Original Article

Ghrelin Attenuates the Inhibitory Effects of Glucagon-Like Peptide-1 and Peptide YY(3-36) on Food Intake and Gastric Emptying in Rats

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Ghrelin stimulates, while glucagon-like peptide-1 (GLP-1) and peptide YY(3-36) [PYY(3-36)] inhibit, food intake and gastric emptying in rats. We determined the dose-dependent effects of a 3-h intravenous infusion of ghrelin at dark onset on food intake in freely feeding rats, and on the inhibitory effects of intravenous infusion of GLP-1 and PYY(3-36) on food intake and gastric emptying. Ghrelin (150 pmol · kg^{-1} · min^{-1}) stimulated food intake by 28% during the infusion period primarily by increasing meal frequency; doses of 15 and 50 pmol · kg^{-1} · min^{-1} had no effect. GLP-1 (15 pmol · kg^{-1} · min^{-1}) inhibited food intake by 35–54%; coinfusion of ghrelin at 50 and 150 pmol · kg^{-1} · min^{-1} attenuated this effect by 60 and 64%, respectively. PYY(3-36) (15 pmol · kg^{-1} · min^{-1}) inhibited food intake by 32%; ghrelin at 15 and 50 pmol · kg^{-1} · min^{-1} attenuated this effect by 54 and 74%, respectively. A 20-min intravenous infusion of ghrelin (15–150 pmol · kg^{-1} · min^{-1}) attenuated GLP-1– and PYY(3-36)-induced inhibition of gastric emptying of saline by 6–29%. Thus, intravenous infusion of ghrelin during the early dark period stimulates food intake in freely feeding rats by increasing meal frequency, and similar doses of ghrelin attenuate gastric emptying and feeding responses to GLP-1 and PYY(3-36). These results suggest that ghrelin may stimulate food intake in part by attenuating the inhibitory effects of GLP-1 and PYY(3-36) on gastric emptying and food intake.
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A growing number of gut-brain peptides have been shown to affect short-term food intake when administered acutely to experimental animals and humans. The 28–amino acid peptide ghrelin stimulates food intake, whereas many other peptides, including cholecystokinin (CCK), glucagon-like peptide-1(7-36) (GLP-1), peptide YY(3-36) [PYY(3-36)], and amylin inhibit food intake (1,2). The extent to which ghrelin interacts physiologically or pharmacologically with the other anorexigenic peptides to affect food intake and energy reserves remains to be determined.

Factors that promote gastric distention by inhibiting gastric emptying can reduce food intake. We have provided evidence that CCK, GLP-1, PYY(3-36), and amylin may inhibit food intake in part by inhibiting gastric emptying because each peptide dose-dependently reduces food intake and gastric emptying with similar potency and efficacy in rats (3–6). Ghrelin has been reported to stimulate both gastric emptying and food intake (2,7,8). If ghrelin increases food intake in part by accelerating gastric emptying, then it would be important to determine whether similar doses of ghrelin stimulate food intake and gastric emptying.

Bolus administration of ghrelin to rodents during the dark period, their active feeding period, has been reported to produce little if any effect on food intake (9–12). Our previous work with anorexigenic peptides suggests that intravenous infusion of these peptides in rats during the early dark period produces a more potent and reliable suppression of feeding than bolus administration (3–6). The effects of intravenous infusion of ghrelin under these conditions remain to be determined.

In the current study, we determined *1*) the dose-dependent effects of a 3-h intravenous infusion of ghrelin at dark onset on food intake and meal patterns in nondeprived rats, *2*) the effects of intravenous infusion of ghrelin under the same conditions on anorexigenic responses to intravenous infusions of GLP-1 and PYY(3-36), and *3*) the effects of a 20-min intravenous infusion of ghrelin on the inhibitory effects of intravenous infusions of GLP-1 and PYY(3-36) on gastric emptying of saline in food-deprived rats.

RESEARCH DESIGN AND METHODS

Male Sprague-Dawley rats (Charles River, Kingston, NY) weighing 340–551 g were housed individually in hanging wire-mesh cages in a room with a 12-h light-dark cycle (lights off at 1700). Animals were provided pelleted rat chow (Labdiet 5001 rodent diet; PMI Nutrition International, Saint Louis, MO) and water ad libitum except as noted in the experimental procedures. The institutional animal care and use committee of the Omaha Veterans Affairs Medical Center approved the experimental protocol.

Peptides. Rat GLP-1 and ghrelin were purchased from Bachem (Torrance, CA). Rat PYY(3-36) was synthesized by solid-phase methodology, using fluorenylmethoxycarbonyl protection strategy, and purified by reverse-phase high-performance liquid chromatography, as described elsewhere (13). Proof of structure was provided by electro-spray mass spectrometry.

Surgical procedures. Rats were surgically implanted with a jugular vein catheter for peptide infusions, as described previously (14). Some rats were also implanted with a stainless steel gastric cannula for instilling saline and retrieving gastric contents, as described previously (15). Venous catheters were plugged with stainless steel wire and kept patent by flushing with 0.2 ml

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CCK, cholecystokinin; GLP-1, glucagon-like peptide-1; PYY, peptide YY.

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of 50% dextrose on alternate days. Animals were allowed at least 1 week to recover from surgery before being subjected to experimental procedures.

Experiments

Dose-dependent effects of intravenous infusion of ghrelin on food intake. For each animal used in these experiments, the jugular vein catheter was connected to a 40-cm length of tubing that passed through a protective spring coil connected between a lightweight harness (IITC, Woodland Hills, CA) worn by the rat and an infusion swivel, which allowed free movement of rats in their home cage. Animals had ad libitum access to ground chow, which was provided fresh each day at 1400. Animals were adapted to experimental conditions for at least 1 week before the start of experiments. In an initial experiment, nondeprived rats ($n = 16$) received a 3-h intravenous infusion of either vehicle (0.15 mol/l NaCl, 0.1% BSA) or ghrelin at 5, 15, or 50 pmol · kg^{-1} · min^{-1}, beginning 15 min before dark onset (1700). Each rat randomly received each dose at intervals of at least 48 h. For each rat, cumulative hourly food intake for 17 h after dark onset was determined from continuous computer recordings of changes in food bowl weight, as described previously (14). Meal parameters (meal size and number of meals) were determined using a minimum intermeal interval criterion of 15 min and a minimum meal size criterion of 50 mg, as recommended by Zorrilla et al. (16). Peptides were administered via a syringe infusion pump (PHD2000; Harvard Apparatus, South Natick, MA); pumps were turned on and off by computer. At the end of the experiment, the patency of jugular vein catheters was determined by intravenous injection of 0.2 ml of the short-acting anesthetic propofol (Abbott Laboratories, North Chicago, IL). A catheter was considered patent if the rat lost consciousness immediately on injection of the anesthetic; only data from such propofol-positive rats were included in statistical analyses. In a separate experiment of identical design, we determined the effects of intravenous infusion of a higher dose of ghrelin (150 pmol · kg^{-1} · min^{-1}).

