Nutritional Neurosciences

Exendin-4, a GLP-1 Receptor Agonist, Interacts with Proteins and Their Products of Digestion to Suppress Food Intake in Rats

Alfred Aziz and G. Harvey Anderson

Department of Nutritional Sciences, Faculty of Medicine, University of Toronto, Toronto, Ontario, Canada M5S 3E2

ABSTRACT This study investigated the hypotheses that dietary proteins suppress food intake partly through the glucagon-like peptide-1 (GLP-1) signaling pathway, and that this effect is mediated by products of protein digestion. The GLP-1 receptor agonist, Exendin-4 (Ex-4) (0.5 μg/rat), was given intraperitoneally to male Wistar rats, and food intake was measured when Ex-4 was given alone or with preloads of intact whey and casein proteins, their hydrolysates and amino acid mixtures (0.5 g·4 mL−1·rat−1). Both Ex-4 and the preloads suppressed food intake (P < 0.05), but the effect of Ex-4 on food intake was reduced when coadministered with the preloads (P < 0.05). Because the effect of Ex-4 was reduced by the protein hydrolysates and by the amino acid preloads, the results support a role for the end products of protein digestion and GLP-1 release in the suppression of food intake in response to protein ingestion. We concluded that the GLP-1 signaling pathway, activated by the release of products of protein digestion, is another mechanism accounting for the reduction of food intake after protein ingestion. J. Nutr. 133: 2326–2330, 2003.

KEY WORDS: • agonist • amino acids • food intake • gut hormone • protein

Protein suppresses food intake more than carbohydrate and fat (1), and several mechanisms have been proposed to account for its satiating effect. Changes in plasma and brain amino acid concentrations have been associated with the modulatory effects of proteins on food intake (2). However, these changes occur relatively late after protein ingestion by rats (3,4) and thus cannot account for the initiation of satiety, which begins as a result of signals arising from the gut. Protein ingestion triggers satiety signals from the gut, primarily through the release of peptide hormones, such as cholecystokinin (CCK) (5,6), which contributes to food intake suppression (7), and also through the release of biologically active encrypted peptides arising from digestion of the protein (8,9). Protein-induced satiety is also mediated through the activation of opioid-like receptors (9,10) by peptides released from digestion of proteins (11–13). The blocking of CCK-A (14,15) and opioid-like receptors results in reversal of food intake suppression caused by proteins (9,10).

Another gut hormone that may account for satiety after protein ingestion is glucagon-like peptide-1 (GLP-1) (7–36), which is released by the endocrine L-cells of the ileum (16,17). GLP-1 secretion has been reported to be particularly sensitive to the ingestion of carbohydrates (18) and some fats (19). Ingestion and intestinal perfusion of proteins and their hydrolys products also stimulate GLP-1 secretion in rats and humans (20–24). However, the role of peripheral GLP-1 in modulating feeding behavior has not been established, in contrast to its activity in the central nervous system (CNS) where it has been shown to suppress food intake (25).

Because bioactive GLP-1 has a very short half-life in plasma due to the rapid degradation by dipeptidyl peptidase IV (DPP IV) (26,27), long-acting GLP-1 analogs such as exendin-4 (Ex-4) have been used to examine the peripheral actions of GLP-1 (28,29). Ex-4 shares 53% amino acid identity with GLP-1 (30), is resistant to the action of DPP IV (31) and is a highly specific agonist for the only GLP-1 receptor identified to date (30,32). Peripheral administration of Ex-4 potently suppresses food intake (33), modulates macronutrient selection in rats (1) and decreases energy intake in healthy humans (34), suggesting that pharmacological activation of the GLP-1 signaling pathway in the periphery modulates the feeding behavior. In addition, we recently reported that whey protein interacts with Ex-4 to suppress food intake in rats (33).

The primary objective of this study was to test the hypotheses that another potential satiety mechanism involved in food intake suppression after protein ingestion arises through the GLP-1 signaling pathway, and that this effect is mediated by the products of protein digestion. Whey and casein were given by gavage as the intact proteins, peptide-containing hydrolysates or free amino acids matching the composition of the proteins in the presence or absence of Ex-4 injections.

