ABSTRACT The hypothesis that peripheral glucagon-like peptide-1 (GLP-1) is a regulator of both food intake and macronutrient selection in rats was tested by administration of its antagonist, exendin 9–39, and its agonist, exendin 4. The effect of exendin 9–39 given intraperitoneally (i.p.) on food intake was measured after carbohydrate, protein or fat preloads, and on choice between a protein-free, high carbohydrate (CHO) diet and a high protein, low carbohydrate (PRO) diet. The effect of exendin 4 on selection between the CHO and PRO diets was also investigated. Exendin 9–39 significantly enhanced food intake suppression occurring after glucose, but not after corn oil or albumin preloads. In diet selection studies, exendin 9–39 selectively decreased intake of only the CHO diet. In contrast, exendin 4 decreased intake of only the PRO diet. Thus, we suggest that peripheral GLP-1 plays a role in the regulation of macronutrient selection as well as food intake in rats. J. Nutr. 131: 2164–2170, 2001.

KEY WORDS: • gut peptide • high protein, low carbohydrate diet • intraperitoneal administration • protein-free, high carbohydrate diet • rats
based on the AIN-93G diet. The proportion of cornstarch and sucrose to casein (57%, based on the data provided by Harlan Teklad, Madison, WI) in the AIN-93G diet was altered to achieve the 0% (CHO) and 50% protein (PRO) diets. The composition (g/kg) of the AIN-93G, PRO and CHO diets was as follows: casein (200, 257 and 0); cornstarch (597, 216.2 and 699.3); L-cystine (3, 0 and 0); and sucrose (100, 41.2 and 133.2), respectively. The same amount of the following ingredients was added to each of the three diets: soybean oil (70), cellulose (50), vitamin mixture (10), mineral mixture (35), choline bitartrate (2.5) and tert-butyl hydroquinone (0.014).

**Nutrient preloads.** Glucose (1.0 g) and egg albumin (0.5 g; Sigma Chemical, St. Louis, MO) were dissolved in deionized water to a total volume of 2.5 mL. Corn oil (1.5 g = ~1.7 mL; Mazola) was added to 0.8 ml water, shaken and given in a two-phase mixture. In experiments involving preloads, all rats received an intragastric preload, in a volume of 2.5 mL, by gavage 30 min before the onset of the dark cycle (1800 h) when food cups were presented. The amount of preloads was chosen on the basis of their equal suppression of food intake during the first 2 h of feeding after gavage (2, 3).

**Peptide treatments.** Both exendin 9–39 and exendin 4 (California Peptide Company, San Jose, CA) were diluted in deionized water and divided into aliquots before being quickly frozen on dry ice. The aliquots were lyophilized in a freeze dryer and the resulting dried peptide stored at −20°C until used. When needed, freeze-dried peptide was allowed to come to room temperature and reconstituted using PBS (Sigma Chemical) at pH 7.4. The reconstituted peptide was used within 1 h of preparation. All injections were given i.p. in a volume of 0.5 mL. Exendin 9–39 was used at doses of 20 and 50 μg, and exendin 4 of 0.5, 10 and 20 μg.

**Procedures.** Before testing, the rats were adapted to the experimental procedures. They were gavaged and/or injected with water and saline, respectively, for 4 d before the adaptation test as follows. On d 1, half of the rats were given a treatment (either preload or injection, or both) and the rest were untreated. On the next day, this testing order was reversed; rats that received treatments on d 1 were left untreated and the rats that received no treatment on the previous day were given treatments. Experimentation began when it was determined that the process of gavaging and/or injecting had no effect on food intake.

Nutrient preloads were provided by gavage at 1730 h. Peptides were injected i.p. at 35 and/or 5 min for 20 μg or 5 min for 50 μg of exendin 9–39 and 35 or 5 min for exendin 4 (0.5–20 μg) before the onset of the dark cycle. At 1800 h, when the dark cycle started, food was provided in a single cup in the studies using the AIN-93G diet or in two cups in selection studies between the CHO and PRO diets. The objective of this series of experiments was to describe the effect of the GLP-1 antagonist, exendin 9–39, on food intake when given either alone or with nutrient preloads, and on selection of the CHO and PRO diets when given alone.

**Experiment 2.** The objective of this series of experiments was to describe the effect of the GLP-1 antagonist, exendin 9–39, on food intake when given either alone or with nutrient preloads, and on selection of the CHO and PRO diets when given alone.

