Dietary Peptides Induce Satiety via Cholecystokinin-A and Peripheral Opioid Receptors in Rats

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ABSTRACT We hypothesized that the digestion of proteins gives rise to peptides that initiate several satiety signals from the gut, and that the signals arising will be dependent on the protein source. The role of peripheral opioid and cholecystokinin (CCK)-A receptors was investigated. Casein, soy protein, and casein and soy hydrolysates were administered to rats by gavage. Food intake was measured over 2 h. The opioid receptor antagonist, naloxone methiodide (1.0 mg/kg) given intraperitoneally (i.p.), increased food intake when given at the same time as the casein preloads, 25 min after the casein preloads and 55 min after the soy protein preloads. The CCK-A receptor antagonist, devazepide (which reverses protein-induced food intake suppression), when given at 0.25 mg/kg, i.p., 60 min before preloads of each of three soy hydrolysates, also blocked suppression of food intake, but the strength and duration of the interaction depended on the preparation. When the two receptor antagonists were both administered with soy or casein preloads, their effects were additive. We conclude that peptides arising from digestion contribute to satiety by independent activation of both opioid and CCK-A receptors. J. Nutr. 132: 2775–2780, 2002.

KEY WORDS: • protein digestion • food intake • cholecystokinin-A receptor • opioid receptor • rats

Protein is more satiating than carbohydrate and fat, and its effect on food intake is more than can be accounted for by its energy content alone (1,2). The mechanism by which proteins trigger food intake regulatory systems is unclear; in the past, it was associated with changes in plasma and brain amino acid concentrations (3). Because plasma and especially brain amino acid concentrations increase relatively late after protein consumption by rats (4,5), it seems more likely that satiety signals arising from protein ingestion begin in the gastrointestinal tract. Peptides released from dietary protein stimulate cholecystokinin (CCK) release (6), which in turn contributes to food intake suppression (7), an effect that can be prevented in part by blocking CCK-A receptors (2,8).

Because we (2,8) as well as others (9) found that devazepide did not completely reverse the anorectic response to proteins, we hypothesized that receptor mechanisms other than that of CCK may be responsive to peptides arising from digestion. Opioid-like receptors are present in the gut, and the digestion of some proteins, including casein (10,11) and wheat protein (12), releases peptides that act on these receptors. The central role of opioid receptors in food intake regulation is well established (13,14), but their role in the periphery has not been studied extensively. Recently, however, it was reported that casein promotes premature meal termination via a mechanism involving both peripheral opioid and CCK receptors (15), suggesting a role for peripheral opioid receptors in satiety.

Therefore, the hypothesis of this study was that the digestion of proteins gives rise to peptides that initiate, at the same time, several satiety signals from the gut, and that the signals arising are dependent on the protein source. To test this hypothesis, we measured the effect of blocking both CCK-A and opioid receptors on the feeding response of rats to casein, soy protein, and their hydrolysates. Casein produces opioid-like active peptides and effects CCK release (11,15), whereas soy protein releases peptides that stimulate CCK release (6), but has not been found to release opioid-like peptides.

MATERIALS AND METHODS

Animals and diets. Male Wistar rats (Charles River, St-Constant, Canada) were housed individually in hanging wire-mesh stainless steel cages in a room with a temperature of 22 ± 1°C and a 12-h light:dark cycle (lights on at 0600 h); they had free access to water throughout and to pelleted diet (Rodent Laboratory Chow 5001; LabChows, Strathroy, Canada) for the first 3 d. On d 3 after arrival, the pelleted diet was removed and replaced with an AIN-93G powdered diet (16) containing either 0 g protein (CHO diet) or 20 g/100 g high protein casein (PRO). The CHO diet was available only from 1800 to 2000 h, and the PRO diet was available from 2000 to 0800 h and then removed. The PRO diet was replaced with the CHO diet for the first 2 h of food intake measurement to eliminate confounding treatment-induced release of CCK with diet-induced release of CCK. The University of Toronto Animal Care Committee approved the
The composition of the nutrient preloads was as reported by the manufacturer for moisture, ash and carbohydrate (Table 1). The composition of the nutrient preloads as well as the drug treatments are given in Table 1. Nitrogen values were also determined in our laboratory by classic Kjeldahl’s nitrogen analysis. Each rat received a preload of pure protein or protein equivalent in a volume of 4 mL deionized water because of the limited solubility of soy protein.