Effects of ghrelin on GLP-1–induced inhibition of food intake. Two experiments of similar design were performed to determine whether intravenous infusion of ghrelin attenuates the anorexia produced by intravenous infusion of GLP-1. In the first experiment, each rat ($n = 16$) randomly received, at intervals of at least 48 h, a 3-h intravenous infusion of vehicle, GLP-1 at 15 pmol · kg^{-1} · min^{-1}, and coadministration of GLP-1 with ghrelin at 15 or 50 pmol · kg^{-1} · min^{-1}. In a second experiment, rats received intravenous infusion of vehicle, GLP-1 at 15 pmol · kg^{-1} · min^{-1}, ghrelin at 150 pmol · kg^{-1} · min^{-1}, and coadministration of GLP-1 and ghrelin at these doses. We previously showed that a 3-h intravenous infusion of GLP-1 inhibits food intake in this rat model with a minimum effective dose of 0.5 pmol · kg^{-1} · min^{-1} and an estimated half-maximal effective dose (ED_{50}) of 23 pmol · kg^{-1} · min^{-1} (5). Hence, the GLP-1 dose (15 pmol · kg^{-1} · min^{-1}) used in this experiment is within the effective dose range.

Effects of ghrelin on PYY(3-36)-induced inhibition of food intake. A single experiment of similar design was performed to determine whether intravenous infusion of ghrelin attenuates the anorexia produced by intravenous infusion of PYY(3-36). Each rat ($n = 16$) randomly received, at intervals of at least 48 h, a 3-h intravenous infusion of vehicle, PYY(3-36) at 15 pmol · kg^{-1} · min^{-1}, and coadministration of PYY(3-36) with ghrelin at 15 or 50 pmol · kg^{-1} · min^{-1}. We previously showed that a 3-h intravenous infusion of PYY(3-36) in this rat model inhibits food intake with a minimum effective dose of 5 pmol · kg^{-1} · min^{-1} and an estimated ED_{50} of 15 pmol · kg^{-1} · min^{-1} (6). Hence, the PYY(3-36) dose (15 pmol · kg^{-1} · min^{-1}) used in this experiment is within the effective dose range.

Effects of ghrelin on GLP-1–induced inhibition of gastric emptying. Four experiments were performed as described previously (5) to determine the effects of a 20-min intravenous infusion of different dose combinations of GLP-1 and ghrelin on volume of saline emptied from the stomach in food-deprived rats. In the first experiment, each rat ($n = 12$) randomly received, at intervals of at least 48 h, a 20-min intravenous infusion of vehicle, GLP-1 (50 pmol · kg^{-1} · min^{-1}), ghrelin (15 pmol · kg^{-1} · min^{-1}), and coinfusion of GLP-1 and ghrelin at these doses. At 10 min after infusion onset, 5 ml of 0.9% saline containing phenol red was instilled into the stomach. Then, 10 min later, gastric contents were collected, the stomach was flushed with 5 ml of 0.9% saline, and the total volume of fluid recovered from the stomach was determined. Concentrations of phenol red in instilled saline and recovered fluid were determined spectrophotometrically to determine gastric emptying. During intervening days between doses, rats had free access to rat chow and liquid diet (1.5 kcal/ml Ensure Plus; Abbott Laboratories, Columbus, OH). In the next three experiments of identical design, rats received dose combinations of 50 pmol · kg^{-1} · min^{-1} each of GLP-1 and ghrelin, 50 and 150 pmol · kg^{-1} · min^{-1} of GLP-1 and ghrelin, respectively, and 17 and 50 pmol · kg^{-1} · min^{-1} of GLP-1 and ghrelin, respectively. At the end of each experiment, the patency of jugular vein catheters was determined by intravenous injection of propofol; only data from such propofol-positive rats were included in statistical analyses. GLP-1 doses used in these experiments were selected based on our previous study showing that GLP-1 inhibits gastric emptying with a minimum effective dose and an estimated ED_{50} of 5 and 19 pmol · kg^{-1} · min^{-1}, respectively (5).

Effects of ghrelin on PYY(3-36)-induced inhibition of gastric emptying. Four experiments of identical design were performed to determine the effects of a 20-min intravenous infusion of different dose combinations of PYY(3-36) and ghrelin on gastric emptying. In the first experiment, each rat ($n = 11$) received intravenous infusion of vehicle as well as PYY(3-36) and ghrelin at 50 and 15 pmol · kg^{-1} · min^{-1}, respectively, and coinfusion of PYY(3-36) and ghrelin at these doses. In the other three experiments, rats ($n = 11$–12) received dose combinations of 50 pmol · kg^{-1} · min^{-1} each of PYY(3-36) and ghrelin; 50 and 150 pmol · kg^{-1} · min^{-1} of PYY(3-36) and ghrelin, respectively; and 17 and 50 pmol · kg^{-1} · min^{-1} of PYY(3-36) and ghrelin, respectively. The PYY(3-36) doses used in these experiments were selected based on our previous study showing that PYY(3-36) inhibits gastric emptying in this rat model with a minimum effective dose and an estimated ED_{50} of 5 and 37 pmol · kg^{-1} · min^{-1}, respectively (13).

Statistical analyses. Values are the means ± SE. Data from the two experiments determining the dose-response effects of ghrelin on food intake were combined and analyzed by one-way repeated-measures ANOVA. Data from experiments on the effects of coadministration of lower doses of ghrelin (15 or 50 pmol · kg^{-1} · min^{-1}) with either GLP-1 or PYY(3-36) on food intake were analyzed by one-way repeated-measures ANOVA. Data from each of the experiments on the effects of each peptide alone, and in combination, on food intake and gastric emptying were analyzed by two-way repeated-measures ANOVA. Paired Student's t tests were used for planned comparisons of treatment means, and $P < 0.05$ was considered statistically significant.

The ability of ghrelin infusion at dark onset to affect food intake, meal size, and meal frequency in an animal may be dependent on baseline feeding patterns in that animal. The relationship between 3-h food intake, mean meal size, and number of meals during vehicle (baseline) infusion (X) and percent change in each of these parameters during ghrelin infusion (Y) was approximated with the exponential equation $Y = ae^{-bX}$, using nonlinear regression analysis to estimate parameters a and b. Similar analyses were performed to assess the ability of ghrelin infusion to affect feeding parameters when coinfused with GLP-1 or PYY(3-36).