**Experiment 2a: effect of exendin 9–39 (20 and 50 μg) on food intake of the maintenance diet.** Two experiments were conducted. Five minutes before the dark cycle (1800 h), rats (n = 21) were injected with either 20 μg (BW = 224 g) or 50 μg (BW = 243 g) of exendin 9–39 or saline. The AIN-93G diet was presented at 1800 h and food intake was measured at 1, 2 and 14 h. The quantity of the preloads was based on our previous studies (2, 3).

**Experiment 2b: effect of exendin 9–39 on suppression induced by nutrient preloads.** Three separate experiments were conducted. Preloads of glucose (1 g; n = 20, BW = 275 g), corn oil (1.5 g; n = 20, BW = 276 g) and chicken egg albumin (0.5 g; n = 20, BW = 294 g) were given by gavage. Rats were injected i.p. with exendin 9–39 (20 μg) 5 min before and immediately after the nutrient preload (1730 h). Thirty minutes later, they were fed the AIN-93G diet.

**Experiment 2c: effect of exendin 9–39 on selection of CHO and PRO diets.** A single dose of exendin 9–39 (50 μg) was injected i.p. 5 min before the rats (n = 14, BW = 394 g) were allowed to select between the CHO and the PRO diet. Because only one injection was given, the dose of the peptide was increased.

**Experiment 3.** The objective of this series of experiments was to describe the effect of the GLP-1 agonist, exendin 4, on food intake when given with a glucose preload, and on selection of the CHO and PRO diets when given with or without glucose preloads.

**Experiment 3a: effect of exendin 4 (0.5 μg) and glucose preload on intake of the AIN-93G diet.** To determine the effect of exendin 4 combined with glucose on consumption of the maintenance diet, either exendin 4 (0.5 μg) or saline was injected at 35 min and a glucose preload (1 g) was given by gavage to all rats (n = 13, BW = 370 g) 30 min before presentation of the AIN-93G diet.

**Experiment 3b: effect of exendin 4 (0.5 μg) and glucose preload on selection of CHO and PRO diets.** Exendin 4 (0.5 μg) or saline was injected i.p. 5 min before an intragastric preload of glucose (1 g) was given (n = 13, BW = 352 g). Thirty minutes later, the rats were allowed to select between the CHO and PRO diets.

**Experiment 3c: effect of exendin 4 on selection of CHO and PRO diets.** In a within-subject design, each rat (n = 13) was given either exendin 4 (0.5, 10 and 20 μg) or saline 5 min before having access to the CHO and PRO diets. Body weight of the rats was 231, 287 and 324 g for the exendin 4 doses of 0.5, 10 and 20 μg, respectively.

**Statistical analysis.** Data were assessed by repeated-measures one-way ANOVA with post-hoc Tukey’s test (Experiment 1) and by paired t test (Experiments 2 and 3). Significance was declared if P < 0.05. Analyses were performed using the Graphpad statistical package (Graphpad Software, San Diego, CA). All data are expressed as mean ± SEM.

**RESULTS**

**Experiment 1: food intake suppression induced by nutrient preloads.** Food intake of the maintenance diet by rats was significantly reduced after all three nutrient preloads compared with water control, during the 0–1 h ([F (3, 44) = 10.2, P < 0.01] and 0–2 h [F (3, 44) = 10.9, P < 0.01]), without differences seen among the three nutrient preloads (Table 1).

During the second hour of feeding, suppression of food intake was stronger after corn oil and albumin preloads than after water or glucose treatment ([F (3, 44) = 4.48, P < 0.01]. No
difference in food intake among the four treatments was found during the rest of the night, 2–14 h [$F (3, 44) = 2.74, P \geq 0.05$], indicating that suppression of food intake by nutrient preloads occurred during early evening feeding.

Experiment 2a: effect of exendin 9–39 (20 and 50 μg) on food intake of the maintenance diet. Neither 20 nor 50 μg of exendin 9–39 affected food intake of the AIN-93G diet at any time (Table 2).

Experiment 2b: effect of exendin 9–39 on food intake suppression induced by nutrient preloads. Exendin 9–39 decreased the consumption of the maintenance diet by rats during the second hour after the glucose gavage (Fig. 1), but not at any time after corn oil and albumin preloads (Table 3).

Experiment 2c: effect of exendin 9–39 on selection of CHO and PRO diets. Baseline food intake and selection patterns were consistent with those of a previous report (15), with rats selecting 32 ± 2% protein over five baseline days. The total food intake was reduced by the peptide treatment during 0–2 and 0–14 h (Fig. 2). The primary effect of exendin 9–39 on the food intake occurred within 2 h after meal initiation and was due to a decreased intake of the CHO diet during the 0–0.5, 0–1 and 0–2 h periods of feeding ($P < 0.05$). A decrease in intake of the PRO diet was apparent only for the cumulative measurement of 0–14 h.