Drugs. Naloxone methiodide (Sigma Chemical, St. Louis, MO) was diluted in saline and used within 1 h of preparation at a dose of 1.0 mg/kg body. The CCK-A receptor antagonist devazepide (donated by ML Laboratories PLC, London, UK) was suspended in a vehicle of methylcellulose (BDH Toronto, Toronto, Canada). The methocel solution was prepared by adding 0.25 g of methyl cellulose powder to 100 g of hot (80°C) deionized water. The methocel solution was stirred for 1 min and allowed to chill to 5°C for 2–3 h. Every 0.5 h, the solution was stirred until it was clear with no visible particles. A glass homogenizer (Tissue Grinder, Pyrex Brand, No.7725; Thomas Scientific, Swedesboro, NJ) was used to mix in devazepide (0.5 g/L). Each rat received 0.25 mg devazepide/kg body, a dose that given alone does not affect food intake. All injections were given intraperitoneally in a volume of 1.0 mL.

Procedures. Before testing, rats were adapted to the experimental procedures as previously described (2). They were gavaged and/or injected with water and saline, respectively, over 4 d before the adaptation test, performed as follows. On d 1, one half of the rats were fed a treatment (preload and injection), whereas the rest were untreated. On the next day, this testing order was reversed. Experimentation began when it was determined that the processes of gavaging and injecting did not affect food intake.

Nutrient preloads and drug treatments were given before access to food at 1800 h and at times described for the individual experiments. Food consumption was measured to the nearest 0.1 g, under red light, at hourly intervals for up to 3 h.

Experiment 1: Effect of 1.0 mg/kg naloxone on food intake

The objective of this experiment was to determine the duration of the effect on food intake of 1.0 mg naloxone/kg. A repeated-measures design was used. Each rat [n = 12, mean initial body weight (BW) = 297 g] was injected with saline on d 1 and 3. Naloxone was injected on d 2. The injections were given 30 min before consumption of the AIN-93G diet (1800 h) after which food intake was measured at 1 and 2 h.

Experiment 2: Effect of naloxone on food intake suppression induced by soy protein or casein and their hydrolysates

A paired t test design was used to determine the effect of drug treatment on food intake after the preloads in Experiment 2a. In this design, one half of the rats were fed the preload alone on d 1, and the other half received preload plus drug. After a washout day, the treatments were reversed and paired comparisons made.

Experiments 2b and 2c used a two-way, factorial design with the effect of naloxone and the effect of the day on which the rats were fed the preloads as the main factors. To eliminate a possible effect of the order of preload administration over the several days of the experiment, the paired treatments were randomized over days. For example, in a set of 16 rats fed paired treatments of four different preloads and preloads plus drug, groups of four rats would be randomly assigned to one of the four paired treatments, and then proceed through each of the remaining three preload treatments given in randomized order. The duration of the experiment was 15 d. Naloxone was administered 25 or 55 min after the preloads.

Experiment 2a: Effect of naloxone administered with preloads. Rats (n = 12, BW = 248 g) received preloads of casein, soy protein and their hydrolysates in a series of four independent studies. Naloxone and preloads were given at 1730 h.

Experiment 2b: Effect of naloxone administered 25 min after preloads. Because naloxone reversed food intake suppression by the hydrolysates but not by the intact proteins, it was given 25 min later (at 1755 h) to allow for digestion of the intact proteins. Rats (n = 16, BW = 248 g) received preloads of soy protein, casein, soy hydrolysate (SH) and casein hydrolysate (CH) with and without naloxone. The treatments were randomized over time.