RESULTS

Dose-dependent effects of intravenous infusion of ghrelin on food intake. A 3-h intravenous infusion of ghrelin at 5, 15, and 50 pmol · kg^{-1} · min^{-1} had no significant effect on food intake or meal parameters (Figs. 1 and 2). Ghrelin at 150 pmol · kg^{-1} · min^{-1} produced a robust and sustained increase in cumulative food intake with a maximum increase of 83% at 1 h decreasing to 14% by 5 h (Figs. 1 and 2*A*). Ghrelin increased food intake by reducing latency to the first meal (25.9 ± 6.3 vs. 42.7 ± 9.2 min, $P < 0.001$) and by increasing the number of meals during the 3-h infusion period by 52% (Fig. 2*B*).

Nonlinear regression analysis showed an inverse exponential relationship between ghrelin-induced increase in food intake during the 3-h infusion period and baseline intake when vehicle was infused in the same animals ($Y = 711\ e^{-0.58X}$, $r^2 = 0.38$, $n = 71$, $P < 0.0001$) (Fig. 3*A*). Similar inverse exponential relationships occurred between *1*) the ghrelin-induced increase in number of meals and the baseline number of meals ($Y = 519\ e^{-1.33X}$, $r^2 = 0.37$, $n = 71$, $P < 0.0001$) (Fig. 3*B*) and *2*) the ghrelin-induced increase in mean meal size and the baseline mean meal size ($Y = 187\ e^{-1.79X}$, $r^2 = 0.29$, $n = 71$, $P < 0.0001$) (Fig. 3*C*). Thus, ghrelin is more effective in stimulating food intake during the early dark period in rats that normally consume less food during this period. Furthermore, in rats that normally consume smaller meals, the ghrelin-induced increase in food intake appears to be caused in part by an increase in meal size, whereas in rats that normally consume fewer meals, the ghrelin-induced increase in food intake appears to be caused in part by an increase in meal frequency.

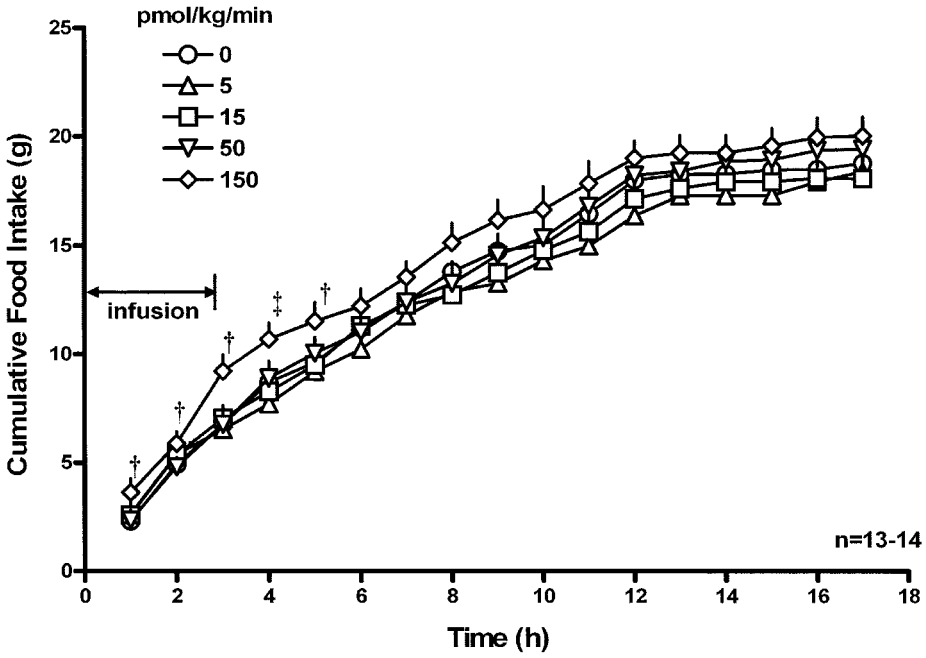

FIG. 1. Effects of intravenous infusion of ghrelin on cumulative food intake. Freely feeding rats received a 3-h intravenous infusion of different doses of ghrelin at dark onset. Data are the means ± SE. †$P < 0.01$, ‡$P < 0.001$ vs. 0 dose.

Effects of ghrelin on GLP-1–induced inhibition of food intake. In an initial experiment, 3-h intravenous infusion of GLP-1 at 15 pmol · kg^{-1} · min^{-1} decreased cumulative food intake during the infusion period by 35% (Fig. 4*A*). When ghrelin (15 pmol · kg^{-1} · min^{-1}) was coinfused with GLP-1 (15 pmol · kg^{-1} · min^{-1}), ghrelin had no effect on GLP-1–induced inhibition of food intake. In contrast, ghrelin coinfusion at a higher dose (50 pmol · kg^{-1} · min^{-1}) attenuated the GLP-1–induced suppression of feeding at 3 h by 60% (Fig. 4*A*). Meal pattern analysis revealed that GLP-1 reduced food intake during the infusion period by decreasing mean meal size by 30% (Table 1). Coinfusion of ghrelin (15 or 50 pmol · kg^{-1} · min^{-1}) did not attenuate this effect of GLP-1 on meal size, nor did ghrelin coinfusion increase the number of meals.

In the next experiment, 3-h intravenous infusion of GLP-1 at 15 pmol · kg^{-1} · min^{-1} decreased cumulative food intake during the infusion period by 54% (Fig. 4*B*). Ghrelin infusion alone at 150 pmol · kg^{-1} · min^{-1} increased cumulative food intake during the infusion period by 29%. When ghrelin and GLP-1 were administered together at these doses, ghrelin attenuated the GLP-1–induced inhibition of feeding at 3 h by 64%. Meal pattern analysis revealed that GLP-1 reduced food intake during the infusion period by decreasing mean meal size by 52% (Table 1). Ghrelin infusion alone had no effect on meal parameters during the infusion period. In contrast, when ghrelin and GLP-1 were coinfused, ghrelin attenuated the GLP-1–induced reduction in average meal size by 22% and increased the number of meals by 38% at 3 h.

Nonlinear regression analysis showed an inverse exponential relationship between the ghrelin-induced increase in food intake when coinfused with GLP-1 and food intake in the same animals when GLP-1 was given alone ($Y = 909\ e^{-0.88X}$, $r^2 = 0.66$, $n = 42$, $P < 0.0001$) (Fig. 5*A*). Similar inverse exponential relationships occurred between *1*) the ghrelin-induced increase in the number of meals when coinfused with GLP-1 and the number of meals in the same animals when GLP-1 was infused alone ($Y = 1{,}480\ e^{-2.11X}$, $r^2 = 0.69$, $n = 42$, $P < 0.0001$) (Fig. 5*B*) and *2*) the ghrelin-induced increase in mean meal size when coinfused with GLP-1 and the mean meal size in the same animals when GLP-1 was infused alone ($Y = 834\ e^{-2.17X}$, $r^2 = 0.59$, $n = 42$, $P < 0.0001$) (Fig. 5*C*). These data indicate that the ability of ghrelin to stimulate food intake, meal size, and meal frequency is dependent on the degree to which each of these meal parameters is respectively reduced by GLP-1 infusion.

