; n = 15. Devazepide (Dev) was injected 15 min before onset of peptone infusion; peptone was infused into duodenum for 2 h beginning 15 min before dark onset (1.1 g/h). *P < 0.05 vs. 0 Dev, 0 peptone; †P < 0.05 vs. 0 Dev, 1.1 peptone; ‡P < 0.05 vs. same Dev dose at 0 peptone.
Infused, indicating that total caloric intake was regulated. Second, devazepide treatment alone stimulated food intake dose dependently during the first 3 h by 30–73%, with a minimal effective dose of 100 µg/kg. Third, the inhibitory effects of an approximate half-maximal dose of peptone (1.1 g/h) on food intake appeared to be partially reversed by devazepide in a dose-related manner, with a minimal effective dose of 30 µg/kg, the lowest dose administered. The 30, 100, and 300 µg/kg doses produced comparable effects (29–47% reversal); the 1,000 µg/kg appeared to produce the largest effect (54–65% reversal). The magnitudes of these devazepide-induced effects were equal to, and in some cases greater than, those produced by the same devazepide doses when administered alone. Devazepide (1,000 µg/kg) treatment before a lower dose of peptone (0.8 g/h) produced comparable results. Together, these results suggest that an essential CCK mechanism plays a significant role in mediating the satiety response to duodenal delivery of protein.

Our results indicate that 2-h duodenal infusions of peptone reduced caloric ingestion to regulate total caloric intake. We previously reported that a similar regulation of caloric intake occurs when glucose is infused into the duodenum under the same experimental conditions (19). These results suggest that in this animal model, caloric ingestion is rapidly controlled to regulate delivery of calories to the small intestine. Duodenal infusion of peptone, therefore, does not appear to change the rate of caloric delivery to the small intestine, but rather the proportion of protein or peptone within the caloric mix. Thus our experimental approach examined whether a partial replacement of "chow" calories (28% protein) with peptone calories in the small intestine affects the degree to which an essential CCK mechanism contributes to the satiety effect of the total caloric load delivered to the small intestine. If peptone calories are less effective in stimulating an essential CCK mechanism than the chow calories they replace, then devazepide would be expected to stimulate feeding less when administered with peptone than when administered alone. The converse would be expected if the peptone calories are more effective in stimulating an essential CCK satiety mechanism than the chow calories they replace. As described above, our results show that devazepide was as effective and, at some doses, more effective in stimulating feeding when administered with peptone than when administered alone. These results support

Table 3. Individual and combined effects of devazepide and duodenal infusion of peptone on meal parameters

<table>
<thead>
<tr>
<th>Dev, µg/kg</th>
<th>Peptone, g/h</th>
<th>Latency, min</th>
<th>MS, g</th>
<th>PMI, min</th>
<th>SR, min/g</th>
<th>Average for All Meals During First 3 h</th>
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<td></td>
<td></td>
<td>No.</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>16.8 ± 7.2</td>
<td>2.8 ± 0.4</td>
<td>66.4 ± 14.1</td>
<td>23.0 ± 2.9</td>
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<tr>
<td>1,000</td>
<td>0</td>
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<td>2.2 ± 0.2</td>
<td>38.5 ± 7.2</td>
<td>20.5 ± 5.0</td>
<td>3.6 ± 0.3*</td>
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<tr>
<td>0</td>
<td>0.8</td>
<td>30.6 ± 10.8</td>
<td>2.6 ± 0.4</td>
<td>114.6 ± 31.5</td>
<td>100.0 ± 49.5*</td>
<td>2.5 ± 0.3</td>
</tr>
<tr>
<td>1,000</td>
<td>0.8</td>
<td>8.4 ± 6.6</td>
<td>2.4 ± 0.3</td>
<td>104.4 ± 34.9</td>
<td>71.8 ± 36.1</td>
<td>3.3 ± 0.4*</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 14. Devazepide was injected 15 min before onset of peptone infusion; peptone was infused into duodenum for 2 h beginning 15 min before dark onset (0.8 g/h). *P < 0.05 vs. 0 Dev, 0 peptone; †P < 0.05 vs. 0 Dev, 0.8 peptone; ‡P < 0.05 vs. same 1,000 Dev dose, 0 peptone.
an important role for CCK in mediating the satiety response to duodenal delivery of peptone.

