CCK-A receptor antagonists have selective effects on nutrient-induced food intake suppression in rats

L. TRIGAZIS, F. J. VACCARINO, C. E. GREENWOOD, AND G. H. ANDERSON

Department of Nutritional Sciences, Faculty of Medicine, University of Toronto, M5S 3E2; and
Department of Psychiatry and Psychology, Centre for Addiction and Mental Health, Toronto, Ontario, Canada M5T 1R8

Trigazis, L., F. J. Vaccarino, C. E. Greenwood, and G. H. Anderson. CCK-A receptor antagonists have selective effects on nutrient-induced food intake suppression in rats. Am. J. Physiol. 276 (Regulatory Integrative Comp. Physiol. 45): R323–R330, 1999.—To provide additional support to the hypothesis that only dietary protein (Pro; chicken egg albumin) and not amino acids (AA; patterned after albumin), carbohydrates (CHO; cornstarch), or fats (Fat; corn oil) produces a satiating effect via CCK receptors, two CCK-A receptor antagonists (PD-140,548 and devazepide) were coadministered with each nutrient. Given alone [4 ml intragastrically (ig)] Pro (1.0 g), AA (1.0 g), CHO (1.4 g), and Fat (2.4 g) suppressed (P < 0.05) food intake on average during the first 2 h of feeding by 1.4 (36%), 1.5 (48%), 1.0 (33%), and 1.2 g (41%), respectively. Devazepide (0.5 mg/kg) and PD-140,548 (1.0 mg/kg) given alone increased food intake during 0–2 h by 0.7 g (18%) and during 0–1 h by 0.5 g (15%), respectively. When coadministered with PD-140,548 (1.0 mg/kg ip), the suppression of food intake caused by Pro was modulated during 0–2 h by 57% (Pro × drug interaction, P < 0.05), but AA-, CHO-, and Fat-induced suppression of feeding was not affected (nutrient × drug interaction, P > 0.05). Devazepide (0.5 mg/kg ip) did not modulate AA-, CHO-, and Fat-induced food intake suppression during any time period (nutrient × drug interaction, P > 0.05). These studies provide additional evidence that CCK-A receptors play a role in Pro (albumin) but not AA-, CHO (cornstarch), or Fat (corn oil)-induced food intake suppression in rats.

dietary protein (Pro) is a strong appetite suppressant (3) and suppresses voluntary food intake (short term) beyond that which is predicted based on the energy content of the preload alone (1). Despite much work in this area, the mechanism by which Pro suppresses voluntary food intake is still unclear, although recent evidence suggests a role for CCK (22).

The peptide CCK, a satiety hormone released on arrival of food in the gut (11, 12), has been extensively studied in the regulation of food intake. Since the initial report of peripherally administered CCK suppressing food intake in rats (6), the satiating effect of exogenously administered CCK has been well established.

A number of CCK receptor antagonists have been used to study the effects of endogenous CCK on satiety (26). These receptor antagonists differ in their structure and thus in their chemical and physical properties. Devazepide (benzodiazepine derivative) (4) has been an effective tool in investigating the role of endogenous CCK as a physiological regulator of feeding because, when administered alone to rats, it induces feeding (9, 15, 18, 19).

Because Pro is the most potent stimulant of CCK release in the rat (5, 11, 12) we began to examine the role of CCK-A receptors in Pro-induced suppression of food intake. We have shown that Pro-induced food intake suppression, but not that of amino acids (AA), carbohydrate (CHO), or fat (Fat), was attenuated by giving concurrently an intragastric load of the macronutrient along with devazepide (22). The oral route of devazepide administration was initially chosen to allow the delivery of both macronutrient and CCK-receptor blocker treatment at the same time and thus minimize stress to the rats. Because this approach leaves uncertain the absorption of the drug, devazepide was also given intraperitoneally to determine the effect on Pro-induced food intake suppression. However, the effect of intraperitoneal administration of devazepide concurrently with gavage of the non-Pro nutrients was not investigated. In addition, it was possible that the reversal of Pro-induced satiety by devazepide was not due to the blockage of peripheral CCK-A receptors but also due to a central effect of devazepide, because this drug easily permeates the blood-brain barrier (BBB) (16) and can bind to other sites involved in food intake control, such as the benzodiazepine receptor (4, 13, 14). Hence, a different class of CCK-A receptor antagonist than devazepide, PD-140,548, a dipeptoid CCK-A receptor antagonist (10), was also used in the present study to provide pharmacological support to our previous observation of the involvement of CCK-A receptors in Pro-induced satiety in the rat.