MATERIALS AND METHODS

Animals and diets. Male Wistar rats (Charles River, Quebec, Canada) were housed individually in hanging wire-mesh stainless...
GLP-1 SIGNALING AND PROTEIN-INDUCED SATIETY

The reconstituted peptide was used within 1 h of reconstitution by use of phosphate-buffered saline (PBS) (Sigma, St. Louis, MO). By the end of the experiment, the osmolality of the preloads was not equal. Exendin-4 was injected i.p. at 0925 h and nutrient preloads were provided by gavage at 0930 h. At 1000 h when the dark cycle started, food was provided. Food consumption was measured under a red light to the nearest 0.1 g with adjustment for spillage at various times.

Experiment 1: Effect of Ex-4 and WP and CP preloads on food intake. The objective of this experiment was to describe the effect of the GLP-1 agonist, Ex-4, on food intake when given with intact protein preloads. Each rat (n = 16, body weight = 243 g) received six treatments in random order: control (water and PBS), Ex-4, WP, CP, Ex-4 and WP, CP, Ex-4 and CP.

Experiment 2: Effect of Ex-4 and WH and CH preloads on food intake. The objective of this experiment was to describe the effect of the GLP-1 agonist, Ex-4, on food intake when given with protein hydrolysate preloads. Each rat (n = 16, body weight = 252 g) received six treatments in random order: control (water and PBS), Ex-4, WH, Ex-4 and WH, CH, Ex-4 and CH.

Experiment 3: Effect of Ex-4 and AAWP and AACP on food intake. The objective of this experiment was to describe the effect of the GLP-1 agonist, Ex-4, on food intake when given with amino acid mixture preloads. Each rat (n = 16, body weight = 288 g) received six treatments in random order: control (water and PBS), Ex-4, AAWP, Ex-4 and AAWP, AACP, Ex-4 and AACP.

Statistical Analysis. In all three experiments, data were assessed by repeated-measures two-way ANOVA to look for main treatment effects and interactions between Ex-4 and the preloads followed by a one-way ANOVA with post hoc Duncan’s test to determine the effect of individual treatments by use of the SAS system (SAS Institute, Cary, NC). Differences were considered significant at P < 0.05.

RESULTS

Experiment 1: Effect of Ex-4 and intact protein preloads on food intake. Both Ex-4 and the intact protein preloads affected food intake (Table 2). Based on the two-way ANOVA, the main effect of Ex-4 was to reduce food intake during 0–1 h (P = 0.009), 0–2 h (P = 0.002), 0–3 h (P = 0.0001) and 0–8 h (P = 0.004), and that of the preloads to also decrease food intake but only during 0–1 h (P = 0.004).

A significant interaction was found between the effect of Ex-4 and the intact protein preloads on food intake during 0–1 h (P = 0.0002), 0–2 h (P = 0.004), 0–3 h (P = 0.06) and 0–8 h (P = 0.04; Table 2). The interaction is clearly seen (Table 2) when the data at each time point were subjected to one-way
ANOVA followed by Duncan’s test for mean comparisons. At these time points Ex-4 had less effect on food intake when given with the protein preloads than when given with the control (Table 2). For example, in the 1st h, Ex-4 alone and compared with control suppressed food intake by 3.0 g. However, when Ex-4 was combined with WP, it suppressed food intake by 1.5 g compared with WP alone, and when given with CP, it had no effect on food intake compared with CP alone (Table 2). Ex-4 suppressed food intake by 37% compared with the control but by only 25% when given with WP, and had no effect when given with CP.

