

Evidence that Intestinal Glucagon-Like Peptide-1 Plays a Physiological Role in Satiety

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A physiological role in satiety is proposed for glucagon-like peptide-1 (GLP-1), secreted by the distal intestine in response to ingested nutrients. Here we report that in rats, ip injection of the GLP-1 receptor (GLP-1-R) antagonist exendin 9-39 (Ex9) elicited hyperphagia, but only at times of day when intake is otherwise low. Furthermore, ip administration of Ex9 attenuated satiety induced by either a voluntarily consumed sucrose meal (by 100%) or an intragastric glucose load (by 40%). To determine whether these effects involve blockade of GLP-1-R in brain or at a peripheral site, we injected Ex9 either centrally (into the third ventricle) or peripherally (ip) prior to GLP-1 injected either centrally or peripherally. Anorexia induced by peripheral GLP-1 was fully blocked by peripheral, but not central, pretreatment with Ex9, whereas the opposite was true for anorexic effect of central GLP-1. Thus, ip Ex9 appears to attenuate satiety via peripheral GLP-1-R blockade. Finally, anorexia induced by ip injection of exendin-4 (a GLP-1-R agonist) was due to both reduced meal size and increased duration between meals. We conclude that GLP-1 released from the intestine in response to ingested nutrients is a physiologically active satiety signal. (*Endocrinology* 150: 1680–1687, 2009)

The delivery of nutrients to the gastrointestinal tract during a meal activates neural and hormonal responses that promote metabolic homeostasis by both limiting the amount of food consumed and enhancing the clearance of glucose and other nutrients from the bloodstream. Gut-derived mediators that inhibit feeding and lead to meal termination are collectively referred to as satiety signals, whereas a separate set of signals known as incretins augment nutrient-induced insulin secretion, thereby enhancing nutrient uptake (1, 2). Prominent among incretin peptides is glucagon-like peptide (GLP)-1, which is secreted by intestinal L cells in response to ingested nutrients. In addition to its incretin effect, systemic administration of GLP-1 or long-acting GLP-1 receptor (GLP-1-R) agonists, such as exendin-4 (Ex4), reduce food intake and body weight, effects that confer therapeutic benefit to humans that receive Ex4 for the treatment of diabetes (1).

These findings raise the possibility that endogenously released GLP-1 serves as both a satiety signal and an incretin, a

combination of functions not previously ascribed to any other molecule. In the current studies, therefore, we wished to determine whether GLP-1 plays a physiological role in satiety sensation, a function suggested but not actually confirmed by previous studies. To accomplish this goal, we sought to determine whether blockade of GLP-1 receptors using the GLP-1-R antagonist, exendin (9-39) (Ex9) either increases spontaneous food intake or attenuates the satiety effect of ingested nutrients. The results of these studies provide clear evidence that endogenous GLP-1 signaling is required for intact satiety sensation.

Because GLP-1 and its receptors are present in both the central nervous system (CNS) and peripheral tissues, the feeding effects of GLP-1 could be mediated at either or both sites. Indeed, GLP-1 reduces food intake after either central or systemic administration (3–5). In the brain, GLP-1 is synthesized exclusively in neurons in the nucleus of the solitary tract (6) that project to the hypothalamus, hindbrain, and other brain areas where GLP-1-R are expressed (7). GLP-1-R expressed by autonomic fibers

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Abbreviations: CCK, Cholecystokinin; CNS, central nervous system; Ex4, exendin-4; Ex9, exendin 9-39; GLP, glucagon-like peptide; GLP-1-R, GLP-1 receptor; IG, intragastric; OXM, oxyntomodulin; 3rd-icv, third-intracerebroventricular.

innervating the gastrointestinal tract, portal vein, pancreatic islets, and other tissues are also targets for mediating the action of gut-derived GLP-1 (8, 9). We therefore sought to determine whether gut-derived GLP-1 exerts its anorexic effects through a peripheral mechanism or by crossing the blood-brain barrier and binding to central GLP-1-R. Our findings that third-intracerebroventricular (3rd-icv) injection of Ex9 did not impair the response to peripheral GLP-1, and conversely, that peripheral Ex9 administration had no effect on the response to 3rd-icv GLP-1, support a model in which meal-related GLP-1 release by the intestine reduces food intake via a peripheral site of action. Combined with our finding that ip Ex4 injection reduces meal size and increases the interval between meals, we conclude that in addition to its function as an incretin peptide, GLP-1 released from the gastrointestinal tract plays a critical physiological role in the detection of satiety by the CNS.

Materials and Methods

Subjects

Naive male Wistar rats (Charles River Laboratories, Inc., Wilmington, MA) were individually housed in ventilated Plexiglass cages in a temperature-controlled room under a 12-h light; 12-h dark cycle. Water and standard rat chow (Purina, St. Louis, MO) were available *ad libitum* except where otherwise noted. All subjects were handled daily and habituated to ip injection of 1.5 ml saline and measurement of food intake on at least three occasions before the experiments began. Body weight and food intake were measured daily. Study procedures were approved by the Animal Care Committee at the University of Washington and conformed to standards described in the Guide for the Care and Use of Laboratory Animals (National Research Council, 1996).