Experiment 2c: Effect of naloxone administered 55 min after preloads. Because naloxone reversed food intake suppression by intact casein, but not by intact soy protein when given 25 min after preloads, it was given at 1755 h, 55 min after the preloads to allow more time for digestion of soy protein. Rats (n = 16, BW = 242 g) received preloads of casein and soy protein with and without naloxone. The treatments were randomized over time.

Table 1

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Casein</th>
<th>CH</th>
<th>Soy protein</th>
<th>SH</th>
<th>SH</th>
<th>SH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein or equivalent</td>
<td>84.8</td>
<td>84.2</td>
<td>90.3</td>
<td>51.4</td>
<td>55.4</td>
<td>54.8</td>
</tr>
<tr>
<td>Moisture</td>
<td>7.0</td>
<td>3.5</td>
<td>6.0</td>
<td>4.0</td>
<td>3.0</td>
<td>4.6</td>
</tr>
<tr>
<td>Ash</td>
<td>15.0</td>
<td>3.8</td>
<td>4.0</td>
<td>14.0</td>
<td>12.1</td>
<td>12.0</td>
</tr>
<tr>
<td>Total N</td>
<td>13.3</td>
<td>13.2</td>
<td>15.8</td>
<td>9.2</td>
<td>9.7</td>
<td>9.4</td>
</tr>
<tr>
<td>Free amino acid N</td>
<td>—</td>
<td>5.6</td>
<td>—</td>
<td>1.8</td>
<td>1.7</td>
<td>3.1</td>
</tr>
<tr>
<td>Peptide N</td>
<td>—</td>
<td>7.4</td>
<td>—</td>
<td>7.4</td>
<td>8.0</td>
<td>6.3</td>
</tr>
<tr>
<td>Peptide N/Total N</td>
<td>—</td>
<td>56.9</td>
<td>—</td>
<td>80.4</td>
<td>82.5</td>
<td>67.0</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>min</td>
<td>min</td>
<td>min</td>
<td>29.0</td>
<td>35.0</td>
<td>24.0</td>
</tr>
</tbody>
</table>

1 Instant Calcium Caseinate, Flavor Force, Sarasota, FL.
2 Casein Hydrolysate Enzymatic, ICN Biomedicals, Aurora, OH.
3 Soybean Protein Isolated, ICN Biomedicals; trypsin inhibitor: 4.9–7.3 mg/g protein.
4 Soy Hydrolysate Enzymatic, ICN Biomedicals; papaic digest of soybean meal.
5 Phytone Peptone, Voigt Global Distribution, Kansas City, MI; papaic digest of soybean meal.
6 Soyton, Voigt Global Distribution; digested by trypsin and chymotrypsin.
7 Calculated as difference between total and free amino acid N. Peptides precipitated with 90% methanol.
8 min, minimal amounts.
Experiment 3: Effect of devazepide on food intake suppression induced by three soy hydrolysates

The aim of this experiment was to determine whether hydrolysates of different composition had the same interaction with CCK-A receptor. The first soy hydrolysate (SH 1) was the same as in Experiment 2. The second soy hydrolysate (SH 2) was chosen to closely match the composition of the first, and the third (SH 3) was chosen for its differences from the other two. A naive set of 12 rats (BW = 236 g) received the hydrolysates (at 1730 h) with or without devazepide (injected at 1630 h). The treatments were randomized over time. The same design was followed as in Experiments 2b and 2c.

Experiment 4: Effect of devazepide and naloxone individually and combined on food intake suppression by SH1 (4a), CH (4b), soy protein (4c) and casein (4d)

A repeated measures 2 × 2 factorial design was used with naloxone and devazepide as the main effects. The effects of naloxone, devazepide and naloxone with devazepide on food intake after four protein preloads were determined. Food intake after the combined treatments was compared with the sum of the food intakes after the treatments alone.

Experiment 4a: Soy hydrolysate. Each rat (n = 16, BW = 306 g) received four treatments in random order. These were SH1, SH1 plus devazepide, SH1 plus naloxone and SH1 plus both devazepide and naloxone. Devazepide was injected at 1630 h; naloxone and preloads were given at 1730 h.

