The GLP-1 agonist exendin-4 reduces food intake in nonhuman primates through changes in meal size

Karen A. Scott and Timothy H. Moran

Department of Psychiatry and Behavioral Sciences, Johns Hopkins University School of Medicine, Baltimore, Maryland

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Scott KA, Moran TH. The GLP-1 agonist exendin-4 reduces food intake in nonhuman primates through changes in meal size. Am J Physiol Regul Integr Comp Physiol 293: R983–R987, 2007. First published June 20, 2007; doi:10.1152/ajpregu.00323.2007.—Exendin-4 (Ex4), a long-acting glucagon-like peptide-1 (GLP-1) receptor agonist, has been shown to reduce food intake and suppress gastric emptying in rodents and humans. In this study we investigated the effects of peripheral administration of Ex4 on food intake and meal patterns in adult male rhesus macaques. Rhesus macaques (n = 4) that had been trained to lever press for food pellets were injected intramuscularly 15 min before the start of their 6-h daily feeding period. Ex4 was given at doses of 0.10, 0.32, 0.56, 1.0, and 3.0 μg/kg. Ex4 suppressed food intake in a dose-dependent manner, with the 3.0 μg/kg dose completely preventing feeding during the 6-h period and the 0.10 μg/kg dose suppressing intake by 17%. Doses of 0.32, 0.56, 1.0, and 3.0 μg/kg caused significant reductions in cumulative intake at all six hourly time points. Ex4 inhibited food intake through a specific effect on meal size. Meal size was significantly reduced in a dose-dependent manner with significant reductions at the 0.32 and 1.0 μg/kg doses (P < 0.05). Day 2 and 3 intakes returned to baseline levels with no compensation for Ex4-induced feeding suppression. Administration of doses of 0.32 and 0.56 μg/kg Ex4 over 5 consecutive days led to sustained reductions in intake with no evidence of compensation. Again, these reductions were due to specific effects on meal size. These results demonstrate that activation of GLP-1 pathways has potent effects on the controls of meal size and overall food intake in a nonhuman primate model.

intestinal peptides; satiety; meal patterns; Macaca mulatta

GLUCAGON LIKE PEPTIDE-1 (GLP-1) is a posttranslational product of preproglucagon that is secreted from L cells in the distal intestine in response to the ingestion of nutrients. GLP-1 stimulates insulin and inhibits glucagon secretion. It also enhances glucose disposal and inhibits gastric emptying. GLP-1 has been shown to inhibit food intake following both central and peripheral administration. The actions of peripherally administered GLP-1 on food intake are brief, because it is rapidly degraded by dipeptidyl-peptidase IV (DPP-IV) (7). Bolus administration produces small and transient suppressions of food intake, whereas continuous intravenous infusions can result in large and sustained decreases (3, 18).

Exendin-4 (Ex4), a 39-amino acid incretin mimetic isolated from the saliva of the Gila monster (Heloderma suspectum), shares a 53% homology with GLP-1 (4, 5, 8, 17). Ex4 functions similarly to GLP-1; however, the effects are much longer lasting, because it is not degraded by DPP-IV. Peripheral administration of Ex4 has been shown to reduce plasma glucose levels, suppress glucagon secretion, slow gastric emptying, and inhibit food intake in rodents. Ex4 has also been demonstrated to reduce plasma glucose in nonhuman primates (5), and exenatide, a synthetic version of Ex4, has been shown to result in reduced glucose levels and food intake, as well as reduced body weight in humans (10). Although Ex4 has been shown to cross the blood-brain barrier in rodents and bind to GLP-1 receptors in the hypothalamus and thalamus (7, 18), the feeding inhibitory actions appears to occur via sensory afferent pathways, since ablation of these pathways by capsaicin eliminates the ability of Ex4 to suppress food intake in mice (19).

The current study was designed to assess the ability of a dose range of Ex4 to affect food intake in nonhuman primates, identify the mode of action of such a suppression, and determine whether repeated administration of Ex4 has lasting effects on food intake.