Drugs

GLP-1 was obtained from Bachem (Torrance, CA) and Ex4 and Ex9 were obtained from California Peptide Research (Napa, CA). Each was dissolved in sterile 0.9% saline.

Effect of GLP-1-R blockade on food intake

To determine whether ip Ex9 increases food intake during the light cycle, rats were split into three weight-matched groups ($n = 6$ –7/group) and were treated with either vehicle or Ex9 at a dose of 30 $\mu\text{g/kg}$ ip or 100 $\mu\text{g/kg}$ ip. On the day of the experiment, food was removed from rats' cages 4 h before injections. ip injections were administered 8 h into the light phase. Food was immediately returned and then intake was measured 1 and 2 h later. To examine the effects of Ex9 at a time when rats consume a relatively large amount of food, we subjected three new groups of rats ($n = 6$ /group) to the same procedure, except that injections were delivered immediately before the start of the dark phase. To assess whether Ex9 affects food intake when delivered during the dark but at a time when spontaneous intake is relatively low, rats were deprived of food for 4 h before dark onset, as in the previous studies, and were given their food back immediately before dark with no injections at that time. One hour into the dark phase, food intake was measured and animals received ip injections of vehicle, 30 $\mu\text{g/kg}$ Ex9, or 100 $\mu\text{g/kg}$ Ex9. Food was returned immediately and intake was measured 1 and 2 h later. This protocol used a within-subject design, in which each rat ($n = 10$) received all three drug conditions on consecutive days in counterbalanced order.

Effect of GLP-1-R blockade on satiety induced by an oral preload

Food was removed from rats' ($n = 11$) cages 4 h before the dark cycle began. The rats were trained to consume 12.5% sucrose solution over 15

min before the onset of the dark phase and were habituated to receiving ip injections immediately after this sucrose intake session. Within 5 days, the amount of sucrose consumed over this 15-min interval was highly reproducible (mean 10.4 ± 0.4 kcal) and thus constituted a reliable oral preload.

On experiment days, rats received either no access to the sucrose (no load condition) or sucrose access as they had during training (load condition). These conditions were presented in counterbalanced order on consecutive days. Immediately before dark phase onset, rats were given ip injections of saline or Ex9 (100 $\mu\text{g/kg}$), and preweighed chow was returned to their cages. Food intake was measured 30, 60, and 120 min later. This study used a mixed between/within-subject design, in which rats received either ip saline ($n = 6$) or ip Ex9 ($n = 5$) after both the no-preload and preload conditions.

Effect of GLP-1-R blockade on satiety induced by an intragastric (IG) preload

One limitation of a study design in which a nutrient preload is provided through voluntary consumption of a sucrose meal is the inability to include a control preload condition for comparison. To address this limitation, we investigated whether the satiating effect of a nutrient load delivered directly into the stomach is dependent on GLP-1-R signaling. Rats ($n = 5$) were fitted with a SILASTIC brand (Dow Corning, Midland, MI) IG catheter according to a technique adapted from Davis and Campbell (10).

After at least 1 wk of recovery from surgery, rats were habituated to IG infusion of warm saline followed by access to vanilla Ensure liquid diet (Abbott, Abbott Park, IL) in their home cages for 2 h during the midlight cycle. Chow was removed from their cages 6 h before the start of infusions. These habituation infusions were administered on three occasions before the experiment.

The infusion and intake test procedure was as follows. Tygon tubing from syringes filled with infusate (see below) was connected to the rats' IG catheters, and a motorized syringe pump (World Precision Instruments, Sarasota, FL) was used to infuse 10 ml at a rate of 1 ml/min (chosen to approximate rats' average rate of ingestion). Five minutes after the end of the infusion, the IG catheters were flushed with 0.5 ml saline to ensure that the infusate was delivered to the stomach in its entirety. The infusion tubing was then disconnected, and each rat received an ip injection. Preweighed bottles of vanilla Ensure liquid diet were made available 5 min after injections. Intake of Ensure 30, 60, and 120 min later provided a measure of satiety induced by the IG infusion.

On experimental days, rats received IG infusions of either saline or 10 kcal glucose (10 ml 25% glucose delivered at 1 ml/min), followed by an ip injection of either saline or the GLP-1-R antagonist Ex9 (100 $\mu\text{g/kg}$). Each rat received all four possible conditions: IG saline/ip saline; IG glucose/ip saline; IG saline/ip Ex9; IG glucose/ip Ex9, presented on consecutive days in counterbalanced order.