**Experiment 2: Effect of Ex-4 and protein hydrolysate preloads on food intake.** Both Ex-4 and the protein hydrolysate preloads affected food intake (Table 3). Based on the two-way ANOVA, the main effect of Ex-4 was to reduce food intake during 0–1 h (P < 0.0001), 0–2 h (P < 0.0001), 0–3 h (P < 0.0001) and 0–8 h (P < 0.0001), and that of the preloads to also decrease food intake during 0–1 h (P = 0.01), 0–2 h (P = 0.01), 0–3 h (P = 0.04) and 0–8 h (P = 0.004). A significant interaction was found between the effect of Ex-4 and the amino acid mixture preloads on food intake at all times, during 0–1 h (P = 0.004), 0–2 h (P = 0.0009), 0–3 h (P = 0.002) and 0–8 h (P = 0.01; Table 4). Unlike expts. 1 and 2, only the interaction at 0–1 h occurred because Ex-4 had less effect on food intake when given with both of the amino acid mixture preloads than when given with the control (Table 4). For example, Ex-4 alone and compared with control suppressed food intake by 4.4 g. However, when Ex-4 was combined with AAWP, it suppressed food intake by 1.6 g compared with AAWP alone, and when given with AACP, by 3.0 g compared with AACP alone (Table 4). Ex-4 suppressed food intake by 44% when given alone, by 22% when given with AAWP and by 34% when given with AACP. At other times, the interaction could be explained by Ex-4 having less effect when given with AAWP than when given alone or with AACP.

**Table 2**
Effect of coadministering Ex-4 and intact protein preloads on AIN-93G diet intake by rats (expt. 1)†

<table>
<thead>
<tr>
<th>Treatment</th>
<th>0–1 h</th>
<th>0–2 h</th>
<th>0–3 h</th>
<th>0–8 h</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>g</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>8.1 ± 0.6a</td>
<td>10.1 ± 0.8a</td>
<td>13.8 ± 0.9a</td>
<td>24.9 ± 0.8a</td>
</tr>
<tr>
<td>Ex-4</td>
<td>5.1 ± 0.4bc</td>
<td>6.4 ± 0.5c</td>
<td>8.2 ± 0.9b</td>
<td>20.2 ± 1.1d</td>
</tr>
<tr>
<td>WP</td>
<td>5.9 ± 0.6b</td>
<td>8.2 ± 1.0b</td>
<td>11.8 ± 1.0a</td>
<td>23.8 ± 1.10b</td>
</tr>
<tr>
<td>Ex-4 + WP</td>
<td>4.4 ± 0.5c</td>
<td>6.3 ± 0.6bc</td>
<td>7.3 ± 0.7b</td>
<td>18.8 ± 0.8c</td>
</tr>
<tr>
<td>CP</td>
<td>5.5 ± 0.4abc</td>
<td>7.8 ± 0.6abc</td>
<td>11.6 ± 0.7a</td>
<td>22.8 ± 1.0abc</td>
</tr>
<tr>
<td>Ex-4 + CP</td>
<td>5.7 ± 0.4abc</td>
<td>7.3 ± 0.5bc</td>
<td>9.3 ± 0.8b</td>
<td>21.8 ± 1.1abc</td>
</tr>
</tbody>
</table>

† Values are means ± SEM, n = 16. Means in a column with different letters differ, P < 0.0001 (one-way ANOVA followed by post hoc Duncan’s test). Abbreviations: CH, casein hydrolysate; Ex-4, exendin-4; WH, whey hydrolysate.

**Table 3**
Effect of coadministering Ex-4 and protein hydrolysate preloads on AIN-93G diet intake by rats (expt. 2)†

<table>
<thead>
<tr>
<th>Treatment</th>
<th>0–1 h</th>
<th>0–2 h</th>
<th>0–3 h</th>
<th>0–8 h</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>g</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>9.4 ± 0.7a</td>
<td>11.7 ± 0.8a</td>
<td>14.6 ± 0.8a</td>
<td>25.5 ± 0.6a</td>
</tr>
<tr>
<td>Ex-4</td>
<td>5.1 ± 0.7c</td>
<td>6.5 ± 0.7d</td>
<td>7.9 ± 1.0c</td>
<td>18.6 ± 1.3c</td>
</tr>
<tr>
<td>WH</td>
<td>7.3 ± 0.5b</td>
<td>9.0 ± 0.7b</td>
<td>11.7 ± 0.8b</td>
<td>22.8 ± 0.6b</td>
</tr>
<tr>
<td>Ex-4 + WH</td>
<td>4.5 ± 0.3c</td>
<td>6.0 ± 0.4d</td>
<td>6.7 ± 0.4c</td>
<td>17.8 ± 0.9c</td>
</tr>
<tr>
<td>CH</td>
<td>6.7 ± 0.4b</td>
<td>8.6 ± 0.6bc</td>
<td>11.7 ± 0.4b</td>
<td>23.2 ± 0.8b</td>
</tr>
<tr>
<td>Ex-4 + CH</td>
<td>5.2 ± 0.5c</td>
<td>7.0 ± 0.6cd</td>
<td>7.8 ± 0.5c</td>
<td>18.5 ± 0.8c</td>
</tr>
</tbody>
</table>