Experiment 4b: Casein hydrolysate. Each rat (n = 16, BW = 356 g) received four treatments in random order as described in Experiment 4a except CH rather than SH was tested.

Experiment 4c: Soy protein. Each rat (n = 16, BW = 255 g) received four treatments in random order. These were soy protein, soy protein plus devazepide, soy protein plus naloxone and soy protein plus both devazepide and naloxone. Devazepide was injected at 1630 h, the preload was given at 1700 h and naloxone was injected at 1755 h.

Experiment 4d: Casein. Each rat (n = 16, BW = 319 g) received four treatments in random order. These were, casein, casein plus devazepide, casein plus naloxone and casein plus both devazepide and naloxone. Devazepide was injected at 1630 h, the preload was given at 1730 h and naloxone was injected at 1755 h.

Statistical analysis. In Experiment 1, the data were analyzed by repeated-measures one-way ANOVA, followed by post-hoc Duncan’s test. In Experiments 2 and 3, statistical analysis was based on the mean difference scores (MDS). MDS were calculated as the difference of time, Student’s t test was used to identify differences in the MDS at 1730 h and naloxone was injected at 1755 h.

RESULTS

Experiment 1: Effect of 1.0 mg/kg naloxone on food intake

There was no difference in food intake during the first 2 h of feeding on d 1, 2 and 3. Food intake (g) was 2.5 ± 0.1, 2.3 ± 0.1 and 2.6 ± 0.2 during 0–1 h; during 0–2 h, it was 3.4 ± 0.2, 3.6 ± 0.2 and 3.7 ± 0.3 for d 1, 2 and 3, respectively. Therefore, 1.0 mg/kg naloxone alone, given intraperitoneally, did not affect food intake.

Experiment 2: Effect of naloxone on food intake suppression induced by soy protein or casein and their hydrolysates

Experiment 2a: Effect of naloxone administered with preloads. Naloxone administered with the preloads of intact casein and soy protein, 30 min before food cup presentation did not increase food intake at any time interval compared with the effect of the preloads alone (Table 2). When given with the CH preload, naloxone increased food intake during 0–1 h (P < 0.01), but not during 1–2 h and 0–2 h, compared with the effect of the CH preload alone. Naloxone administered at the same time as the SH preload increased food intake during 0–1 h (P < 0.05) and the cumulative time of 0–2 h (MDS = 0.42 ± 0.16 g; P < 0.05), but not during 1–2 h, compared with the effect of the SH preload alone.

Experiment 2b: Effect of naloxone administered 25 min after preloads. Naloxone, given 25 min after the intact casein preload, increased food intake during 0–1 h (P < 0.01) (Table 2), 0–2 h (MDS = 0.76 ± 0.27 g; P < 0.05), but not during 1–2 h, compared with the effect of the casein preload alone. When given 25 min after intact soy protein, or soy and casein hydrolysates, naloxone did not affect food intake at any time interval.

Experiment 2c: Effect of naloxone administered 55 min after preloads. Naloxone given 55 min after the intact soy protein preload but not the casein preload increased food intake during 0–2 h (P < 0.01) (Table 2), 1–2 h (MDS = 0.45 ± 0.20 g; P < 0.05) and 0–2 h (MDS = 0.91 ± 0.14 g; P < 0.01), compared with the effect of the soy protein preload alone.

Experiment 3: Effect of devazepide on food intake suppression induced by three soy hydrolysates

Devazepide administered 90 min before food cup presentation reversed the food intake suppression due to SH 1 only