Effect of central Ex9 on central vs. peripheral GLP-1-induced anorexia

To verify that the dose of Ex9 selected for 3rd-icv injection attenuates the anorexic effect of central GLP-1, rats were stereotactically implanted with a 26-gauge cannula (Plastics One, Roanoke, VA) aimed at the third cerebral ventricle. Cannula placement was verified before the start of the experiment through the measurement of a sympathetically mediated increase in plasma glucose 60 min after 3rd-icv injection of 210 μg of 5-thio-D-glucose. After recovering from surgery for at least 1 wk, rats were fasted overnight before the experiment. Overnight fasting was chosen on the basis of previous studies showing reliable effects of 3rd-icv GLP-1 on fasting-induced food intake. Each rat was assigned to one of two groups: icv saline or icv Ex9 ($n = 6$ –7/group). One hour before the onset of the dark phase, rats were given an icv injection of saline or Ex9 (20 μg in 2 μl) followed by a second icv injection of saline or GLP-1 (3 μg in 2 μl) within 30 min before dark onset. Food was returned immediately before dark and intake was measured 30 and 60 min later. The GLP-1 injection conditions were presented in counterbalanced order and separated by 1 wk.

To determine whether blockade of central GLP-1-R can block the anorexic effects of ip GLP-1, rats with 3rd-icv cannulas were assigned to one of two weight-matched groups: icv vehicle or icv Ex9 ($n = 5$ –7/group). On experimental days, food was removed 4 h before the dark cycle. Thirty minutes before the start of the dark phase, rats received a 3rd-icv injection of vehicle or Ex9 (20 μg in 2 μl). Immediately before dark onset, saline or GLP-1 (100 $\mu\text{g}/\text{kg}$ ip) was administered and food was returned to the cages. Food intake was measured 30 min later. Each rat received both the saline and GLP-1 ip injection conditions, separated by at least 2 d.

Effect of peripheral Ex9 on central vs. peripheral GLP-1-induced anorexia

To test whether the dose of ip Ex9 (100 $\mu\text{g}/\text{kg}$) used in studies investigating the role of GLP-1 in satiety, described above, can block the effect of the same dose of ip GLP-1 (100 $\mu\text{g}/\text{kg}$) given in the last experiment, food was removed from rats' cages 4 h before the dark cycle began. On the day of the experiment, an ip injection of vehicle or Ex9 was followed by a second ip injection of saline or GLP-1. After the second ip injection, food was returned to the rats' cages. Intake was measured 30 and 60 min later. This study used a between-subject design, such that each rat was placed into one of four groups ($n = 12$ –13/group): saline/saline, Ex9/saline, saline/GLP-1, or Ex9/GLP-1.

To ask whether the dose of ip Ex9 that we used in our preload experiments blocks the effects of central GLP-1 on intake, we split 3rd-icv cannulated rats into two groups, one receiving icv saline and the second receiving icv GLP-1. Rats were fasted overnight before the experiment. On the day of the study, ip saline or Ex9 (100 $\mu\text{g}/\text{kg}$) injections were delivered 15 min before icv injections of saline or GLP-1 (3 μg). The icv injections took place within 30 min before the onset of the dark phase. Immediately before dark, food was returned to the cages, and food was weighed again 30 min later. An additional group of rats received ip Ex9 45 min (rather than 15 min) before 3rd-icv GLP-1 injections to determine whether more prolonged exposure to a systemically administered GLP-1-R antagonist influences the feeding effect of centrally administered GLP-1. The ip conditions were presented in counterbalanced order and separated by one week.

Meal pattern analysis

To determine the size and frequency of meals, rats ($n = 6$) were housed for 7 d in calorimetry chambers equipped with the Feed-Scale system (Columbus Instruments, Columbus, OH) for continuous monitoring of food intake. The rats were given several days to habituate to these chambers before the experiment began. On experimental days, food was not available during the 4 h before the onset of the dark phase. With order of injection condition counterbalanced across animals, rats were injected with saline or Ex9 (1 $\mu\text{g}/\text{kg}$ ip) 5 min before the dark phase, and food was returned after the injections. Conditions were separated by 2 d.

Analysis of meal patterns was performed with a customized computer program (Visual Basic, Microsoft, Redmond, WA) using the following criteria. An individual meal was defined as the consumption of at least 0.2 g of chow separated from the end of the previous meal by at least 10 min. Average meal size was calculated by dividing the number of individual meals by the total amount of food consumed over the test period. We defined meal duration as the time from the beginning to the end of a single meal, and the intermeal interval was defined as the time from the end of one meal to the beginning of the next.

Statistical analysis

The effects of Ex9 on food intake, meal frequency, and meal size were assessed by one-tailed paired-samples Student's *t* tests. The effect of Ex9 on the size of individual meals during the test period was analyzed by within-subject two-way ANOVA (drug $\times$ meal), and the least significant differences test was used for *post hoc* pairwise comparisons. $P < 0.05$ was taken to be significant.

In all other experiments, the data were analyzed via two-way ANOVA (between subjects, within subjects, or mixed design, depending on the study), and least significant differences tests were used to perform *post hoc* pairwise comparisons. $P < 0.05$ was taken to be significant.