MATERIALS AND METHODS

Four individually housed adult male rhesus monkeys (Macaca mulatta), weighing between 8 and 15 kg, were used in this study. Monkeys were maintained on a 12:12-h light-dark cycle (7:00 AM to 7:00 PM) in an environmentally controlled room with ad libitum access to water. Food in the form of 1 g of nutritionally complete pellets (Bioserv, Frenchtown, NJ) was provided for 6 h per day. Food pellets were available in response to lever pressing on a fixed ratio reinforcement (FR) schedule beginning at 12:00 PM each day. The FR schedule was individually determined for each animal to prevent pouching of pellets. FR ratios ranged from FR3 to FR15 lever presses per pellet. All procedures were reviewed and approved by the Johns Hopkins University Animal Care and Use Committee.

Individual day exendin-4 injections. In initial experiments, monkeys received an intramuscular injection of 0.1, 0.32, 0.56, 1.0, and 3.0 μg/kg Ex4 (Bachem, King of Prussia, PA) or saline vehicle 15 min before the onset of food access (11:45 AM). The order of Ex4 administration was 3.0, 1.0, 0.32, 0.1, and 0.56 μg/kg. At the time of injection, monkeys had been fasted for ~18 h (end of the feeding program on the previous day). At least 5 days were allowed between Ex4 injections to eliminate any effects from previous Ex4 injections and to monitor food intake on subsequent days. Intake was computer monitored on all days as a time stamp of each pellet delivery. Intakes were compared at hourly intervals and as meal patterns.

Multiple-day Ex4 injections. For this experiment, individual doses of Ex4 were given for 5 consecutive days. Again, doses were administered 15 min before onset of the feeding program. The Ex4 doses used in this study were 0.32 and 0.56 μg/kg. Intakes were monitored for 3 additional days following each series of the Ex4 injection. Intakes were compared at hourly intervals and as meal patterns. For the consecutive day experiment, monkeys were weighted the day before the 5 consecutive days and at the end of the 6-h feeding period on the day of the fifth dose.

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**Meal patterns.** One-gram food pellets were available in response to lever press. Intake data were analyzed for total intake at hourly intervals, meal patterns, and latency to the first meal. Meals were defined as the acquisition of at least five pellets preceded and followed by a period of at least 10 min without feeding. These meal parameters accounted for >95% of the pellets received.

*Data analyses.* For statistical analyses, the mean of the three saline baseline levels for each monkey served as the saline control values. Data for hourly intakes and for each meal pattern variable were analyzed using SigmaStat (v3.10). Total intakes and meal pattern data were analyzed using repeated-measures ANOVA, analyses of simple effects, and Student-Newman-Keuls comparisons. Data are means ± SE.

**RESULTS**

Food intake was reduced significantly for all doses of Ex4 at all time points during the 6-h feeding period in a dose-dependent manner (Fig. 1; \( P < 0.001 \)), with the exception of the 0.10 \( \mu g/kg \) dose at the 1-h time point. The 3.0 \( \mu g/kg \) dose completely prevented food intake in all monkeys, and at the 1.0 \( \mu g/kg \) dose, one monkey did not eat. Lower doses produced partial suppressions that were maintained for the full 6-h feeding period. At the 6-h time point, the 0.1 \( \mu g/kg \) dose completely prevented food intake in all monkeys, and at the 1.0 \( \mu g/kg \) dose, one monkey did not eat. Lower doses produced partial suppressions that were maintained for the full 6-h feeding period. The 0.32 \( \mu g/kg \) dose significantly reduced 6-h cumulative intake compared with the 0.1 \( \mu g/kg \) dose (\( P = 0.005 \)), as did the 0.32, 0.56, 1.0, and 3.0 \( \mu g/kg \) doses (\( P < 0.001 \)). The 0.32 \( \mu g/kg \) dose significantly reduced 6-h cumulative intake compared with the 0.1 \( \mu g/kg \) dose (\( P < 0.005 \)), as did the 0.56, 1.0, and 3.0 \( \mu g/kg \) doses (\( P < 0.001 \)). In addition, the 3.0 \( \mu g/kg \) dose significantly reduced cumulative intake compared with the 0.1 (\( P < 0.001 \)), 0.32 (\( P < 0.001 \)), and 0.56 \( \mu g/kg \) doses (\( P = 0.009 \)).