Table 2

<table>
<thead>
<tr>
<th>Preload</th>
<th>Treatment</th>
<th>Time of naloxone administration after preloads, min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein Preload</td>
<td>N2</td>
<td>2.25 ± 0.24</td>
</tr>
<tr>
<td>Casein</td>
<td>N2 + Preload</td>
<td>1.86 ± 0.23</td>
</tr>
<tr>
<td></td>
<td>MDS</td>
<td>0.39 ± 0.24</td>
</tr>
<tr>
<td>Soy protein Preload</td>
<td>N</td>
<td>1.58 ± 0.23</td>
</tr>
<tr>
<td>Soy protein</td>
<td>N + Preload</td>
<td>1.44 ± 0.22</td>
</tr>
<tr>
<td></td>
<td>MDS</td>
<td>0.14 ± 0.29</td>
</tr>
<tr>
<td>CH Preload</td>
<td>N</td>
<td>1.81 ± 0.15</td>
</tr>
<tr>
<td></td>
<td>MDS</td>
<td>0.82 ± 0.26</td>
</tr>
<tr>
<td>SH 1 Preload</td>
<td>N</td>
<td>0.95 ± 0.15</td>
</tr>
</tbody>
</table>
|                | MDS         | 0.35 ± 0.12                                           

1 Data are means ± SEM, n = 16.
2 Naloxone 1.0 mg/kg (intraperitoneal).
3 MDS, mean difference score (preload + naloxone − preload).
4 P < 0.05; ** P < 0.01.
over the cumulative time of 0–3 h (P < 0.05) compared with the hydrolysate alone. It reversed food intake suppression by SH 2 during 0–1 h (P < 0.01), 0–2 h (P < 0.01) and 0–3 h (P < 0.01) and by SH 3 during 0–1 h (P < 0.05), 2–3 h (MDS = 0.61 ± 0.25 g; P < 0.05) and 0–3 h (P < 0.01) (Table 3).

**Experiment 4: Effect of devazepide and naloxone individually and combined on food intake suppression by SH1 (4a), CH (4b), soy protein (4c) and casein (4d)**

The data for this series of experiments are summarized for h 1 of feeding in Table 4. Data from h 2 are not tabulated but are provided in the text when not consistent with h 1 data.

**Experiment 4a: Soy hydrolysate.** Both devazepide and naloxone treatments increased food intake after the CH preload. Devazepide increased food intake during 0–1 h (P < 0.01) (Table 4) and 0–2 h (P < 0.05), and naloxone increased food intake during 0–2 h (P < 0.05) compared with the preload alone.

A significant interaction occurred between the two treatments on food intake during 0–2 h (P = 0.003). Food intakes were 1.7 ± 0.2 g after SH 1 preload alone, 2.9 ± 0.4 g after SH 1 and naloxone, 3.1 ± 0.3 after SH 1 and devazepide, and 2.9 ± 0.2 after SH 1 was given with both naloxone and devazepide. The interaction occurred because the combination enhanced food intake compared with control by 1.2 g, which was less than the 2.6 g expected from the sum of the effects of each treatment alone.

**Experiment 4b: Casein hydrolysate.** Both devazepide and naloxone treatments increased food intake after the CH preload. Devazepide increased food intake during 0–1 h (P < 0.01) (Table 4) and 0–2 h (P < 0.05), and naloxone increased food intake during 0–1 h (P < 0.05) and 0–2 h (P < 0.05) compared with the preload alone.

There was no interaction between the two treatments, indicating that the effect of the combined treatments was the result of the additive effect of the individual treatments. The increase in food intake during 0–1 h by the combined treatments was 1.2 g compared with control, whereas the sum of the increase by the two treatments individually was 1.3 g.