† Values are means ± SEM, n = 16. Means in a column with different letters differ, P < 0.0001 (one-way ANOVA followed by post hoc Duncan’s test). Abbreviations: CH, casein hydrolysate; Ex-4, exendin-4; WH, whey hydrolysate.

**Table 4**
Effect of coadministering Ex-4 and amino acid mixture preloads on AIN-93G diet intake by rats (expt. 3)†

<table>
<thead>
<tr>
<th>Treatment</th>
<th>0–1 h</th>
<th>0–2 h</th>
<th>0–3 h</th>
<th>0–8 h</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>g</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>10.1 ± 0.4a</td>
<td>14.9 ± 0.8a</td>
<td>19.6 ± 0.9a</td>
<td>28.3 ± 0.6a</td>
</tr>
<tr>
<td>Ex-4</td>
<td>5.7 ± 0.5d</td>
<td>8.1 ± 0.6a</td>
<td>9.9 ± 0.9d</td>
<td>22.0 ± 0.8c</td>
</tr>
<tr>
<td>AAWP</td>
<td>7.3 ± 0.4c</td>
<td>10.8 ± 0.7c</td>
<td>14.4 ± 0.9c</td>
<td>24.3 ± 0.6b</td>
</tr>
<tr>
<td>Ex-4 + AAWP</td>
<td>5.7 ± 0.4d</td>
<td>8.4 ± 0.6d</td>
<td>10.9 ± 1.0d</td>
<td>21.4 ± 0.9c</td>
</tr>
<tr>
<td>AACP</td>
<td>8.6 ± 0.5b</td>
<td>12.8 ± 0.7b</td>
<td>16.9 ± 0.9b</td>
<td>27.2 ± 0.6a</td>
</tr>
<tr>
<td>Ex-4 + AACP</td>
<td>5.7 ± 0.4d</td>
<td>7.2 ± 0.5a</td>
<td>9.4 ± 0.9d</td>
<td>20.6 ± 0.8c</td>
</tr>
</tbody>
</table>

† Values are means ± SEM, n = 16. Means in a column with different letters differ, P < 0.0001 (one-way ANOVA followed by post hoc Duncan’s test). Abbreviations: AACP, amino acid mixture formulated after casein; AAWP, amino acid mixture formulated after whey; Ex-4, exendin-4.

**DISCUSSION**

The results of this study support the hypothesis that the GLP-1 signaling pathway is another satiety mechanism contributing to the suppression of food intake after protein ingestion. Free amino acids appear to provide the signal that results in the activation of GLP-1 receptors.

There were two reasons for testing the effect of Ex-4 on the rats’ feeding response after preloads of two sources of intact proteins, protein hydrolysates and amino acid mixtures. First, we wanted to provide further evidence that a mechanism explaining protein-induced suppression of food intake involved the GLP-1 signaling pathway. We previously reported
an interaction between whey and Ex-4 in the suppression of food intake (33), but have not tested any other protein source. Second, we wanted to determine the components of proteins that are responsible for the effects of proteins on food intake by this pathway.