Effects of ghrelin on PYY(3-36)-induced inhibition of food intake. A 3-h intravenous infusion of PYY(3-36) at 15 pmol · kg^{-1} · min^{-1} decreased cumulative food intake during the infusion period by 32% (Fig. 4*C*). When ghrelin was coinfused with this dose of PYY(3-36), ghrelin at 15 and 50 pmol · kg^{-1} · min^{-1} attenuated the PYY(3-36)-induced inhibition of food intake at 3 h by 54 and 74%, respectively. Meal pattern analysis revealed that PYY(3-36) and ghrelin at these doses had no reliable effect on either mean meal size or number of meals during the 3-h infusion period (Table 1).

Nonlinear regression analysis showed an inverse exponential relationship between the ghrelin-induced increase in food intake when coinfused with PYY(3-36) and food intake in the same animals when PYY(3-36) was given alone ($Y = 779\ e^{-0.72X}$, $r^2 = 0.84$, $n = 26$, $P < 0.0001$) (Fig. 5*D*). Similar inverse exponential relationships occurred between *1*) the ghrelin-induced increase in the number of meals when coinfused with PYY(3-36) and the number of meals in the same animals when PYY(3-36) was infused alone ($Y = 2{,}830\ e^{-2.65X}$, $r^2 = 0.76$, $n = 26$, $P < 0.0001$) (Fig. 5*E*) and *2*) the ghrelin-induced increase in mean meal size when coinfused with PYY(3-36) and mean meal size in the same animals when PYY(3-36) was infused alone ($Y = 250\ e^{-1.29X}$, $r^2 = 0.27$, $n = 26$, $P < 0.01$) (Fig. 5*F*). These data indicate that the ability of ghrelin to stimulate food intake, meal size, and meal frequency is dependent on the

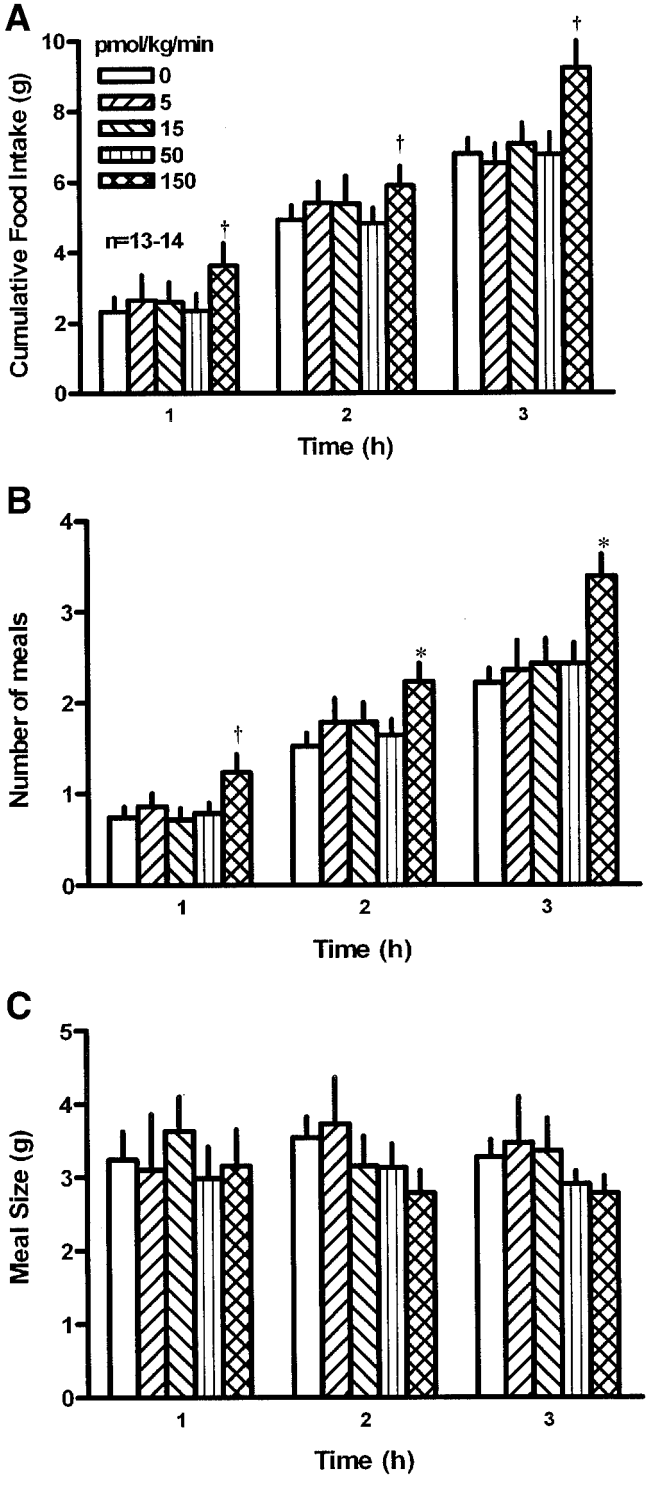

FIG. 2. Effects of intravenous infusion of ghrelin on food intake (*A*), number of meals (*B*), and mean meal size (*C*) during a 3-h infusion period. Data are from experiments described in Fig. 1. **P* < 0.05, †*P* < 0.01 vs. 0 dose.