Our results clearly show that the threshold dose for a devazepide-induced stimulation of food intake was lower when devazepide was administered with peptone. There are several possible explanations for this. One explanation is that devazepide and peptone may have actually produced independent, opposing effects on food intake, and it was the peptone-induced reduction in food intake, rather than a peptone-induced increase in CCK secretion, that increased devazepide's ability to stimulate food intake at the lower doses. Previous studies suggest that devazepide's ability to increase food intake is inversely related to baseline intake. Another possible explanation is that chow calories, when not diluted with peptone, may have produced a significantly larger CCK concentration at CCK-AR sites responsible for CCK's satiety effect, such that a larger dose of the competitive antagonist devazepide was required to block binding of CCK to these receptors. Another possible explanation is that duodenal delivery of nutrients may stimulate more than one CCK-dependent satiety mechanism. Chow calories may have primarily stimulated one mechanism that required a large dose of devazepide to block, whereas peptone may have stimulated yet another mechanism that required a smaller devazepide dose to block. Indeed, there is now good evidence that CCK contributes to the production of satiety both by acting peripherally through an intestinal-vagal mechanism and by acting centrally as a neurotransmitter or neuromodulator within the brain food intake control system (9). There is also strong evidence that in the rat, protein is a more potent stimulus of CCK secretion from intestinal mucosal cells than either carbohydrate or fat (5). Thus it is reasonable to speculate that in the absence of peptone infusion, delivery of chow calories to the small intestine primarily stimulated a central CCK-dependent satiety mechanism that required a relatively high devazepide dose to block, and, in the presence of peptone infusion, delivery of a mixture of peptone and chow calories to the small intestine stimulated not only this central CCK-dependent satiety mechanism, but also an intestinal CCK-dependent satiety mechanism that required a lower dose of devazepide to block. It remains to be determined whether CCK-AR antagonists that do not penetrate the blood-brain barrier attenuate the anorexic response to duodenal delivery of peptone.

If a nutrient-induced anorexia is mediated by an essential CCK-AR-mediated mechanism, then it would seem reasonable to assume that the nutrient would produce effects on meal patterns similar to those produced by exogenous CCK, and that devazepide would specifically attenuate the nutrient-induced effects on meal patterns. We previously reported that when CCK-8 (10 nmol·kg⁻¹·h⁻¹) was infused intravenously for 3 h at dark onset to simulate a possible nutrient-induced secretion of intestinal CCK, CCK-8 suppressed feeding by decreasing meal frequency, and devazepide (1000 µg/kg) completely blocked this effect (18). However, coadministration of devazepide and CCK-8 also resulted in a mean meal size that was significantly larger than that observed with CCK-8 alone. Similar results were observed with coadministration of devazepide and a lower dose of CCK-8 (3 nmol·kg⁻¹·h⁻¹). In the present study the 0.8 g/h dose of peptone decreased mean meal size and increased satiety ratios. Devazepide (1,000 µg/kg) did not significantly reverse these effects but rather increased meal frequency. The larger 1.1 g/h dose of peptone increased first meal latency and decreased mean meal size. Of the range of devazepide doses (30, 100, 300, 1,000 µg/kg) that attenuated the anorexia produced by this dose of peptone, only the 1,000 µg/kg dose appeared to significantly attenuate the peptone-induced reduction in mean meal size. However, this devazepide dose also decreased latency to first meal and increased meal frequency, whereas the 100 µg/kg dose decreased both first meal latency and first postmeal interval. These intermeal effects of devazepide when administered with peptone were similar to those produced by devazepide when administered alone. Thus CCK-8 and peptone did not produce similar effects on meal patterns in our separate studies with different groups of animals, and devazepide did not specifically attenuate peptone-induced effects on meal patterns. These results suggest the possibility that devazepide and peptone may have produced independent, opposing effects on feeding.

We are not aware of any study that has specifically addressed the question of whether it is correct to assume that a devazepide-induced attenuation of a specific nutrient-induced effect on meal patterns is a sufficient or necessary criterion for establishing a role for CCK in mediating the nutrient-induced anorexia. A closer examination of data from our own studies performed in different groups of animals at different times suggests that an animal's strategy for adjusting meal size and frequency in response to a treatment may depend in part on whether the animal tends to eat smaller or larger meals under baseline conditions. For example, it appears that the anorexic responses to CCK-8 and duodenal nutrient infusions were generally associated with a decrease in meal frequency when baseline mean meal sizes were relatively small and with a decrease in mean meal size when baseline meal sizes were relatively large. Similarly, the stimulatory effect of devazepide on food intake appeared to be associated with an increase in meal frequency when baseline mean meal sizes were relatively large and with an increase in mean meal size and frequency when meal sizes were reduced. Thus we urge caution when attempting to compare results from our different experiments, because baseline meal patterns differed significantly across our studies. Differences may have been due in part to differences in the age or size of animals and/or in the work required for animals to obtain food when tethered to infusion swivels. Nevertheless, an attempt was made to control such factors within individual experiments by having each animal receive all treatments randomly during a relatively short period of time. Thus the question remains whether it is correct to
assume that a devazepide-induced attenuation of a specific nutrient-induced effect on meal patterns within an experiment is a sufficient or necessary criterion for establishing a role for CCK in mediating the nutrient-induced anorexia. We are not aware of any study that has systematically addressed this issue with respect to the possible role of endogenous CCK or any other endogenous factor in control of food intake. With respect to CCK’s role, at least two types of experiments would be useful. One would determine the individual and combined effects of devazepide and intravenous CCK-8 infusion on meal patterns in rats that exhibit significant differences in baseline meal sizes. The other would determine in a similar manner the individual and combined effects of CCK-8 and other factors thought to have independent, opposing effects on food intake. Given the paucity of evidence supporting the validity of this criterion, we urge caution in its use.