Thus the objective of this study was to determine the effect of the CCK-A receptor antagonists devazepide and PD-140,548, administered intraperitoneally, on Pro- and non-Pro (AA, CHO, and Fat)-induced food intake suppression. The effects of PD-140,548 on food intake have yet to be reported.

MATERIALS AND METHODS

Animals

Male Wistar rats (Charles River Breeding Labs, St. Constant, Quebec, Canada), purchased at a body weight of 170 ± 10 g, were used in all experiments. They were housed individually in hanging stainless steel wire mesh cages and maintained on a 10:14-h light-dark cycle (lights on 0800–1800) with controlled room temperature (22 ± 1°C). Water was provided ad libitum from spouts connected to an automated watering system, but food was presented at the onset of the dark period (1800) and food cups removed at 0800. Rats that ate less than 1.0 g in the first hour of feeding were
eliminated from the study. The University of Toronto Animal Care Committee approved this study.

**Diet**

Two powder diets were used as previously described (22). The 0% Pro diet contained 80.3% cornstarch, 10% fat in the form of corn oil (Mazola), 5% fiber (cellulose), 3.5% minerals (AIN-76), 1% vitamins (AIN-76A), and 0.2% choline bitartrate. The 25% Pro diet was formulated by adding casein (high-Pro casein containing 87.5% Pro) to the 0% Pro diet at the expense of the cornstarch component. Cornstarch (Nacan; Toronto, Canada) and corn oil (Mazola; Best Foods Canada) were obtained from a local supplier (Fernandes Food Market, Toronto, ON, Canada) and all other ingredients were obtained from Teklad Test Diets, Madison, WI. The diet was presented in 250-ml glass food cups (7.6 cm high) equipped with stainless steel screen inserts and spill-proof lids (4.5 cm diameter opening). The 0% Pro diet was given to rats from 1800 to 2000 and was replaced with the 25% Pro diet until 0800 during all experiments.

**Drugs**

The CCK-A receptor antagonist devazepide (L-364,718) \( [(3S)-(1H-2,3-dihydro-1-methyl-2-oxo-5-phenyl-1H-1,4-benzodiazepine-3-yl)-1H-indole-2-carboxamide] \) was a gift from Merck, Sharp and Dohme (West Point, PA). Because devazepide is fat soluble, it was dissolved in saline. The drugs were given intraperitoneally 30 min before food presentation. The CCK-A receptor antagonist devazepide (L-364,718) \( \text{and} \) PD-140,548 \( \text{were} \) given in a 0.25% Methocel solution by gavage to restrained rats. All nutrient treatments were administered in a total volume of 4 ml/rat, and all drug treatments were given in a volume of 1 ml/rat.

For experiments using devazepide, a Methocel stock solution and a drug stock suspension were prepared on each experimental day. First, the Methocel stock solution (0.25%) was prepared by dispersing methylcellulose powder in hot deionized water (80°C). After it was stirred (1 min), it was chilled to 5°C for ~3 h. The solution was ready for use when it became clear and had no particles visible. Second, to ensure accurate and uniform dispersal of the devazepide dosage, a drug stock suspension (0.5 mg/ml for gavage delivery or 0.5 mg/ml for intraperitoneal injection) was prepared in a glass homogenizer (Tissue Grinder, Pyrex no. 7725; Thomas Scientific, Swedesboro, NJ). The appropriate amount of drug was added to the glass homogenizer. A suspension of the drug was prepared by adding a few drops of Methocel solution with repeated up and down movements of the glass pestle. More Methocel solution was added until the appropriate volume was reached.