### Table 1

Food intake of the maintenance diet by rats preloaded with water, glucose, corn oil or albumin

<table>
<thead>
<tr>
<th>Time, h</th>
<th>Water, 2.5 mL</th>
<th>Glucose, 1 g</th>
<th>Corn oil, 1.5 g</th>
<th>Albumin, 0.5 g</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–1</td>
<td>4.3 ± 0.4b</td>
<td>2.1 ± 0.3b</td>
<td>2.7 ± 0.3b</td>
<td>2.7 ± 0.3b</td>
</tr>
<tr>
<td>1–2</td>
<td>1.2 ± 0.3b</td>
<td>1.9 ± 0.3a</td>
<td>0.6 ± 0.2b</td>
<td>1.0 ± 0.3b</td>
</tr>
<tr>
<td>0–2</td>
<td>5.5 ± 0.4a</td>
<td>3.9 ± 0.4b</td>
<td>3.3 ± 0.3b</td>
<td>3.7 ± 0.4b</td>
</tr>
<tr>
<td>2–14</td>
<td>19.8 ± 0.5</td>
<td>19.7 ± 0.6</td>
<td>18.6 ± 0.4</td>
<td>19.6 ± 0.6</td>
</tr>
<tr>
<td>0–14</td>
<td>25.2 ± 0.5a</td>
<td>23.6 ± 0.8b</td>
<td>21.9 ± 0.5c</td>
<td>23.3 ± 0.7b</td>
</tr>
</tbody>
</table>

1 Values are means ± SEM, $n = 14$. Values with different letters in a row are significantly different at $P < 0.05$.

2 The rats, deprived of food for 10 h daily (0800–1800 h), were preloaded with water, glucose (1.0 g), corn oil (1.5 g) or chicken egg albumin (0.5 g) in a volume of 2.5 mL given by gavage at 1730 h and 30 min later were fed the AIN-93G diet for 14 h.

### Table 2

Effect of exendin 9–39 (20 and 50 μg) on rats’ consumption of the maintenance AIN-93G diet

<table>
<thead>
<tr>
<th>Treatment</th>
<th>0–1</th>
<th>1–2</th>
<th>2–3</th>
<th>0–3</th>
<th>3–14</th>
<th>0–14</th>
</tr>
</thead>
<tbody>
<tr>
<td>20 μg</td>
<td>2.1 ± 0.2</td>
<td>1.1 ± 0.2</td>
<td>1.8 ± 0.2</td>
<td>5.0 ± 0.3</td>
<td>18.7 ± 0.5</td>
<td>23.6 ± 0.4</td>
</tr>
<tr>
<td>Saline</td>
<td>2.2 ± 0.2</td>
<td>0.9 ± 0.2</td>
<td>1.7 ± 0.2</td>
<td>4.8 ± 0.3</td>
<td>19.0 ± 0.6</td>
<td>23.8 ± 0.5</td>
</tr>
<tr>
<td>50 μg</td>
<td>1.9 ± 0.1</td>
<td>1.3 ± 0.2</td>
<td>1.6 ± 0.2</td>
<td>4.8 ± 0.2</td>
<td>19.6 ± 0.6</td>
<td>24.4 ± 0.5</td>
</tr>
<tr>
<td>Saline</td>
<td>2.1 ± 0.1</td>
<td>1.1 ± 0.2</td>
<td>1.8 ± 0.2</td>
<td>5.1 ± 0.3</td>
<td>20.0 ± 0.5</td>
<td>25.0 ± 0.5</td>
</tr>
</tbody>
</table>

1 Values are means ± SEM, $n = 21$.

2 The rats, deprived of food for 10 h daily (0800–1800 h), were injected intraperitoneally with exendin 9–39 (20 and 50 μg) or saline at 1755 h and 5 min later given the AIN-93G diet for 14 h.
feeding intervals as well as over the total day (0–14 h). Exendin 4 (20 μg) decreased total food intake by ~50% and increased intake of the CHO diet during 0.5–1, 1–2 and 0–2 h, resulting in reduction by about half in intake of the CHO diet for the 0–14 h. In contrast, intake of the PRO diet was decreased by ~70% in the first 2 h of feeding, and cumulative intake (0–14 h) was reduced by 65%. The rats given the highest dose of exendin 4 (20 μg) were lethargic and for some rats administered the 10 μg dose, a decrease in activity was apparent.