What appears to be consistent for each of the nutrients tested in our studies (glucose, oleic acid, peptone) is that nutrient-induced anorexia may be more sensitive to reversal by devazepide at lower rates of nutrient delivery to the small intestine. This would be consistent with the idea that in freely feeding rats, CCK plays a partial, indispensable role in mediating the satiety response to duodenal delivery of these nutrients at relatively low delivery rates and that larger delivery rates produce a greater stimulation of redundant CCK-independent satiety mechanisms. Peptone-induced anorexia, compared with the anorexias produced by glucose and oleic acid, does appear, however, to be more sensitive to reversal by devazepide at higher doses on its dose-response curve. This may mean that peptone produces either a greater release and action of CCK to produce satiety or a lesser stimulation of redundant CCK-independent satiety mechanisms.

As discussed in the introduction, the few studies that have investigated the effects of duodenal delivery of protein or protein digestive products on food intake have produced contradictory results. The present study demonstrates that duodenal infusion of Bacto Peptone, a partial hydrolysate of beef gelatin, decreased food intake dose dependently in freely feeding rats. The approximate half-maximal dose of peptone for suppression of feeding (0.07 kcal/min) was within the range of caloric rates of gastric emptying (0.05–0.3 kcal/min) previously reported for rats ingesting liquid (4, 10) and solid diets (8). These results support the hypothesis that physiological rates of delivery of protein to the small intestine can produce satiety.

No previous study has used CCK-AR antagonists to examine the role of CCK in mediating the anorexia produced by duodenal infusion of intact protein or peptone. CCK receptor blockade has been reported to have no effect on the anorexia produced by duodenal infusion of a single dose of L-phenylalanine in sham-feeding adult rats (22); however, L-phenylalanine is thought not to stimulate intestinal secretion of CCK in rats (13). Thus the present study is the first to provide evidence consistent with the hypothesis that an essential CCK mechanism plays a significant role in mediating the satiety response to duodenal infusion of protein. The results of our studies using devazepide to examine the role of CCK in mediating the satiety response to duodenal delivery of oleic acid, glucose, and peptone are similar to those of Trigazis et al. (16), who examined the effects of devazepide on anorexic responses to gastric loads of protein, amino acids, carbohydrate, and fat. In their study, devazepide also appeared to reverse the anorexia produced by each nutrient. However, only with protein did the reversal appear to be greater than that produced by devazepide alone. Thus the authors concluded that their results support the hypothesis that CCK plays an important role in protein-, but not amino acid-, carbohydrate-, or fat-induced suppression of food intake. There are several reasons why this conclusion may not be correct. First, the finding that a significant statistical interaction was observed for protein but not for the other nutrients could have been due to the fact that, for some unknown reason, devazepide treatment alone did not significantly increase food intake in the protein experiment such as it did in most of the experiments with the other nutrients. It should indeed be easier to demonstrate statistically that a devazepide-induced reversal of a nutrient-induced anorexia is significantly greater than that produced by devazepide treatment alone, if the devazepide-alone treatment has no stimulatory effect on food intake. Furthermore, as discussed here and in our recent papers (18, 20), we urge caution when interpreting the meaning of the significance of treatment interactions in this type of study. Second, our work suggests that devazepide may be more effective in reversing the anorexia produced by duodenal delivery of glucose and oleic acid when delivered at doses that are on the lower end of their dose-response curves. Nutrient dose-response curves were not generated in the study of Trigazis et al. (16), and devazepide was tested only with single doses of carbohydrate, fat, and amino acids that produced relatively large suppressions in food intake. And finally, the conclusions of Trigazis et al. imply that if devazepide has no effect on a nutrient-induced anorexia, then CCK plays no role in mediating the anorexia produced by the nutrient. However, if CCK is one of several redundant satiety mechanisms triggered by a nutrient load, then CCK receptor blockade alone may have little if any effect on food intake, and it would therefore be inappropriate under these circumstances to single out the CCK mechanism as being unimportant. The experimental approach used in the present study and that of Trigazis et al. only tests for an “essential” CCK mechanism, not a redundant CCK mechanism, which may be triggered by some doses of some nutrients.

Perspectives

Our results suggest the presence of satiety mechanisms sensitive to the delivery of protein digestive products to the small intestine. The relative importance of protein conformation, degree of protein digestion, and amino acid composition in stimulating these
mechanisms remains to be determined. Our results further suggest that CCK plays a significant, necessary role in mediating the satiety response to duodenal delivery of protein. It is not clear where or how endogenous CCK might be acting to produce satiety because CCK is found throughout the brain and in neurocrine and endocrine cells in the gut and because central as well as peripheral administration of CCK–8 inhibits food intake and devazepide, the CCK-A receptor antagonist used in the present study, easily penetrates the blood-brain barrier (9).

This work was supported by the Medical Research Service of the Department of Veterans Affairs and National Institute of Diabetes and Digestive and Kidney Diseases Grant DK-52447.

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Received 26 October 1998; accepted in final form 26 February 1999.

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