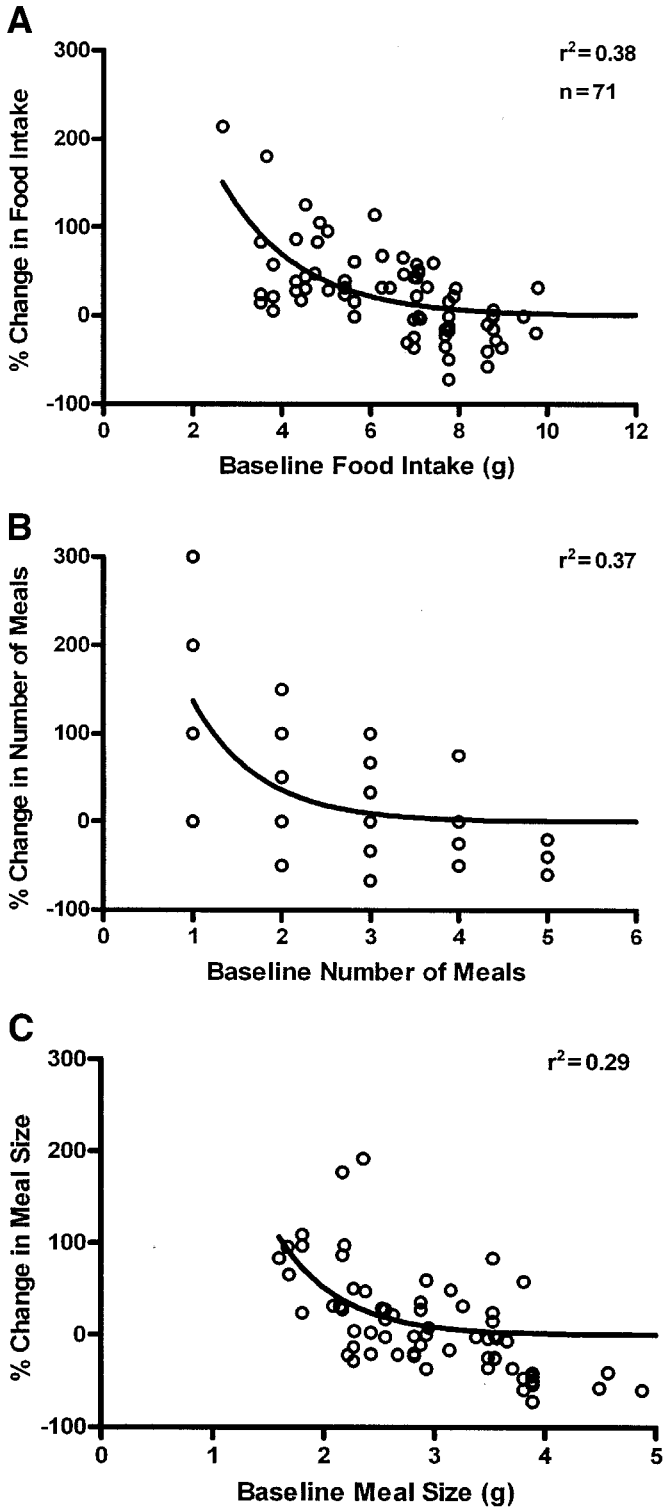

FIG. 3. Ghrelin-induced changes in meal parameters during the 3-h infusion period as a function of baseline meal parameters when vehicle was infused in the same animals. Data are from experiments described in Fig. 2. *A*: Percent change in food intake versus baseline food intake ($Y = 711\ e^{-0.58X}$, $r^2 = 0.38$, $n = 71$, $P < 0.0001$). *B*: Percent change in number of meals versus baseline number of meals ($Y = 519\ e^{-1.33X}$, $r^2 = 0.37$, $n = 71$, $P < 0.0001$). *C*: Percent change in mean meal size versus baseline mean meal size ($Y = 187\ e^{-1.79X}$, $r^2 = 0.29$, $n = 71$, $P < 0.0001$).

degree to which each of these meal parameters is respectively reduced by PYY(3-36) infusion.

Effects of ghrelin on GLP-1–induced inhibition of gastric emptying. In an initial experiment, a 20-min intravenous infusion of GLP-1 alone at 17 pmol · kg^{-1} · min^{-1} inhibited gastric emptying of saline by 53% (Fig. 6*A*). Intravenous infusion of ghrelin alone at 50 pmol · kg^{-1} · min^{-1} increased gastric emptying slightly by 14%. When given together at these doses, ghrelin attenuated GLP-1–induced inhibition of gastric emptying by 27%. In the next experiment, intravenous infusion of GLP-1 alone at 50

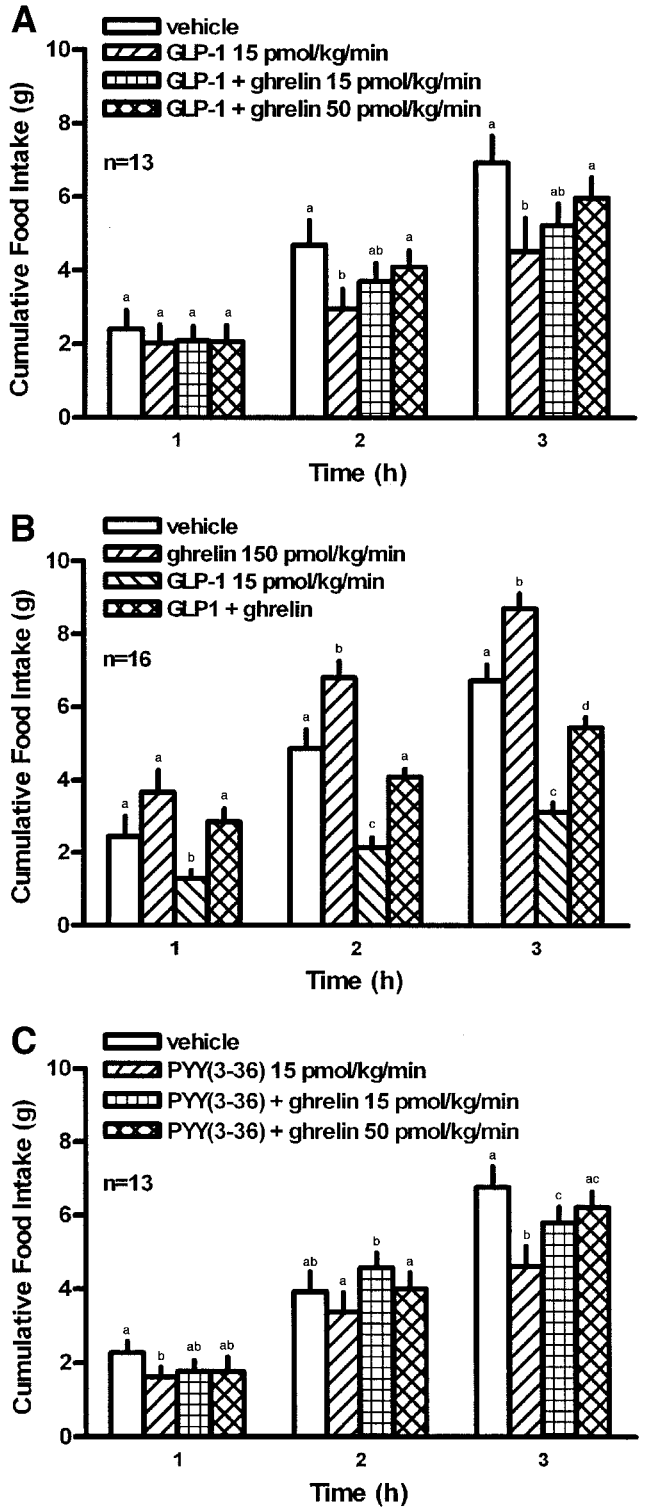

FIG. 4. Effects of a 3-h intravenous infusion of different dose combinations of ghrelin with either GLP-1 (*A* and *B*) or PYY(3-36) (*C*) on 3-h food intake. Values with different letters are different ($P < 0.05$).