For experiments using PD-140,548, a drug stock solution (1 mg/ml) was prepared as follows. One milliliter of saline was added to the vial containing 5 mg of the drug (desiccated form). This solution was then transferred to a test tube with a Pasteur pipette. This procedure was repeated four times to ensure the entire drug was removed from the vial and to bring the total volume in the test tube up to 5 ml. The appropriate amount of drug stock solution was added to a separate test tube for each rat of which 1 ml was drawn into a syringe for intraperitoneal injection.

**Design**

Five experiments were conducted. Experiments 1A and 1B investigated the effect of devazepide and PD-140,548, respectively, on food intake. Experiment 2 was designed to describe the effect of PD-140,548 on Pro-induced food intake suppression. Experiments 3–5 examined the effect of devazepide and PD-140,548 on AA-, CHO-, and Fat-induced food intake suppression, respectively. All experiments used a within-subject design.

For experiments 1A and 1B, the design was as follows. On day 1, one-half the rats were given drug treatment (1 dose) and the remaining rats were given vehicle (control). On day 2, rats that previously received vehicle now received drug treatment and vice versa. The difference in food intake between control and drug treatments generated a mean difference score for food intake for each rat. This procedure was repeated for the next dose of drug after a 2-day washout period.

For experiments 2–5, the four treatments of control, nutrient, CCK-A blocker, and nutrient + CCK-A blocker were administered in a repeated-measures design over 4 treatment days with a 1-day washout between treatment days. On the basis of the evidence that Pro is the most potent secretagogue of CCK in rats (11, 12), the 0% Pro diet was given for the first 2 h of voluntary food intake measurement (1800–2000) to eliminate confounding treatment-induced release of CCK with drug-induced release of CCK. For the remainder of the feeding period, rats received the 25% Pro diet.

Experiment 1. To determine the effect of the CCK-A receptor antagonists alone on food intake, rats were injected with the drug and voluntary food intake was subsequently measured. In experiment 1A, the effect of three doses of devazepide (0.1, 0.3, 0.5 mg/kg) on voluntary food intake was compared with control intake in 14, 15, and 16 rats, respectively. In experiment 1B, two doses of PD-140,548 (0.5 and 1.0 mg/kg) were each given to 17 rats.

On test days, treatments [consisting of a control (drug vehicle), nutrient alone, CCK-A receptor antagonist alone, and nutrient plus CCK-A receptor antagonist] were given at 1730, one-half hour before food presentation. Food intake was measured at 1, 2, and 14 h after introduction of food cups (1800). Nutrient treatments consisted of chicken egg albumin (Grade II, Sigma) that contained 85% Pro (N × 6.25), AA mixture formulated on the basis of the AA profile of albumin (3), CHO (cornstarch), or Fat (Mazola corn oil). Preloads were given in a 0.25% Methocel solution by gavage to restrained rats. All nutrient treatments were administered in a total volume of 4 ml/rat, and all drug treatments were given in a volume of 1 ml/rat.
Experiment 2. Although devazepide (intragastric and intraperitoneal) was shown to block Pro-induced food intake suppression (22), this study was required to extend evidence for a Pro-specific role of CCK-A receptors by determining the effect of a different class of CCK-A receptor antagonist, namely PD-140,548. Each rat (n = 17) received four treatments: the control (intraperitoneal saline followed by the intragastric Methocel solution), PD-140,548 (1.0 mg/kg ip), Pro (1.0 mg/g), and Pro (intragastric) with PD-140,548 (intragastric).

Experiments 3–5. Previously, we showed that devazepide did not modulate AA-, CHO-, or Fat-induced suppression of food intake (22). However, because the drug was coadministered intragastrically with the nutrient preload, this may have affected the bioavailability and pharmacokinetics of the drug. Thus, the purpose of these experiments was to determine the effect of devazepide or PD-140,548 when given intraperitoneally on the food intake suppressive effect of an AA, CHO, or Fat preload.