**DISCUSSION**

The hypothesis that peripheral GLP-1 is a regulator of both food intake and diet selection is supported by these studies. Both the agonist and antagonist decreased food intake. Exendin 9–39, the antagonist, had a primary effect on response to the carbohydrate component of the diet, whereas exendin 4 had a primary effect on response to the protein component of the diet. Evidence for this nutrient-specific action was provided by both the preload and diet selection experiments. The effect of exendin 9–39, given either centrally or peripherally, on macronutrient selection has not been reported. As shown by the present results, rats given an i.p. injection of exendin 9–39 decreased consumption of the CHO diet but not the PRO diet when allowed to select from these two choices (Fig. 2). Because the antagonist suppressed intake of the single diet after glucose but not corn oil or egg albumin preloads, these results suggest that blocking GLP-1 activity leads to a selective feeding response to the carbohydrate portion of the diet.

Central injection of exendin 9–39 increases food intake in sated rats (9,16). In contrast, peripheral injection decreased food intake (Figs. 1, 2). At present, no explanation can be offered for this apparent contradiction in response between centrally and peripherally administered exendin 9–39.

The effect of exendin 4, the agonist, on macronutrient selection has not been reported. Given centrally, exendin 4, like GLP-1, decreases food intake (12,17,18). In the present study, rats injected i.p. with exendin 4 decreased food intake, but at least at a low dose, they selectively suppressed intake of the PRO diet.

Several aspects of the design of the present studies require comment with respect to interpretation of the data. First, the evidence provided herein for peripheral GLP-1 involvement in macronutrient selection is indirect. Because of the short half-life of GLP-1, we used an agonist and an antagonist as treatments, assuming that the vagus is involved in the transmission of GLP-1-mediated satiety signals from the gut to the brain (19). Indeed, intraportal injections of physiologic and pharmacologic doses of GLP-1 have been shown to facilitate the afferent impulse discharge rate of the hepatic vagus (20). Because this same report did not find exendin 9–39 and exendin 4 to affect the discharge rate of the vagus, there is some uncertainty about their mechanisms of action and therefore the extent to which their effects on food intake reflect the action of peripheral GLP-1 (20).

Second, the primary purpose of the preload studies was to determine whether there was an interaction between the antagonist, exendin 9–39 and the composition of the preloads given the highest dose of exendin 4 (20 μg) were lethargic and for some rats administered the 10 μg dose, a decrease in activity was apparent.

**FIGURE 2**

Effect of exendin 9–39 on selection between a protein-free, high carbohydrate (CHO) diet and a high protein, low carbohydrate (PRO) diet in rats food deprived for 10 h daily. Exendin 9–39 (50 μg) or saline was injected intraperitoneally 5 min before rats were allowed to select between the CHO and PRO diets at 1800 h. **Upper panel:** intake of the CHO diet; **middle panel:** intake of the PRO diet; **lower panel:** total food consumption (intake of the CHO diet + intake of the PRO diet).

Values are means ± SEM, n = 14; *P < 0.05 and **P < 0.01.
affecting subsequent food intake. We previously showed that preloads of protein or carbohydrate given to rats modify both total food intake and diet choice (1,21,22). In addition, we showed that suppression of food intake by preloads can be modified by agonists and antagonists. For example, injection of the CCK-A antagonist devazepide blocks the food intake suppression induced by protein (chicken egg albumin) preloads in rats (2,3). Because individual macronutrients vary in their stimulation of GLP-1 release, it was predicted that concurrent treatment with the peptides would provide an indication of which of the macronutrients utilizes GLP-1 in altering food intakes. In the present study, exendin 9–39 caused a further reduction in food intake from the AIN diet after the glucose, but not after the albumin or corn oil preloads (Fig. 1, Table 3). Therefore, these results support the hypothesis of a nutrient-specific interaction between the nutrient source and the effect of GLP-1 on food intake. Why exendin 4 also enhanced the effect of a glucose preload on food intake from the AIN diet remains unexplained. It was expected that the agonist would have an effect opposite to that of the agonist.

The goal of Experiment 1 was to define the dose of each of the macronutrients that, when given as preloads, would decrease food intake to a similar extent. As reported, this was achieved (Table 1). The importance of utilizing macronutrient doses that suppressed food intake similarly in early feeding is directed by consideration of treatment effect and sample size. For example, if the same quantities of each macronutrient were given on a weight basis, protein would suppress food intake more than carbohydrate and fat, and carbohydrate more than fat (3). Thus the magnitude of response to the drug treatment may be modified by the extent of satiety caused by the macronutrient. This in turn would influence the sample size required to test the interaction between preload composition and drug treatment. We chose to standardize the early feeding response to each of the macronutrients, thereby allowing the use of the same sample size and the same group of rats for all tests. Of course it remains possible that the treatment effect would be different if higher quantities of each preload were used to provide a greater degree of satiation in the rats at the start of the feeding period.