**TABLE 3**

<table>
<thead>
<tr>
<th>Time, h</th>
<th>Treatment</th>
<th>SH 2</th>
<th>SH 3</th>
<th>SH 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–1</td>
<td>Preload + D</td>
<td>0.86 ± 0.18</td>
<td>1.15 ± 0.25</td>
<td>1.29 ± 0.18</td>
</tr>
<tr>
<td></td>
<td>Preload</td>
<td>0.57 ± 0.16</td>
<td>0.68 ± 0.17</td>
<td>0.67 ± 0.14</td>
</tr>
<tr>
<td></td>
<td>MDS</td>
<td>0.28 ± 0.16</td>
<td>0.47 ± 0.19*</td>
<td>0.62 ± 0.17**</td>
</tr>
<tr>
<td>0–2</td>
<td>Preload + D</td>
<td>2.34 ± 0.47</td>
<td>2.52 ± 0.31</td>
<td>2.72 ± 0.21</td>
</tr>
<tr>
<td></td>
<td>Preload</td>
<td>1.54 ± 0.19</td>
<td>1.83 ± 0.26</td>
<td>1.87 ± 0.20</td>
</tr>
<tr>
<td></td>
<td>MDS</td>
<td>0.80 ± 0.41</td>
<td>0.68 ± 0.33</td>
<td>0.85 ± 0.20**</td>
</tr>
<tr>
<td>0–3</td>
<td>Preload + D</td>
<td>3.77 ± 0.40</td>
<td>4.41 ± 0.46</td>
<td>4.54 ± 0.26</td>
</tr>
<tr>
<td></td>
<td>Preload</td>
<td>2.84 ± 0.28</td>
<td>3.12 ± 0.34</td>
<td>3.47 ± 0.30</td>
</tr>
<tr>
<td></td>
<td>MDS</td>
<td>0.93 ± 0.32*</td>
<td>1.29 ± 0.36**</td>
<td>1.07 ± 0.30**</td>
</tr>
</tbody>
</table>

1. Data are means ± SEM, n = 12.
2. Soy Hydrolysate Enzymatic, ICN Biomedicals, Aurora, OH.
3. Phytone Peptone, Voigt Global Distribution, Kansas City, MI.
5. Devazepide 0.25 mg/kg (intraperitoneal).
6. MDS, mean difference score (preload + devazepide − preload alone).

**Experiment 4c: Soy protein.** Both devazepide and naloxone increased food intake after the intact soy protein preload. Devazepide increased food intake during 0–1 h (P < 0.01) (Table 4), 1–2 h (P < 0.05) and 0–2 h (P < 0.01), and naloxone enhanced feeding during 0–1 h (P < 0.05) compared with the preload alone.

There was no interaction between the two treatments. The increase in food intake during 0–1 h by the combined treatments was 0.7 g compared with control, whereas the sum of the increase by the two treatments alone was 0.9 g.

**Experiment 4d: Casein.** Both devazepide and naloxone increased food intake after the intact casein preload. Devazepide increased food intake during 0–1 h (P < 0.01) (Table 4) and 0–2 h (P < 0.05), and naloxone enhanced feeding during 0–1 h (P < 0.05) compared with the preload alone.

There was no interaction between the two treatments on food intake during any of the time intervals. The increase in food intake during 0–1 h by the combined treatments was 0.7 g compared with control, which was equal to the sum of the increase by the two treatments individually.

**DISCUSSION**

The results of this study support the hypothesis that the digestion of food proteins gives rise to peptides that initiate a multiplicity of satiety signals from the gut. We showed that suppression of food intake by casein and soy protein involves peptides released from their digestion and is mediated through both CCK-A and opioid receptors. When the receptor antagonists were combined, their effect on food intake in h 1 of feeding was greater than the effect of each alone, suggesting that their effects were additive.

Mediation of satiety signals induced by casein and its hydrolysate through these mechanisms was expected. Previous in vivo studies have shown the presence of opioid activity in casein (11). Also, it was demonstrated recently that intact casein promotes premature meal termination in meal-fed rats, most likely through the mechanism that involves peripheral opioid and CCK receptors (15). However, there are no reports describing opioid activity of soy protein.

The role of CCK-A receptors in food intake suppression caused by proteins is well established (2,8,18). Thus, the primary aim of this study was to investigate the effect on feeding behavior of giving an antagonist of the opioid receptor with and without devazepide, a CCK-A receptor antagonist. Because the chosen dose of naloxone did not affect food intake (Experiment 1), we concluded that increases in food intake after naloxone was given with protein preloads would be the result of blockage of the receptor and not the effect of the drug alone. To be able to compare protein sources of animal origin with those from plants, we used casein and soy protein and their hydrolysates. Some early work showed that soy protein inhibited the adenylate cyclase activity in hybrid cell homogenates, but, unlike wheat or milk proteins, this inhibition was not naloxone-reversible (19), suggesting the lack of opioid peptides in soy protein. Our study suggests, however, that there is opioid-like activity arising from both proteins, and that these are involved in food intake regulation. Naloxone increased food intake by 83% when given with CH, by 58% with SH, by 21% with intact casein and by 59% with intact soy protein in h 1 of feeding (Table 2).