In experiment 3A, the four treatments given to 14 rats consisted of AA (patterned after the Pro content of egg albumin; 1.0 g), devazepide (0.5 mg/kg), AA in combination with devazepide, and the control (intraperitoneal Methocel solution). In experiment 4A and experiment 5A, CHO (cornstarch; 1.4 g) and Fat (corn oil; 2.4 g) were the nutrients administered with devazepide to 14 and 17 rats, respectively. In experiments 3B, 4B, and 5B, the same four treatments as in part A of the experiments were given to 14, 21, and 20 rats, respectively, but PD-140,548 (1.0 mg/kg) was the CCK-A receptor antagonist used.

Statistical Analysis

Results are expressed as means ± SE. For experiment 1, a paired t-test was performed. For experiments 2–5, statistical evaluation was performed by a two-way repeated-measures ANOVA (nutrient × CCK-A receptor antagonist) to assess the independent effects of the two variables (nutrients decreasing food intake and the CCK-A receptor antagonist increasing food intake) and to test for interactive effects between them. This was followed by a one-way ANOVA to allow comparisons of treatment means. A post hoc test (Duncan’s multiple-range test) was performed following significant treatment effects. A probability level of P < 0.05 was accepted for the purpose of declaring statistically significant effects of treatments.

RESULTS

Experiment 1A: Effect of Devazepide on Food Intake

The 0.1 mg/kg dose caused higher food intake compared with the control only during 0–2 h by 11% (t = 2.57, P < 0.05) (Fig. 1A). The 0.3 mg/kg dose caused food intake to be higher during 0–1 h by 39% (t = 3.20, P < 0.01), during 0–2 h by 29% (t = 5.14, P < 0.01), and during 0–14 h by 6% (t = 3.30, P < 0.01). The 0.5 mg/kg dose caused higher food intake during 0–2 h by 18% (t = 2.62, P < 0.02) and during 0–14 h by 10% (t = 4.93, P < 0.01).

Experiment 1B: Effect of PD-140,548 on Food Intake

Compared with the control, only the 1.0 mg/kg dose of PD-140,548 caused food intake to be higher during 0–1 h by 15% (t = 2.42, P < 0.05) (Fig. 1B). This dose was subsequently used in the next experiment where Pro was coadministered with this receptor antagonist.
A significant interaction was found between Pro and PD-140,548 during 0–2 h \( \left[ F \left( 1,13 \right) = 264.5, P < 0.01 \right] \). Comparisons of individual treatment means by one-way ANOVA followed by a post hoc Duncan’s showed that the effect of Pro was seen primarily in the absence of PD-140,548. For example, during 0–2 h Pro treatment alone significantly suppressed food intake compared with both the vehicle control and PD-140,548 treatments. When PD-140,548 was coadministered with Pro, rats ate significantly more food than after Pro treatment alone but not different from that produced by PD-140,548 treatment. Thus PD-140,548 prevented the reduction in food intake caused by Pro treatment alone.