TABLE 1
Effects of 3-h intravenous infusion of ghrelin with GLP-1 or PYY(3-36) on mean meal size and number of meals during the infusion period in 13–16 rats

Treatment	Meal size (g)	No. of meals
Experiment 1		
Vehicle	3.0 ± 0.3[a]	2.5 ± 0.3[a]
GLP-1 (15 pmol · kg^{-1} · min^{-1})	2.1 ± 0.4[b]	2.2 ± 0.3[a]
GLP-1 + ghrelin (15 pmol · kg^{-1} · min^{-1})	2.1 ± 0.3[b]	2.7 ± 0.3[a]
GLP-1 + ghrelin (50 pmol · kg^{-1} · min^{-1})	2.6 ± 0.3[b]	2.5 ± 0.3[a]
Experiment 2		
Vehicle	3.3 ± 0.5[a]	2.4 ± 0.2[a,b]
Ghrelin (150 pmol · kg^{-1} · min^{-1})	3.6 ± 0.3[a]	2.7 ± 0.2[b,c]
GLP-1 (15 pmol · kg^{-1} · min^{-1})	1.5 ± 0.1[b]	2.1 ± 0.2[a]
GLP-1 + ghrelin	1.9 ± 0.1[c]	2.9 ± 0.2[c]
Experiment 3		
Vehicle	2.5 ± 0.2[a]	2.8 ± 0.2[a]
PYY(3-36) (15 pmol · kg^{-1} · min^{-1})	2.1 ± 0.6[a]	2.7 ± 0.3[a]
PYY(3-36) + ghrelin (15 pmol · kg^{-1} · min^{-1})	2.1 ± 0.3[a]	3.1 ± 0.3[a]
PYY(3-36) + ghrelin (50 pmol · kg^{-1} · min^{-1})	2.2 ± 0.2[a]	3.0 ± 0.2[a]

Values with different letters within experiments are different ($P < 0.05$).

pmol · kg^{-1} · min^{-1} inhibited gastric emptying by 76% (Fig. 6*B*). Ghrelin infusion alone at 15 pmol · kg^{-1} · min^{-1} had no effect on gastric emptying. When given together at these doses, ghrelin slightly attenuated the GLP-1–induced inhibition of gastric emptying by 6%. In the next experiment, GLP-1 infusion alone at 50 pmol · kg^{-1} · min^{-1} inhibited gastric emptying by 56%, and ghrelin infusion alone at 50 pmol · kg^{-1} · min^{-1} increased gastric emptying by 14% (Fig. 6*C*). When given together at these doses, ghrelin attenuated the GLP-1–induced inhibition of gastric emptying by 13%. In the next experiment, GLP-1 infusion alone at 50 pmol · kg^{-1} · min^{-1} inhibited gastric emptying by 53%, and ghrelin infusion alone at 150 pmol · kg^{-1} · min^{-1} increased gastric emptying by 10% (Fig. 6*D*). When given together at these doses, ghrelin attenuated the GLP-1–induced inhibition of gastric emptying by 29%. Overall, these data show that intravenous infusion of ghrelin dose-dependently attenuated GLP-1–induced inhibition of gastric emptying.

Effects of ghrelin on PYY(3-36)-induced inhibition of gastric emptying. In an initial experiment, PYY(3-36) infusion alone at 17 pmol · kg^{-1} · min^{-1} inhibited gastric emptying by 41%, and ghrelin infusion alone at 50 pmol · kg^{-1} · min^{-1} had no significant effect on gastric emptying (Fig. 7*A*). When given together at these doses, ghrelin attenuated PYY(3-36)-induced inhibition of gastric emptying by 15%. In the next experiment, PYY(3-36) infusion alone at 50 pmol · kg^{-1} · min^{-1} inhibited gastric emptying by 48%, and ghrelin infusion alone at 15 pmol · kg^{-1} · min^{-1} had no effect on gastric emptying (Fig. 7*B*). When given together at these doses, ghrelin had no affect on the PYY(3-36)-induced inhibition of gastric emptying. In the next experiment, PYY(3-36) infusion at 50 pmol · kg^{-1} · min^{-1} alone inhibited gastric emptying by 48%, and ghrelin infusion alone at 50 pmol · kg^{-1} · min^{-1} had no effect on gastric emptying (Fig. 7*C*). When given together at these doses, ghrelin attenuated the PYY(3-36)-induced inhibition of gastric emptying by 21%. In the next experiment, PYY(3-36) infusion alone at 50 pmol · kg^{-1} · min^{-1} inhibited gastric emptying by 37%, and ghrelin infusion alone at 150 pmol ·