The effect of Ex-4 on food intake was markedly reduced when given with the preloads (Tables 2–4). The statistical interaction between Ex-4 and the preloads implies that the effects of the drug and preload treatments were not independent. If they were, the conclusion would be that the treatments resulted in the activation of two independent satiety signaling pathways. Thus the combination effect would be expected to approximate the sum of the effect of each of the treatments. Because the effect of Ex-4 was reduced when administered with the preloads, the data suggest that GLP-1, whose secretion is presumably stimulated by the preloads (20–23), competed with its agonist Ex-4 on the GLP-1 receptor and thus reduced the effect of Ex-4 on food intake.

The effect of oral GLP-1 concentrations in humans adds support to a proposed role for GLP-1 in food intake suppression after protein ingestion. Protein consumption equivalent to that in 352 g of turkey meat elicits a transient peak in plasma GLP-1 after 30 min, followed by a steady rise over 3 h (21). Similarly, both whey and casein preloads increase plasma GLP-1 in humans (23). An increase in plasma GLP-1 has been found after ileal perfusion of peptides, which are oligopeptides obtained by digestion of proteins in vitro (24), and an oral amino acid load (25 g) produces a sharp and rapid increase in plasma GLP-1 in humans (17). The effect of protein ingestion on GLP-1 secretion is less certain in rats. Ileal perfusion of peptides increases GLP-1 secretion in isolated vascularity perfused rat ilea, but neither intact proteins nor amino acid mixtures elicits a GLP-1 response (20). We are unaware of reports of the plasma GLP-1 response to oral protein or amino acid ingestion by rats.

Only indirect evidence for GLP-1 action in suppressing food intake after protein ingestion arises from these studies for three reasons. First, Ex-4 is a pharmacological GLP-1 analog, and therefore cannot be assumed to describe the role of peripheral endogenous GLP-1 in food intake regulation. Second, the modes of action of Ex-4 on food intake may include other actions in addition to the activation of GLP-1 receptors on vagal afferents and the relay of satiety signals by the vagus nerve to the CNS (3). Direct activation of the GLP-1 receptors in brain areas involved in the regulation of feeding behavior may be another mode of action. GLP-1 is known to access the brain through either transport across the blood–brain barrier (BBB) (37,38), or through brainstem regions that are BBB free (39). However, it is not known whether Ex-4 does likewise. In addition, GLP-1 suppresses gastrointestinal motility (40) and thus the feeding response to Ex-4 might also be explained by delayed gastric emptying. However, the latter is unlikely to be the sole explanation of the feeding response after Ex-4 because the effect of Ex-4 on food intake depended on the source of the preload (Tables 2–4). For example, the effect of Ex-4 was reduced more during 0–1 h when given with CP than when given with WP (Table 2), whereas the opposite was observed with the amino acid mixture preloads (Table 4).

To support direct evidence for the involvement of GLP-1 in the postnutrient regulation of feeding behavior requires the use of either exogenous GLP-1 or GLP-1 receptor antagonist. The use of exogenous GLP-1 fails to show any effect because of the rapid degradation of the peptide by DPP IV (25). On the other hand, the GLP-1 receptor antagonist Ex 9-39 is available and does not suppress food intake in rats (1), suggesting that this may be an appropriate antagonist for investigating the role of peripheral endogenous GLP-1 in macronutrient-induced satiety. An appropriate dose would be expected to attenuate food intake suppression caused by proteins, their hydrolysates and amino acid mixtures.

The reduction in the effect of Ex-4 (and thereby the present involvement of GLP-1) was dependent on the type and protein source of the preloads (Tables 2–4). It is difficult to explain these differences other than to observe that there are other mechanisms that also account for food intake suppression after protein ingestion. For example, CP is more slowly digested than WP (36), and peptides arising from its digestion suppress food intake through other gut peptides, that is, CCK and opioid peptides (9). One explanation for the stronger effect of AAWP on Ex-4 might be that it contains a higher ratio of tryptophan to large neutral amino acids (≈40%) than AACP (36), which would contribute more to the conversion of the CNS of tryptophan to serotonin, a neurotransmitter involved in the suppression of appetite (2).

In summary, our results suggest that the GLP-1 signaling pathway is among the mechanisms involved in the suppression of food intake after protein ingestion and that the pathway is activated as a result of the release of free amino acids during digestion.

LITERATURE CITED

2330