Experiment 3A: Coadministration of AA and Devazepide and Food Intake

Table 2 shows the individual and combined effects of AA and devazepide treatments on cumulative food intake. Repeated-measures two-way ANOVA showed significant main effects of AA treatment in lowering food intake during 0–1 h \( \left[ F \left( 1,13 \right) = 264.5, P < 0.01 \right] \), 0–2 h \( \left[ F \left( 1,13 \right) = 84.0, P < 0.01 \right] \), and 0–14 h \( \left[ F \left( 1,13 \right) = 12.6, P < 0.01 \right] \) and of devazepide treatment in increasing food intake during 0–2 h \( \left[ F \left( 1,13 \right) = 16.5, P < 0.01 \right] \) and 0–14 h \( \left[ F \left( 1,13 \right) = 15.7, P < 0.01 \right] \). A significant interaction was not found \( (P > 0.05) \) between AA and devazepide during any time period. Comparisons of individual treatment means by one-way ANOVA followed by a post hoc Duncan’s showed that the effect of the AA treatment on food intake was similar whether or not devazepide was present. For example, during 0–2 h, AA treatment alone significantly suppressed food intake compared with both the vehicle control and devazepide treatments. When devazepide was coadministered with AA, rats ate a similar amount of food as after AA treatment but ate less food than after devazepide treatment. The effect of AA treatment appeared to be attenuated by devazepide during 0–14 h, because devazepide coadministered with AA increased food intake to levels that were significantly greater than those produced by AA treatment but not different from those produced by devazepide treatment. However, the magnitudes of the devazepide effect \( (\text{devazepide} + \text{AA}) – \text{AA} \) were not statistically different from those produced by devazepide alone \( (\text{devazepide} – \text{control}) \), as indicated by nonsignificant interaction between devazepide and AA. Thus devazepide did not prevent the reduction in food intake caused by AA treatment alone.

Table 3. Experiment 3B: effect of AA and PD-140,548 on food intake

<table>
<thead>
<tr>
<th>Time, h</th>
<th>Control</th>
<th>AA</th>
<th>AA + Drug</th>
<th>Drug</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–1</td>
<td>0.6 ± 0.1†</td>
<td>0.6 ± 0.1†</td>
<td>0.6 ± 0.1†</td>
<td>2.3 ± 0.1*</td>
</tr>
<tr>
<td>0–2</td>
<td>2.0 ± 0.3*</td>
<td>2.0 ± 0.3</td>
<td>2.0 ± 0.3*</td>
<td>2.3 ± 0.1*</td>
</tr>
<tr>
<td>0–14</td>
<td>21.5 ± 0.4†</td>
<td>21.5 ± 0.4†</td>
<td>23.1 ± 0.6†</td>
<td>24.3 ± 0.5*</td>
</tr>
</tbody>
</table>

| Values are mean food intake in grams ± SE; \( n = 14 \) rats (body wt 251 ± 6 g). Control, Methocel (0.25%, 1 ml ip and 4 mg ig). Amino acids (AA; 1.0 g ig), patterned after chicken egg albumin. Drug, devazepide (0.5 mg/kg ip). Means with different superscripts for a given time period are significantly different from each other by Duncan’s new multiple-range test \( (P < 0.05) \).

Experiment 4A: Coadministration of CHO and Devazepide and Food Intake

Table 4 shows the individual and combined effects of CHO and devazepide treatments on cumulative food intake. Repeated-measures two-way ANOVA showed a significant main effect of AA treatment in lowering food intake during 0–1 h \( \left[ F \left( 1,13 \right) = 62.7, P < 0.01 \right] \) and devazepide \( (0.5 \text{mg/kg ip}) \) was not statistically different from each other by Dunn’s new multiple-range test \( (P < 0.05) \).
0–2 h \(F(1,13) = 35.4, P < 0.01\). The main effect of devazepide treatment was to cause higher food intake during 0–1 h \(F(1,13) = 4.4, P = 0.05\) and approached significance during 0–2 h \(F(1,13) = 3.9, P = 0.07\) and during 0–14 h \(F(1,13) = 4.0, P = 0.07\). No significant interaction \((P > 0.05)\) was found for any time period. Comparisons of individual treatment means by one-way ANOVA followed by a post hoc Duncan's showed that the effect of the CHO treatment on food intake was similar whether or not devazepide was present. For example, during 0–2 h CHO treatment alone significantly suppressed food intake compared with both the vehicle control and devazepide treatments. When devazepide was coadministered with CHO, rats ate a similar amount of food as after CHO treatment but ate less food than after devazepide treatment. Thus devazepide did not prevent the reduction in food intake caused by CHO treatment alone.