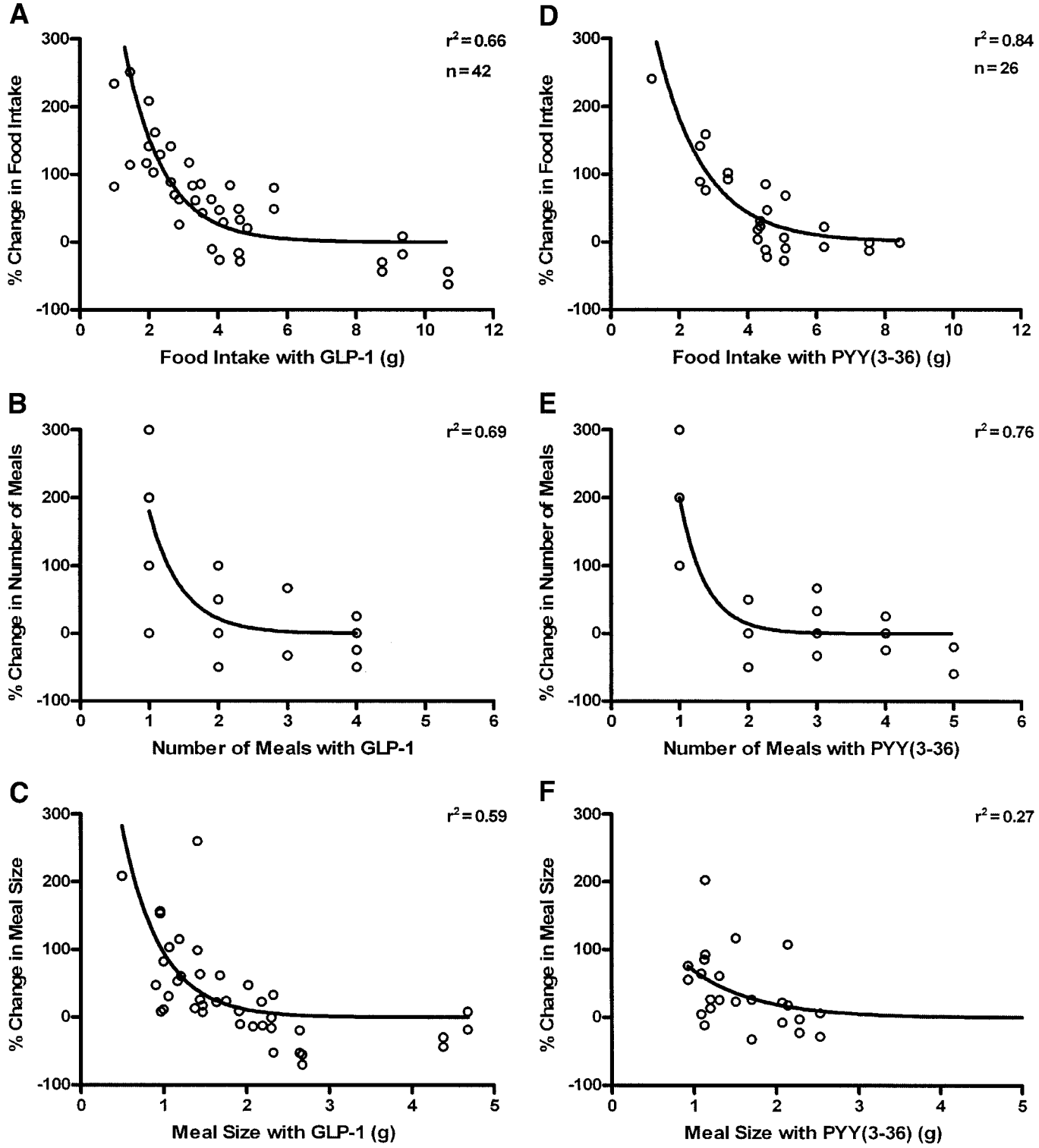

FIG. 5. Ghrelin-induced changes in meal parameters during the 3-h infusion period in animals coinfused with either GLP-1 or PYY(3-36). Data are from experiments described in Fig. 4 and Table 1. *A*: Percent change in food intake produced by ghrelin + GLP-1 vs. food intake with GLP-1 alone ($Y = 909^{-0.88X}$, $r^2 = 0.66$, $n = 42$, $P < 0.0001$). *B*: Percent change in number of meals produced by ghrelin + GLP-1 vs. number of meals produced with GLP-1 alone ($Y = 1{,}480\ e^{-2.11X}$, $r^2 = 0.69$, $n = 42$, $P < 0.0001$). *C*: Percent change in mean meal size produced by ghrelin + GLP-1 vs. mean meal size produced with GLP-1 alone ($Y = 834\ e^{-2.17X}$, $r^2 = 0.59$, $n = 42$, $P < 0.0001$). *D*: Percent change in food intake produced by ghrelin + PYY(3-36) vs. food intake with PYY(3-36) alone ($Y = 779\ e^{-0.72X}$, $r^2 = 0.84$, $n = 26$, $P < 0.0001$). *E*: Percent change in number of meals produced by ghrelin + PYY(3-36) vs. number of meals produced with PYY(3-36) alone ($Y = 2{,}830\ e^{-2.65X}$, $r^2 = 0.76$, $n = 26$, $P < 0.0001$). *F*: Percent change in mean meal size produced by ghrelin + PYY(3-36) vs. mean meal size produced with PYY(3-36) alone ($Y = 250\ e^{-1.29X}$, $r^2 = 0.27$, $n = 26$, $P < 0.01$).

$kg^{-1} \cdot min^{-1}$ had no effect on gastric emptying (Fig. 7*D*). When given together at these doses, ghrelin attenuated the PYY(3-36)-induced inhibition of gastric emptying by 24%. Overall, these data show that intravenous infusion of ghrelin dose-dependently attenuated PYY(3-36)-induced inhibition of gastric emptying.

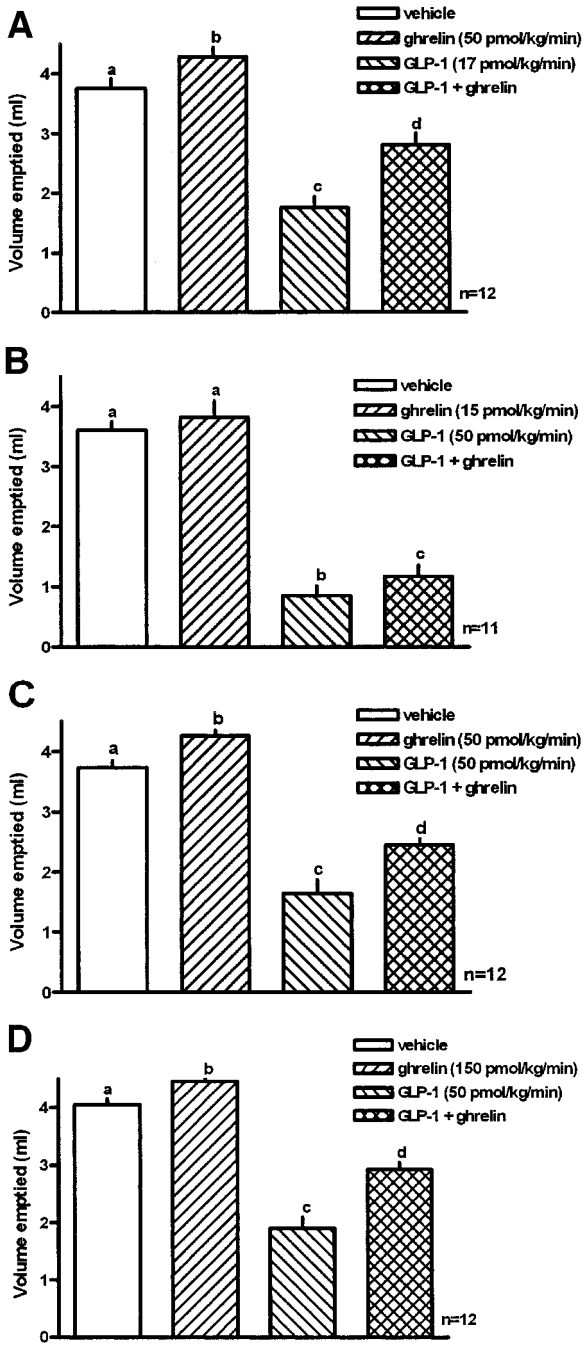

FIG. 6. Effects of intravenous infusion of different dose combinations of ghrelin and GLP-1 (*A–D*) on gastric emptying of saline. Peptides were infused intravenously for 20 min in rats that were food deprived for 18 h. At 10 min after infusion onset, 5 ml of saline containing phenol red was instilled intragastrically, followed by collection of gastric contents 10 min later. Values with different letters are different ($P < 0.05$).