Third, although the two-choice diet selection experiments provided evidence that the agonist and antagonist influenced the rats’ choice of protein vs. carbohydrate, the effect of these peptides on the rats’ response to fat remains undetermined. The preload studies with exendin 9–39 and corn oil suggest that fat might be little or no interaction between fat and GLP-1 in the regulation of food intake. This observation must be interpreted with caution because fat stimulates GLP-1 secretion (23–25), and the effect of GLP-1 on insulin secretion and lipogenesis in adipose tissue (26) indicates that it may play a role in fat metabolism and intake. We recently found that a corn oil preload resulted in a transient enhancement (0–2 h) of the anorectic effect of GLP-1 (200 ng) injected into the hypothalamic paraventricular nucleus (PVN) (27).

Fourth, the doses of exendin 9–39 and exendin 4 may have been inappropriate. As noted earlier, there have been no reported studies of the effect of exendin 9–39 on food intake and very few reports of the effect of exendin 4 given periph-

FIGURE 3 Effect of exendin 4 (0.5 µg) and glucose preloads (1 g) on consumption (g) of AIN-93G diet by rats food deprived for 10 h daily. Exendin 4 (0.5 µg) or saline was injected intraperitoneally at 35 min and an intragastric gavage of glucose at 30 min before presentation of the AIN-93G at 1800 h. Values are means ± SEM, n = 13; **P < 0.01.

FIGURE 4 Effect of exendin 4 (0.5 µg) and glucose preloads (1 g) on selection between a protein-free, high carbohydrate (CHO) diet and a high protein, low carbohydrate (PRO) diet in rats food deprived for 10 h daily. Exendin 4 (0.5 µg) or saline was injected intraperitoneally at 5 min before an intragastric preload of glucose (1g) at 1730 h. The rats were allowed to select between the CHO and PRO diets at 1800 h. Upper panel: intake of the CHO diet; middle panel: intake of the PRO diet; lower panel: total food consumption (intake of the CHO diet + intake of the PRO diet). Values are means ± SEM, n = 13; *P < 0.05 and **P < 0.01.
diets. Carbohydrate preloads and decreased carbohydrate intake of diabetic fatty Zucker rats when given i.p. twice daily (9). However, central injection of exendin 9–39 also failed to affect food intake from a single diet in food-deprived rats (9). For exendin 4, the dose of 0.5 μg was based on a report that 0.1–100 μg/rat caused a dose-dependent decrease in the food intake of diabetic fatty Zucker rats when given i.p. twice daily (18). Yet, no comment has been made previously of the more general adverse effect of these doses on behavior.

Although the results of this study suggest that peripheral GLP-1 may be a component of macronutrient-specific feeding behavior, the physiologic mechanism remains undetermined. Two aspects of GLP-1 action might provide some evidence that GLP-1 could modify macronutrient-specific feeding behavior. First, blocking GLP-1 action with exendin 9–39 would be expected to modify glucose metabolism. The primary metabolic role of GLP-1 appears to be in the regulation of carbohydrate metabolism in the periphery through its effects on glucose-dependent insulin release, inhibition of glucagon secretion and stimulation of glycogenesis in the liver and muscle (29–33). Glucose metabolism is believed to be one of the regulators of food intake (1).

Second, GLP-1 may slow the rate of protein digestion and absorption by decreasing gastric acid secretion, gastric emptying and pancreatic secretion (34–36). A prolonged presence of protein and peptides in the intestine would be predicted to prolong the release of CCK in rats (37,38). Because CCK mediates the satiating effects of proteins (2), it is possible that exendin 4 indirectly stimulates a decrease in protein intake via this mechanism.

In support of the hypothesis that GLP-1 functions in macronutrient-specific feeding responses, we also found that the administered i.p. by others in food intake studies in rats (17,18).
effect on food intake of GLP-1 injected into the PVN was influenced by the macronutrient content of the food consumed. Carbohydrate enhanced, protein blocked and corn oil had a transient effect on the suppression of food intake caused by GLP-1 in the PVN (27).

In summary, the results obtained indicate that exendin 9–39 affected the feeding response after preloads of glucose, but not after protein or fat. When the rats were allowed a choice between the carbohydrate and protein diets, exendin 9–39 reduced intake of the carbohydrate diet, whereas exendin 4 reduced intake of the protein diet. Therefore, peripheral GLP-1 might have a role in the regulation of macronutrient-regulated feeding behaviors as well as in the regulation of total food intake in rats.

LITERATURE CITED