Experiment 4B: Coadministration of CHO and PD-140,548 and Food Intake

Table 5 shows the individual and combined effects of CHO and PD-140,548 treatments on cumulative food intake. Repeated-measures two-way ANOVA showed a significant main effect of CHO treatment in lowering food intake during 0–1 h \(F(1,20) = 64.7, P < 0.01\), 0–2 h \(F(1,20) = 48.8, P < 0.01\), and 0–14 h \(F(1,20) = 10.1, P < 0.01\). A significant main effect of PD-140,548 treatment in increasing food intake was observed during 0–2 h \(F(1,20) = 13.1, P < 0.01\) and approached significance during 0–1 h \(F(1,20) = 4.1, P = 0.06\). No significant interaction \((P > 0.05)\) was found for any time period. Comparisons of individual treatment means by one-way ANOVA followed by a post hoc Duncan's showed that the effect of the CHO treatment on food intake was similar whether or not PD-140,548 was present. For example, during 0–2 h CHO treatment alone significantly suppressed food intake compared with both the vehicle control and PD-140,548 treatments. When PD-140,548 was coadministered with CHO, rats ate a similar amount of food as after CHO treatment but ate less food than after PD-140,548 treatment. Thus PD-140,548 did not prevent the reduction in food intake caused by CHO treatment alone.

Experiment 5A: Coadministration of Fat and Devazepide and Food Intake

Table 6 shows the individual and combined effects of Fat and devazepide treatments on cumulative food intake. Repeated-measures two-way ANOVA showed a significant main effect of Fat treatment in lowering food intake during 0–1 h \(F(1,16) = 25.6, P < 0.01\), 0–2 h \(F(1,16) = 82.6, P < 0.01\), and 0–14 h \(F(1,16) = 259.5, P < 0.01\) and of devazepide treatment in causing higher food intake during 0–2 h \(F(1,16) = 4.7, P < 0.05\) and 0–2 h \(F(1,16) = 17.3, P < 0.01\) and approached significance during 0–14 h \(F(1,16) = 4.5, P = 0.051\). No significant interaction \((P > 0.05)\) was found for any time period. Comparisons of individual treatment means by one-way ANOVA followed by a post hoc Duncan's showed that the lowering of food intake after Fat administration was not affected by the presence of devazepide. For example, during 0–14 h Fat treatment alone significantly suppressed food intake compared with both the vehicle control and devazepide treatments. When devazepide was coadministered with Fat, rats ate a similar amount of food as after Fat treatment but ate less food than after devazepide treatment. The effect of Fat treatment appeared to be attenuated by devazepide during 0–2 h because devazepide coadministered with Fat increased food intake to levels that were significantly greater than those produced by Fat treatment. However, the magnitudes of the devazepide effect \([(\text{devazepide} + \text{Fat}) - \text{Fat}]\) were not statistically different from those produced by devazepide alone (devazepide – control), as indicated by nonsignificant interaction between devazepide and Fat. Thus devazepide did not prevent the reduction in food intake caused by Fat treatment alone.

Experiment 5B: Coadministration of Fat and PD-140,548 and Food Intake

Table 7 shows the individual and combined effects of Fat and PD-140,548 treatments on cumulative food intake.
intake. Repeated-measures two-way ANOVA showed a significant main effect of Fat treatment in lowering food intake during 0–1 h \[F(1,19) = 45.4, P < 0.01\], 0–2 h \[F(1,19) = 57.9, P < 0.01\], and 0–14 h \[F(1,19) = 229.6, P < 0.01\]. PD-140,548 did not affect food intake during any time period. No significant interaction \((P > 0.05)\) between Fat and PD-140,548 was found for any time period. Comparisons of individual treatment means by one-way ANOVA followed by a post hoc Duncan’s showed that the effect of the Fat treatment on food intake was similar whether or not PD-140,548 was present. For example, during 0–2 h Fat treatment alone significantly suppressed food intake compared with both the vehicle control and PD-140,548 treatments. When PD-140,548 was coadministered with Fat, rats ate a similar amount of food as after Fat treatment but ate less food than after PD-140,548 treatment. Thus PD-140,548 did not prevent the reduction in food intake caused by Fat treatment alone.