On the days following Ex4-induced feeding suppressions, intake returned to baseline levels in all cases. There were no sustained effects over multiple days even for the 3 \( \mu g/kg \) dose that completely prevented food intake. There were also no increases in food intake over baseline levels on the days following Ex4 administration.

Ex4-induced reductions in food intake were predominantly expressed as changes in meal size. Meal size was reduced as a function of dose (\( P < 0.001 \)), with significant reductions at the 0.32, 0.56, and 1.0 \( \mu g/kg \) doses (Fig. 2). Meal size was not significantly affected at the 0.1 \( \mu g/kg \) dose. Meal number was only affected at doses of Ex4 that produced overall suppressions of food intake >75%, the 1.0 and 3.0 \( \mu g/kg \) doses (Fig. 3). As noted above, the 1.0 \( \mu g/kg \) dose prevented food intake in one animal, and the 3.0 \( \mu g/kg \) dose prevented feeding in all animals. Ex-4 tended to decrease meal duration, although the effect was not significant (\( P = 0.18 \)). There was no effect of Ex-4 on ingestion rate within meals. Ingestion rate under vehicle conditions was ~3 pellets/min and ranged from 2.46 to 3.65 pellets/min with Ex-4 administration. Ex4 tended to decrease meal duration, although the effect was not significant (\( P = 0.18 \)). There was no effect of Ex-4 on ingestion rate within meals. Ingestion rate under vehicle conditions was ~3 pellets/min and ranged from 2.46 to 3.65 pellets/min with Ex-4 administration. There was no dose-related effect on eating rate (\( P > 0.5 \)). Finally, there was no significant effect of Ex4 on the latency to the first meal (\( P = 0.26 \)).

Intermediate doses of 0.32 and 0.56 \( \mu g/kg \) were chosen to test the efficacy of repeated injections of Ex4. As shown in Fig. 4, the 0.32 \( \mu g/kg \) Ex4 dose reduced food intake at all six hourly time points (\( P < 0.001 \)) on all 5 days of administration. There were no differences in intake across the treatment days, indicating no loss of efficacy with continued treatment. Body weights were not significantly reduced over the 5 treatment days at this Ex4 dose. Intakes on the 3 days following Ex4 administration returned to baseline levels and did not differ from those on the saline baseline day preceding the 5 days of Ex4 administration. As shown in Fig. 5, there was an overall treatment effect of the 0.32 \( \mu g/kg \) dose of Ex4 on

![Fig. 1. Dose effects of exendin-4 (Ex4) on cumulative food intake. Ex4 reduced food intake in a dose-dependent manner over the 6-h feeding period. Intakes at all time points were significantly reduced by all doses of Ex4 (\( P < 0.001 \)), with the exception of 0.10 \( \mu g/kg \) at the 1-h time point.](image1)

![Fig. 2. Daily meal size following Ex4 administration. Ex4 reduced meal size in a dose-dependent manner, with significant differences from saline at the 0.32, 0.56, 1.0, and 3.0 \( \mu g/kg \) doses (\( P < 0.05 \)). No meals were taken at the 3.00 \( \mu g/kg \) dose.](image2)

![Fig. 3. Meal number following doses of Ex4. Ex4 did not affect number of meals taken until the dose of 1.0 \( \mu g/kg \), which inhibited food intake altogether in 1 animal (*). The dose of 3.0 \( \mu g/kg \) eliminated meals in all animals.](image3)
meal size ($P = 0.002$), but the only pairwise significant differences were seen between postinjection day 3 and Ex4 treatment days 2 and 3 ($P < .025$). Meal number was not significantly affected on treatment days compared with those on either saline baseline days or postinjection days.