Results

GLP-1-R blockade increased food intake

Administration of either of two different doses of the GLP-1-R antagonist Ex9 (30 $\mu\text{g}/\text{kg}$ ip and 100 $\mu\text{g}/\text{kg}$ ip) during the light phase, when spontaneous food intake is relatively low, increased food intake relative to vehicle [$F(2,17) = 6.08$, $P < 0.05$] during the 2 h after treatment (Fig. 1A). In contrast, injection of either dose of Ex9 immediately before the onset of the dark phase, at which point rats normally begin to eat a large meal, had no effect on food intake (Fig. 1B). By comparison, when Ex9 was given 1 h after dark onset, food consumption was increased during the 1 h after ip injection of both doses of Ex9 [$F(2,18) = 3.82$, $P < 0.05$] (Fig. 1C), similar to the effect observed during mid-light cycle (Fig. 1A).

GLP-1-R blockade attenuated nutrient preload-induced satiety

To determine whether spontaneously consumed nutrients reduce subsequent food intake via a GLP-1-dependent mechanism,

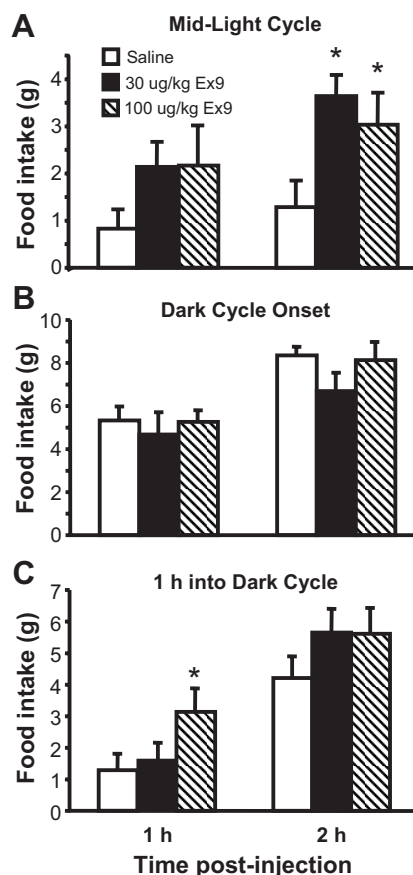


FIG. 1. Ex9 effect on food intake. Food intake was measured in rats at 1 and 2 h after ip injection of either saline vehicle or Ex9 (30 or 100 $\mu\text{g}/\text{kg}$) during the middle of the light phase (A); immediately before the dark phase (B), or 1 h into the dark phase (C). Data are mean $\pm$ SEM. *, $P < 0.05$ vs. vehicle.

we asked whether the GLP-1-R antagonist, given immediately before dark cycle onset, blunts the satiating effect of a voluntarily consumed sucrose preload. When rats were injected with saline, the sucrose preload reduced chow intake over the next 2 h ($P < 0.05$) (Fig. 2). Whereas Ex9 (100 $\mu\text{g/kg}$ ip) had no effect when delivered in the absence of a preload, the effect of the sucrose preload to reduce intake was completely prevented by antagonist treatment [preload $\times$ Ex9 interaction, $F(1,8) = 22.35$, $P < 0.01$] (Fig. 2).

IG infusion of a 10-kcal glucose preload significantly suppressed subsequent liquid diet intake relative to IG saline during a 2-h test session ($P < 0.05$). When Ex9 (100 $\mu\text{g/kg}$ ip) was administered immediately after the IG infusion ended, the satiating effect of the glucose preload (relative to IG saline) was attenuated (by 40%, Fig. 3) compared with the ip saline condition [preload $\times$ Ex9 interaction, $F(1,4) = 9.03$, $P < 0.05$].

Central Ex9 did not block peripheral GLP-1-induced anorexia

We determined that 3rd-icv pretreatment with Ex9 at a dose of 20 μg strongly attenuated the ability of 3rd-icv GLP-1 to reduce food intake in previously fasted rats, such that the anorexic effect of 3rd-icv GLP-1 was no longer significant at 30 min [Ex9 $\times$ GLP-1 interaction, $F(1, 11) = 5.95$, $P < 0.05$] (Fig. 4A) and was completely blocked by 3rd-icv Ex9 pretreatment after 1 h (data not shown). By contrast, the effect of systemic GLP-1 (100 $\mu\text{g/kg}$ ip) to reduce food intake (by $\sim 50\%$) was not altered by 3rd-icv Ex9 pretreatment at the same dose [main effect of GLP-1, $F(1, 10) = 55.15$, $P < 0.001$; interaction with Ex9, $P = \text{NS}$] (Fig. 4B).