**DISCUSSION**

The results from this study provide additional evidence that CCK-A receptors play a role in Pro (albumin)-, but not AA (patterned after albumin)-, CHO (cornstarch)-, or Fat (corn oil)-induced food intake suppression in rats. Blocking CCK-A receptors by PD-140,548, given intraperitoneally, reversed Pro-induced reduction of food intake. In contrast, intraperitoneal administration of either PD-140,548 or devazepide failed to modulate AA-, CHO-, or Fat-induced suppression of feeding. These results are consistent with our previous report when these nutrients were coadministered intragastrically with devazepide (22).

Because the CCK-A receptor antagonists increased food intake, proof of the drugs’ effect in blocking the suppression of food intake by the nutrient resided in obtaining a significant interaction (nutrient × drug) between the main effects of the treatments. Examination of the interaction between drug and nutrient treatment was critical to determine whether the reversal of nutrient-induced food intake suppression by CCK receptor blockade reflected either a causal relationship between the receptor antagonists blocking the action of the nutrients at the same receptor sites or if it reflected independent, opposing effects of the CCK-A receptor antagonists increasing food intake and the nutrients suppressing food intake.

Although PD-140,548 was ineffective when given intragastrically (data not shown) and it provides a weaker response when given intraperitoneally \((0.8 \text{ g/1.4 g} = 57\%, \text{ present study})\) than devazepide \((1.1 \text{ g/1.4 g} = 79\%)\) (22) in modulating Pro-induced suppression of food intake \((0–2 \text{ h})\), it has provided additional pharmacological evidence for a selective role of CCK-A receptors in Pro-induced suppression of food intake.

The hypothesis that CCK release is involved in Pro-induced satiety is also supported by this study. Although casein is a potent secretagogue of CCK (12), albumin is a much more soluble Pro. For this reason albumin has been consistently used in this laboratory.
The present study provides evidence that CHO does not suppress food intake to the same extent as Pro when given on an equicaloric basis. A 1.4-g dose of CHO was required to suppress 0–2 h food intake (33%) to the same extent as 1.0 g of Pro (36%). This observation provides additional evidence that specific and sensitive macronutrient systems exist to regulate food intake, suggesting that Pro is operating through different feeding mechanisms from CHO.

The finding that a CCK-A receptor antagonist does not attenuate the appetite suppressive effects of fat is consistent with some (7, 8), but not all, of the literature (24). These inconsistencies may be due to fat type and route of administration. First, corn oil has no (11, 12) or little (5) effect on raising plasma CCK levels, whereas oleic acid has a potent effect on elevating endogenous CCK (11) in rats. The release of CCK may explain in part the ability of a CCK receptor blocker to attenuate some types of fats while having no effect on others. Second, the route of fat administration may be important. Fat in the present study was administered intragastrically, which may evoke several satiety signals arising from gastric, pre-, and postabsorptive mechanisms. Thus the blockade of those signals arising specifically from CCK binding to receptors may have been insufficient to eliminate the entire satiety response elicited by the intragastric administration of fat. In contrast, the effect of oleic acid on sham feeding, where intestinal satiety signals are predominant, may be more readily blocked by a CCK receptor antagonist (24). It remains to be determined if the observation in the present study is dependent on the use of corn oil as the fat source.

Results of the dose response experiments 1A and 1B support the notion that endogenous CCK is a satiety signal, because both CCK-A receptor antagonists increased feeding when administered alone. If CCK is important in producing satiety, then CCK-receptor blockade should stimulate feeding by attenuating the effect of CCK at these receptors.