Because we hypothesized that the digestive process, through the release of specific peptides, is a determinant of the effect of protein on satiety, we used both intact proteins and protein hydrolysates in these studies. Two separate lines of evidence showed the importance of peptides in satiety. First, when we...
compared three SH to determine their interaction with CCK-A receptors, all three suppressed food intake via CCK-A receptors, but the strength and duration of their interaction with receptors depended on the preparation. Devazepide most strongly blocked the effect of SH 2 on food intake compared with SH 1 and SH 3 (Table 3). Available information about the composition of tested hydrolysates does not offer an explanation for the observed differences. Carbohydrate content, which may play a role in food intake suppression via the CCK-A receptor (20), was similar in all three preparations. The origin of the enzyme used for hydrolysis and peptide content were also considered. Despite similarities in the origin of the enzyme used in their preparation and the peptide and carbohydrate contents of SH 1 and SH 2, these were the two hydrolysates with the weakest and strongest interactions with the receptor, respectively, suggesting that differences in peptide composition or in availability of active peptide in the three hydrolysates are responsible for the effect of devazepide on feeding response.

A second line of evidence that the peptides liberated from digestion are active in food intake suppression arises from the data showing that the effect of naloxone on food intake depends on both the time of drug administration and the form of protein. When given at the same time as CH and SH, naloxone increased food intake, but had no effect on food intake suppression by the intact proteins (Table 2). When naloxone was given 25 min after the preload, food intake was increased only after the intact casein, but naloxone had an effect on soy protein only when given 55 min after the preload (Table 2). A delayed response to the intact protein because of the receptor, respectively, suggesting that differences in peptide composition or in availability of active peptide in the three hydrolysates are responsible for the effect of devazepide on feeding response.

Evidence of independence of the effect of digestion products on satiety via the two receptors is provided by the data (Table 4). Both naloxone and devazepide increased food intake after the preloads. The absence of a significant interaction between the treatments during h 1 of feeding, when the greatest suppression of food intake occurs after the preloads (1), supports the view that the receptors are independently affected by peptides arising from protein digestion. The additive effect of the treatments is clear because the sum of the increases after each of the devazepide and naloxone treatments was very close to the increases after concurrent administration of the antagonists. A strong interaction between naloxone and devazepide occurred only with the SH during the cumulative 2 h of feeding. This was because of the weaker than expected response when the treatments were combined. It can not be determined whether the reduced response was due to interference of one receptor antagonist with the other.

An explanation for the effect of naloxone administration on the feeding response to the proteins and their hydrolysates may be provided by examination of the receptor types involved. In the central nervous system, there are multiple opioid receptor types and subtypes acting in the elicitation and maintenance of feeding by opioid agonists (23). By choosing an antagonist that does not cross the blood brain barrier, we were able to study its effect in the periphery. However, because naloxone methiodide is not a selective antagonist, it is unclear which types or subtypes of opioid receptors are involved.

That receptor type is a consideration is illustrated by studies of the interaction of casein with receptor types. All major milk derived opioids are characterized by mu binding activity with the exception of alpha-casein-derived exorphins that show selectivity for delta-receptors (24). However, alpha-casein-derived exorphins have not been associated with food intake. Mu-receptors have been shown to affect gastrointestinal transit (25). At the present time, it appears that the most likely mechanism through which casein exorphins, or at least beta-casomorphin, affect food intake is by delay of gastric emptying and intestinal transit via their binding to the mu-receptors (26).

In summary, these results show that both casein and soy protein provide satiety signals through opioid and CCK-A receptors. Furthermore, they emphasize the importance of protein digestion in producing peptides that are functional in the stimulation of satiety.

**LITERATURE CITED**