Peripheral Ex9 did not block CNS GLP-1-induced anorexia

The anorexic response to peripheral GLP-1 (100 $\mu\text{g/kg}$ ip) was fully blocked by the same dose of peripheral Ex9 (100 $\mu\text{g/kg}$ ip) used in our preload experiments [Ex9 $\times$ GLP-1 interaction, $F(1,46) = 9.13$, $P < 0.01$] (Fig. 5A). By contrast, pretreatment with the same dose of ip Ex9 had no effect on the anorexic response to 3rd-icv GLP-1, regardless of whether the antagonist

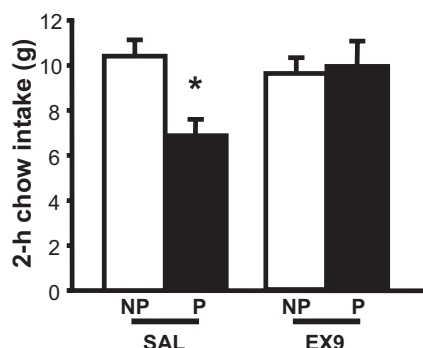


FIG. 2. Effect of GLP-1-R blockade on oral preload-induced satiety. After training rats to receive access to a sucrose solution for 15 min at the same time each day, chow intake was measured after either no preload (NP) or an orally consumed sucrose preload (P). Preloads were followed immediately by ip injection of either saline (SAL) or Ex9 (100 $\mu\text{g/kg}$), after which standard chow was returned to the cage and intake measured over the subsequent 2 h period. Data are mean $\pm$ SEM. *, $P < 0.05$.

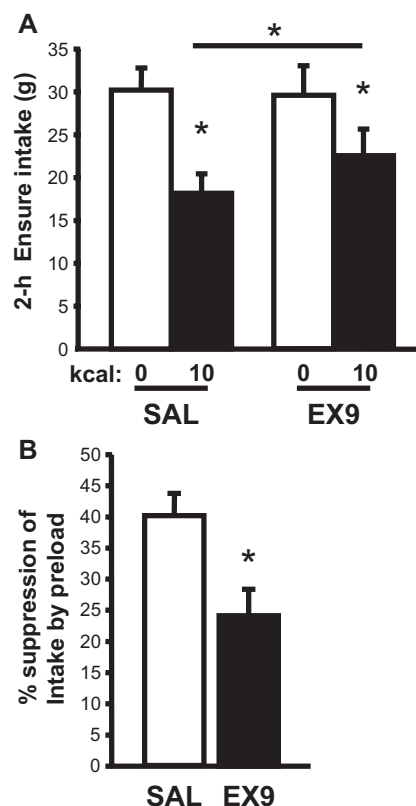


FIG. 3. Effect of GLP-1-R blockade on IG preload-induced satiety. A, Intake of a palatable liquid diet was measured after IG infusion of either saline (SAL) or glucose, followed immediately by ip injection of either saline or Ex9 (100 $\mu\text{g/kg}$). B, The percentage of intake suppression induced by the glucose preload shown in A differed after ip saline vs. ip Ex9. Data are mean $\pm$ SEM. *, $P < 0.05$.

was delivered 15 or 45 min before the central GLP-1 injection ($P < 0.05$) [main effect of GLP-1, $F(1,8) = 12.65$, $P < 0.01$; interaction with Ex9, $P = \text{NS}$] (Fig. 5B).

Ex4 reduced meal size in the rat

To determine whether anorexia mediated by GLP-1-R stimulation involves reductions of meal size (as expected for a satiety signal), meal frequency, or both, we monitored food intake continuously after ip injection of Ex4 (1 $\mu\text{g/kg}$) or saline vehicle immediately before the onset of the dark phase. Ex4 reduced food intake over the next 4 h [$t(5) = 7.56$, $P < 0.001$] (Fig. 6A), and during this time, average meal size was decreased by drug treatment [$t(5) = 3.11$, $P < 0.05$] (Fig. 6B), whereas the number of meals taken was not significantly different across conditions (Fig. 6C). A closer analysis of meal patterns shows that each of the first four meals taken after the onset of the dark phase was reduced in both size [meal $\times$ drug interaction, $F(6, 30) = 3.02$, $P < 0.05$, *post hoc* $P < 0.05$] (Fig. 6D) and duration [$t(5) = 2.09$, $P < 0.05$] (Fig. 6E) after Ex4 administration, whereas meals taken thereafter were similar in size across conditions. Moreover, the time required to complete the first four meals after Ex4 was increased by 50% compared with vehicle treatment [vehicle mean 2.36 ± 0.61 h vs. Ex4 mean 3.58 ± 0.56 h, $t(5) = -2.71$, $P < 0.05$], due largely to an Ex4-induced increase in the average time between those meals [62%, $t(5) = -2.47$, $P < 0.05$] (Fig. 6F).