The 0.56 μg/kg dose had a much more robust sustained effect on cumulative intake and meal size. Cumulative intake was suppressed at all time points across all 5 days of Ex4 administration ($P < 0.001$) compared with both saline baseline and postinjection days (Fig. 6). Again, intakes on postinjection days did not differ from that on the saline baseline day (Fig. 6). Meal size was significantly affected by Ex4 across the 5 days of treatment ($P < 0.05$) (Fig. 7). Meal number was not affected by Ex4 and did not differ among treatment days, saline baseline, and recovery days following injections. Five days of Ex4 administration at this dose resulted in a significant loss of body weight (0.47 kg, 4%; $P < .05$).

**DISCUSSION**

These studies demonstrate that peripheral injection of the GLP-1 agonist Ex4 reduces food intake in nonhuman primates and does so in a dose-related fashion. Reductions in intake lasted through the daily 6-h period of food access. The reductions in food intake that occurred following Ex4 administration were due to reductions in meal size, not meal number. Ex4 produced comparable reductions in food intake across days without apparent loss of efficacy. Following Ex4 treatment, food intake returned to baseline levels without evidence of compensation. At the higher dose employed, 5-day administration resulted in significant weight loss.

The doses that effectively inhibited food intake in the monkeys were in the range of those used clinically for glycemic control. Synthetic Ex4, exenatide, is prescribed at doses of 5 or 10 μg for twice daily subcutaneous injection. Based on a human weight of 70 kg, this would be similar to the 0.32 μg/kg dose administered to the nonhuman primates in this study. Our original choice of higher doses was based on rodent literature, in which doses effective in reducing food intake (ED50) were estimated to be 2 g/kg and higher (2, 12, 21). The effective feeding doses are in the range of those that have been shown to reduce plasma glucose levels in diabetic rhesus monkeys (21) with an ED50 estimated at ~0.25 μg/kg.
Exenatide is marketed as Byetta. Product information identifies common side effects as nausea, vomiting, headache, a jittery feeling, and acid stomach. Nausea is reported to be most common when treatment is initiated but decreases over time in most patients. Even at the higher doses that essentially prevented food intake, we did not observe any signs of nausea, vomiting, or malaise in the monkeys. Monkeys were sitting up in their cages and normally interactive with staff. They simply seemed uninterested in acquiring food. At lower doses that reduced meal size, we observed reductions in meal durations that were comparable to the reductions in meal size such that there were no significant changes in eating rate, a measure that can be associated with malaise such as that induced by cytokines (16).

The effects of Ex4 on food intake and meal patterns that we observed are similar to those demonstrated in response to prolonged intravenous infusion of GLP-1 in rats. Chelikani et al. (3) have demonstrated that 3-h intravenous GLP-1 infusions resulted in dose-related suppression of food intake that last throughout an 18-h feeding period. Decreases in food intake were expressed primarily as decreases in meal size. At higher doses, decreases in meal number were also evident. The feeding reductions were dependent on the prolonged infusions, since intravenous bolus infusions of similar doses were ineffective in decreasing food intake. Ex4, a GLP-1 analog resistant to DPP-IV degradation, would maintain elevated levels of agonist activity similar to those attained with intravenous infusions of the naturally occurring peptide.

The feeding inhibitory action of Ex-4 is expressed primarily as reductions in meal size. Similar modes of action have been demonstrated for other gastrointestinal peptides such as cholecystokinin (CCK) (20) and the lower intestinal peptide PYY(3-36) (14). The sustained actions throughout a 6-h daily feeding period of Ex4 are similar to those that have been demonstrated for a degradation-resistant CCK analog (13) and PYY(3-36) (14) in primates. However, although consistent reductions across days were evident for both the CCK analog and Ex4, reported results from multiple-day PYY(3-36) administration have been mixed with one report of no efficacy beyond the first day with bolus injections (14) and some sustained but reduced inhibitions with twice daily PYY(3-36) intravenous infusions (9).

The site of action of Ex4 for reducing food intake is likely to be peripheral, as noted in the Introduction. GLP-1 receptors are expressed within the nodose ganglion (15), and GLP-1 has to be peripheral, as noted in the Introduction. GLP-1 receptors

were attenuated by ablation of the vagal-brainstem-hypothalamic pathway. Brain Res 1044: 127–131, 2005.


References