PD-140,548 was not as potent as devazepide in increasing food intake when administered intraperitoneally. For example, PD-140,548 (intragastrically) did not affect food intake (data not shown) and a 1.0 mg/kg ip dose of PD-140,548 increased 0–1 h food intake by 15% (experiment 1B). However, only a 0.5 mg/kg dose of devazepide increased food intake by 24% (data not shown) and by 18% (experiment 1A) when administered intragastrically and intraperitoneally, respectively, during the same time frame. There are two possible explanations for this observation. First, because PD-140,548 is a dipeptoid, it is likely ineffective when administered intragastrically (due to possible degradation by the low pH in the stomach) as verified by the inability of PD-140,548 given intragastrically to affect food intake. Devazepide's benzodiazepine nucleus, on the other hand, is more stable and thus less susceptible to degradation by changes in pH, as indicated by its effectiveness in increasing food intake when given intragastrically (22). Second, if the location of the CCK-A receptors blocked by the antagonists is in the gut (possibly on the vagus nerve) (28) then the intraperitoneal route would be less efficient than the intragastric route in delivering the CCK-A receptor antagonist to the proposed site of action.

Although all doses of devazepide increased food intake, the effect of dose was not clear and not linear. For example, in experiment 1A, 0.1, 0.3, and 0.5 mg/kg ip devazepide increased 0–2 h food intake by 11, 29, and 18%, respectively, compared with the control. It is evident that the ability of devazepide to increase food intake when administered alone does not indicate its ability to reverse Pro-induced suppression of food intake. For example, the 0.1 mg/kg dose increased 0–2 h food intake by 18% when administered alone, but when coadministered with Pro it was ineffective in reversing Pro-induced food intake suppression (data not shown). In contrast, the 0.5 mg/kg dose also increased food intake by 15% when administered alone, but modulated Pro-induced food intake suppression by 79% during this same time period (22). It thus appears that the mechanisms by which devazepide normalizes the reduction in food intake by Pro and increases food intake when administered alone may be distinct. Quite possibly, devazepide blocks peripheral CCK-A receptors to reverse Pro-induced food intake suppression yet increases food intake by acting centrally. This is plausible because devazepide is a lipid-soluble drug that can cross the BBB, and it has been shown to bind to non-CCK receptors, such as the benzodiazepine receptor, that are known to stimulate feeding (4, 14). As well, devazepide can block serotonin receptors that normally are involved in appetite suppression (2, 4). The 0.1 mg/kg dose may have been adequate to stimulate central mechanisms of feeding but inadequate to block CCK-A receptors in the periphery and thus Pro-induced suppression of feeding.

In summary, this study provides additional evidence that CCK-A receptors play a role in mechanisms by which Pro (albumin), but not AA, CHO (cornstarch), or Fat (corn oil), suppresses food intake.

Perspectives

The regulation of food intake involves a multitude of signals arising from both pre- and postabsorptive sites. It is unlikely that each site acts independently of the other; rather the various signals that initiate the appropriate satiety responses act in concert to fine tune the food intake regulatory response.

The modulation of the food intake suppressive response after administration of a Pro preload by a specific CCK-A receptor antagonist emphasizes a preabsorptive mechanism and suggests a probable role for CCK in mediating this response. Furthermore, the modulation of only the Pro and not the AA response suggests that intact Pro, or possibly some peptide produced in the digestion of Pro, is required to trigger the mechanism involving CCK-A receptors, perhaps directly or through the release of CCK. Although the site of action appears to be peripheral CCK-A receptors, the source of endogenous CCK (endocrine, neurocrine, paracrine) remains to be determined (17).
Although a rat model was used in this work to investigate the role of CCK-A receptors in Pro-induced food intake suppression, the results of this study may be relevant to other species. Despite the fact that fat releases CCK in humans, dietary Pro is also a potent stimulant of CCK release, and endogenous CCK has been shown to function as a satiety signal, limiting meal size in many species. Even if the finding that CCK receptors are involved in the satiety response of Pro cannot be extrapolated to humans, at the very least, the general principle that a Pro-sensitive system in food intake regulation exists is supported.

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Address for reprint requests: G. H. Anderson Dept. of Nutritional Sciences, FitzGerald Bldg., 150 College St. Toronto, Ontario, Canada M5S 3E2.

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REFERENCES