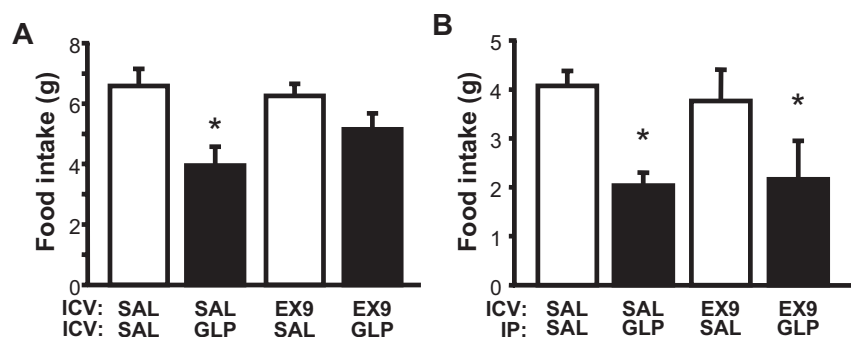


FIG. 4. Effect of central GLP-1-R blockade on central vs. peripheral GLP-1-induced anorexia. Food intake was measured in rats that were pretreated with a 3rd-icv injection of either saline (SAL) or Ex9 (20 µg) and then given either a 3rd-icv injection of saline or GLP-1 (3 µg) (A) or an ip injection of saline or GLP-1 (100 µg/kg) (B). Data are mean $\pm$ SEM. *, $P < 0.05$.

Discussion

The pharmacological effect of GLP-1-R agonists to reduce food intake is well established, but the question of whether GLP-1 plays a physiological role as a satiety signal has not been addressed by previous studies. In the current work, we provide strong, direct support for the conclusion that endogenous meal-related GLP-1 plays a physiological role in the control of food intake. First, we confirmed that food intake suppression induced by the GLP-1-R agonist Ex4 arises from a combination of reduced meal size and increased intermeal interval. Conversely, systemic blockade of GLP-1-R with Ex9 increases food intake in rats, an indication that endogenous GLP-1 signaling is a factor that limits intake under normal feeding conditions. Furthermore, systemic administration of Ex9 completely blocked satiety induced by an orally consumed sucrose preload. A second preload study used a strategy in which satiety is induced by an involuntary IG glucose infusion, which allowed us to include the control condition of a nonnutritive preload for comparison. This experiment confirmed the effect of systemic GLP-1-R blockade to attenuate nutrient preload-induced satiety, although the effect was less pronounced than when a sucrose preload was consumed voluntarily. Taken together, our results constitute direct evidence that endogenous GLP-1 released from the GI tract in response to meals plays a physiological role in satiety.

Although many peptide hormones can reduce food intake, most do not meet the criteria, first described by Gibbs (11) and

colleagues (2, 12), to be considered a physiologically relevant satiety signal. These criteria stipulate that the circulating level of a putative satiety signal must be increased by food intake, have a rapid onset and brief duration of action, and reduce meal size. Its anorexic effects must not be caused by nausea or malaise, and if the signal is thought to act humorally, it must reduce food intake when delivered at physiological doses. Finally, blockade of the signal must increase food intake. Although many gut peptides reduce food intake when delivered pharmacologically, cholecystikinin (CCK) remains the only molecule known to fulfill each of these criteria and to therefore be considered

a true satiety signal (2).

It is instructive to compare GLP-1 with CCK with respect to these criteria. It has long been clear that GLP-1 is secreted from intestinal L cells in response to food intake and that its effects, although rapid in onset, are short-lived in part because the active form of GLP-1 is rapidly degraded by the circulating protease dipeptidyl peptidase IV (1). CCK is also released rapidly upon food ingestion and has a short duration of action (13). Second, although high pharmacological doses of GLP-1-R agonists cause viscerosensory stress, evidenced by their ability to support conditioned taste aversion, like CCK, lower GLP-1 doses can effectively reduce food intake without nonspecific or aversive effects (14, 15). These observations suggest that at physiological levels, GLP-1's effects on feeding are not due to the sensation of gastrointestinal malaise. A third similarity between the actions of GLP-1 and CCK is evidence that GLP-1's effects on food intake are not humorally mediated but are instead mediated by the vagus nerve. As for CCK, either vagotomy or vagal sensory ablation by capsaicin blocks GLP-1-R stimulation-induced anorexia (16–18). The final criterion, that blockade of the signal must increase food intake, has been met for GLP-1 with the results of the studies described here. These findings parallel previous work in which the CCK-A receptor antagonist devazepide was shown to increase food intake on its own and also to blunt satiety induced by intraduodenal nutrient infusion, observations that were critical in the determination that CCK is indeed a physiologically relevant satiety signal (19–21).

These considerations buttress the proposal that GLP-1 can be added to CCK as the only gut peptides that are clearly documented to participate in satiety. Combined with previous evidence identifying GLP-1 as an endogenous regulator of meal-induced insulin secretion, our findings suggest a unique, physiological role for GLP-1 as both an incretin and a satiety signal.

The very short plasma half-life of GLP-1 (<2 min) is among several findings that support a local enteric action of endogenous, gut-derived GLP-1, rather than an action in the CNS. In addition, the GLP-1-albumin

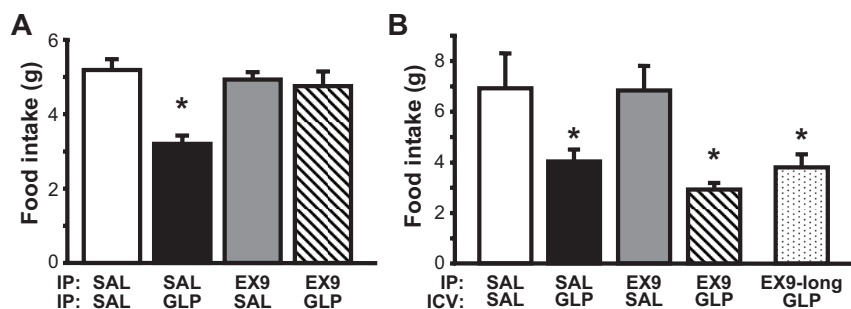


FIG. 5. Effect of peripheral GLP-1-R blockade on central vs. peripheral GLP-1-induced anorexia. Food intake was measured in rats that received an ip injection of either saline (SAL) or Ex9 (100 µg/kg) before either an ip injection of saline or GLP-1 (100 µg/kg) (A) or 3rd-icv injection of saline or GLP-1 (3 µg) (B). The icv GLP-1 injection was given either 15 or 45 min (Ex9-long) after ip injection. Data are mean $\pm$ SEM. *, $P < 0.05$.

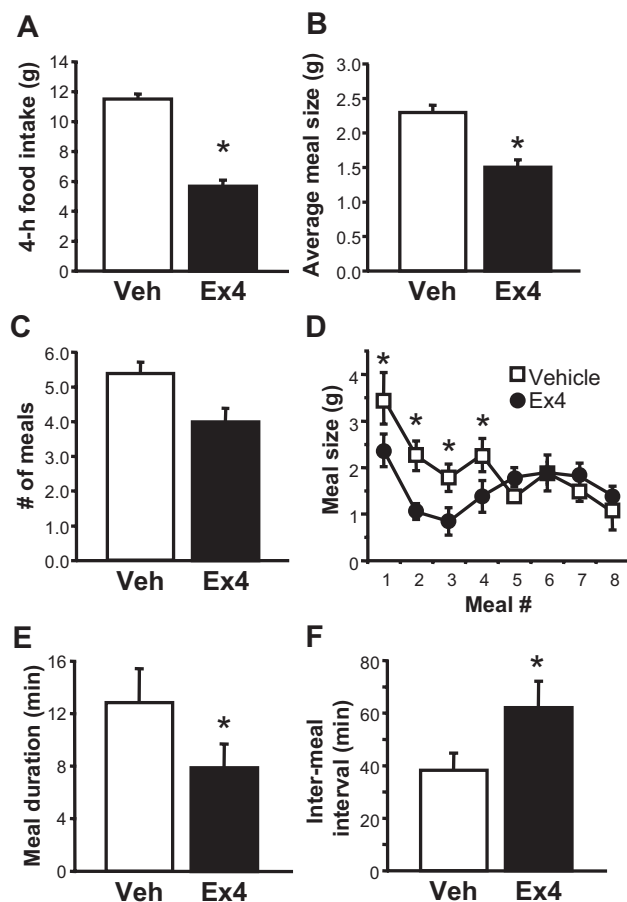


FIG. 6. Effect of Ex4 on meal pattern. Effect of ip injection of vehicle (Veh) or Ex4 at dark cycle onset on feeding parameters measured during the first 4 h of the dark phase, including food intake (A), average meal size (B), and number of meals (C). D, Effect of Ex4 on meal size varied over time, affecting both the duration (E) and intermeal interval (F) of the first four meals taken. Data are mean $\pm$ SEM. *, $P < 0.05$.

fusion protein Albugon, which does not cross the blood-brain barrier, reduces feeding when administered systemically (22), and vagal sensory ablation prevents peripherally administered GLP-1- or Ex4-induced anorexia in rodents (17, 18), findings that imply a role for vagal afferent fibers in its feeding effects. To investigate whether our findings with the GLP-1-R antagonist are the result of peripheral or central GLP-1-R blockade, we asked whether peripherally administered Ex9 blocks the anorexic effects of central as well as peripheral GLP-1-R stimulation. We found that ip injection of Ex9 increased spontaneous food intake and blocked the satiety effects of IG-infused or orally consumed nutrients and ip GLP-1, yet had no effect on anorexia induced by 3rd-icv GLP-1 administration. These observations are compatible with a peripheral site of Ex9 action in these studies. The converse is true as well; central GLP-1-R blockade was not sufficient to impair the response to peripheral GLP-1 treatment. Similarly, Barrera *et al.* (23) found that 3rd-icv delivery of the GLP-1-R antagonist des His1, Glu8 exendin-4 did not block the anorexic effects of peripheral GLP-1 treatment, and Hayes and Grill (24) have shown that caudal brain stem application of Ex9 does not reverse the inhibitory effects of IP Ex4 on either food intake or gastric emptying. Taken together,

these findings implicate a physiological role for peripheral endogenous GLP-1 and its receptors in the satiety response to ingested nutrients. This conclusion does not rule out the possibility that the CNS GLP-1 system also plays a role. Indeed, caudal brain stem GLP-1-R blockade impairs the satiety response to an orally consumed preload (25). Thus, activation of peripheral GLP-1-R may in turn increase GLP-1 signaling in the CNS (presumably involving activation of GLP-1 neurons in the nucleus of the solitary tract), and this sequence of events may contribute to satiety. This model resembles a mechanism proposed for the incretin effect of peripheral GLP-1-R stimulation and, therefore, warrants further investigation (26).

In the present study, we found that in freely feeding animals, peripherally administered Ex9 increased intake when delivered in the mid-light phase when baseline food intake is low but not when delivered immediately before the dark phase when spontaneous intake is high. Rather than being driven by factors related to circadian rhythm or ambient light, these differences in the feeding response to GLP-1-R blockade appear to be related to differences in recent food consumption and/or baseline spontaneous food intake at those times. This interpretation is based on our finding that Ex9 delivered 1 h after the onset of the dark phase, when animals have already taken their first large meal of the night and baseline intake is low, resulted in increased food intake. These findings provide additional support for the hypothesis that endogenous GLP-1 plays a more dominant role in satiety at times when food intake is low and is less important when animals are highly motivated to eat.

We also observed a seemingly greater effect of GLP-1-R blockade to attenuate preload-induced satiety when a preferred food was consumed voluntarily than when nutrients were infused into the stomach. Whereas it is tempting to speculate about the light that these findings cast on the physiological role of GLP-1 in satiety, differences in experimental design (*e.g.* glucose *vs.* sucrose preload, involuntary *vs.* voluntary consumption of the preload, test meal of Ensure *vs.* chow, test meal administered during light *vs.* dark phase) may also have contributed to the outcome. Thus, additional studies are warranted to clarify whether the role played by GLP-1 in satiety is influenced by the nutrient composition or caloric content of foods, the route or timing of ingestion, or other factors.

One limitation to the interpretation of our experiments with Ex9 is that GLP-1 is not the sole ligand for GLP-1-R. Oxyntomodulin (OXM), a peptide closely related to GLP-1 that is also released by the L cells of the distal intestine, also binds to GLP-1-R, albeit with reduced affinity (27). Like GLP-1, OXM is secreted in response to food intake and reduces feeding when delivered pharmacologically (28). Whether peripherally delivered OXM reduces food intake via peripheral GLP-1-R activation has not been determined, and available data implicate an as-yet-unidentified receptor in at least some OXM effects (29). Moreover, OXM binds and activates GLP-1-R with more than 50-fold weaker affinity (27) and more than 300-fold lower efficacy (30) compared with GLP-1. Although these estimates derive from studies in heterologous cell systems, these data suggest that plasma OXM levels, although typically greater than levels of GLP-1, are insufficiently high to explain the satiety action of GLP-1-R signaling. Lastly,

available evidence points to a central mechanism for OXM effects on feeding. For example, Dakin *et al.* (28) reported that peripheral OXM-induced anorexia was blunted by intrahypothalamic (arcuate nucleus) Ex9 pretreatment. Therefore, although we cannot exclude the possibility that some portion of the Ex9 effects that we observed is attributable to endogenous OXM action, we view this possibility as unlikely.

Our finding that Ex4 reduces meal size in rats is consistent with the findings that iv administration of GLP-1 reduces meal size in rats and that Ex4 reduces meal size in nonhuman primates (31, 32). In addition, we found the intermeal interval was also prolonged by Ex4 administration. Thus, pharmacologic GLP-1-R stimulation appears to induce satiety (the decision to terminate a meal) as well as promote satiety (the interval after a meal that precedes the emergence of hunger and the onset of the next meal). In this context, we note that our preload experiments using Ex9 also support a role for endogenous GLP-1 in satiety because the design of those studies examines preload-induced satiety more so than satiation. The preloads in those experiments took place before the test meal and so were not designed to enhance satiation during the test meal. Thus, the effect of GLP-1-R blockade in these experiments may be attributed to an effect on satiety. Although we acknowledge the limitations inherent in studies that measure pharmacological effects of GLP-1R ligands, we conclude that the relevant literature combined with our studies using both agonists and antagonists supports the hypothesis that GLP-1 plays a role in both satiety and satiation.

A priority for future research will be to clarify the mechanisms by which peripheral GLP-1-R mediates its effects on feeding behavior. GLP-1's incretin effects have been hypothesized to occur via a local action on afferent autonomic fibers in the gut and/or hepatic portal vein, as opposed to a direct action on β -cells (33). Although the present studies did not investigate the specific sites of action for GLP-1's satiety effects, our findings combined with available literature support a similar model. This analysis raises the intriguing possibility that both satiety and incretin effects of GLP-1 are mediated by a single common pathway involving autonomic communication between the gut and brain.

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