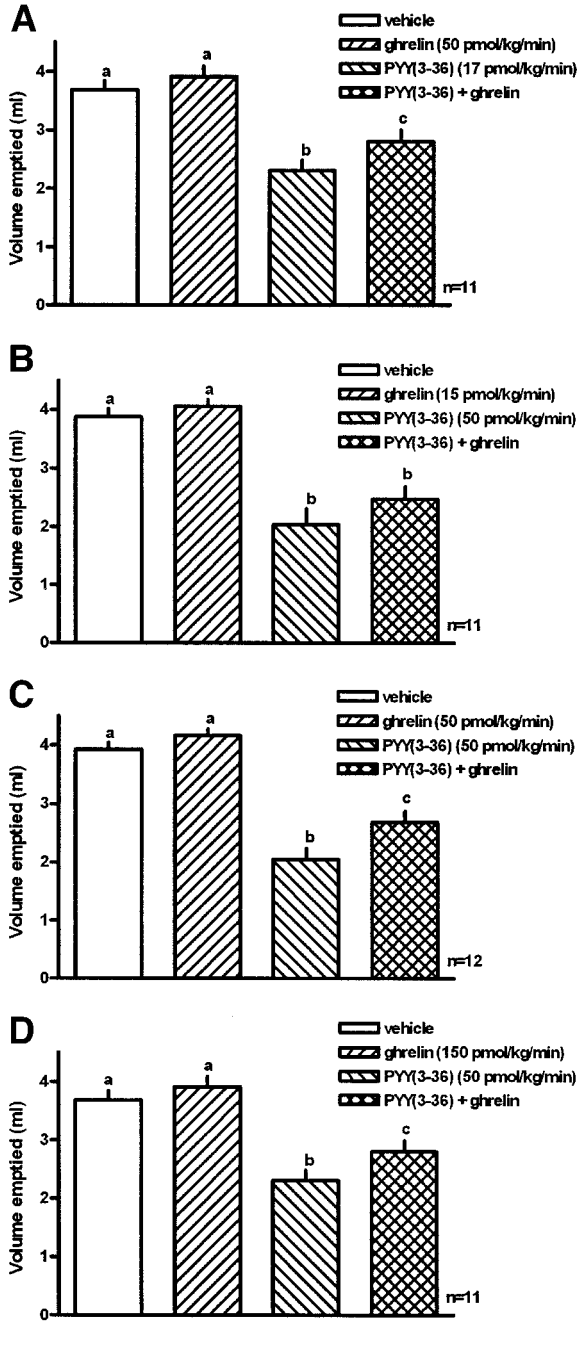

FIG. 7. Effects of intravenous infusion of different dose combinations of ghrelin and PYY(3-36) (*A–D*) on gastric emptying of saline. Experiments are as described in Fig. 5. Values with different letters are different ($P < 0.05$).

DISCUSSION

Our results demonstrate several important properties of the effects of ghrelin on food intake and gastric emptying. First, intravenous infusion of ghrelin during the early dark period in freely feeding rats produces a robust stimulation of food intake by primarily increasing meal frequency. Second, ghrelin dose-dependently attenuates anorexic responses to intravenous infusions of GLP-1 and PYY(3-36). Third, ghrelin dose-dependently attenuates the inhibitory effects of intravenous infusion of GLP-1 and PYY(3-36) on gastric emptying. Fourth, similar doses of ghrelin attenuate gastric emptying and feeding responses to GLP-1 and PYY(3-36). These results suggest that ghrelin may stimulate food intake in part by attenuating the inhibitory effects of GLP-1 and PYY(3-36) on gastric emptying and food intake.

Bolus administration of ghrelin to rodents during the dark period, their active feeding period, has been reported to produce little if any effect on food intake (9–12). Our previous work suggests that intravenous infusion of the anorexigenic peptides CCK-8, amylin, PYY(3-36), and GLP-1 during the early dark period produces a more potent and reliable suppression of feeding in rats than bolus administration (3–6). No previous study has exam-

ined the effects of intravenous infusion of ghrelin under these conditions. In the current study, a wide range of ghrelin doses (5, 15, 50, and 150 pmol · kg^{-1} · min^{-1}) was administered intravenously to freely feeding rats in their home cages during the first 3 h of the dark period, and only the highest dose (150 pmol · kg^{-1} · min^{-1}) significantly increased food intake. In this same experimental model, 3-h intravenous infusion of the various anorexigenic peptides suppresses feeding at much lower doses. The reason for this difference in potency between ghrelin and the anorexigenic peptides may be attributable to a "ceiling" effect that limits the extent to which food intake can be increased by ghrelin during the early dark period, when rats are actively eating. Indeed, in the current study, ghrelin-induced stimulation of food intake was related in an inverse exponential manner to baseline food intake. We also found that lower doses of ghrelin (15 and 50 pmol · kg^{-1} · min^{-1}) can significantly increase food intake when baseline intake is decreased by coinfusion of GLP-1 and PYY(3-36). It remains to be determined whether orexigenic intravenous doses of ghrelin are "physiological" with respect to their ability to reproduce the plasma levels of endogenous ghrelin that precede spontaneous meals.

Our results demonstrate that ghrelin dose-dependently attenuates anorexic responses to exogenous GLP-1 and PYY(3-36) and that exogenous GLP-1 attenuates the orexigenic response to ghrelin administration. Adams et al. (11) reported that intraperitoneal injection of PYY(3-36) during the early light period in mice attenuated the orexigenic response to intraperitoneal injection of ghrelin; however, the single dose of ghrelin that was tested in this study failed to attenuate anorexic responses to intraperitoneal injection of a range of doses of PYY(3-36). These discrepancies might be explained by differences in the method and timing of peptide administration (intraperitoneal versus intravenous, light versus dark cycle), number of ghrelin doses tested, or species studied. Nevertheless, the current study provides evidence that ghrelin can interact with GLP-1 and PYY(3-36) to produce additive, opposing effects on food intake. Whether the endogenous peptides interact in this manner to affect food intake and energy balance remains to be determined.

In the current study, ghrelin infusion during the dark period increased food intake in freely feeding rats primarily by reducing latency to the first meal and increasing meal frequency. Ghrelin produces similar effects on meal latency and frequency when administered by intracerebroventricular injection to rats during the early light period (17). These results are consistent with the hypothesis that ghrelin is a hunger signal involved in meal initiation (18,19). In support of this idea, plasma immunoreactive ghrelin has been reported to increase before meals in humans (18,19). However, recent evidence suggests that a premeal increase in plasma ghrelin may be a learned response to an anticipated meal rather than a hunger signal that initiates spontaneous meals (20). Our results also show that exogenous ghrelin can increase meal size as well as meal frequency in rats that normally eat smaller meals or that eat smaller meals in response to coinfusion of GLP-1 or PYY(3-36). Thus, a role for ghrelin as a hunger signal remains to be established.

Factors that promote gastric distention by inhibiting gastric emptying can reduce food intake, and, conversely, factors that reduce gastric distention by accelerating gastric emptying could potentially increase food intake. In the current study, intravenous infusion of ghrelin alone had no effect or only slightly increased gastric emptying of saline. This was likely attributable to the fact that saline emptying was already rapid and near complete in the baseline control condition, when vehicle was infused. In contrast, we were able to demonstrate a significant stimulatory effect of ghrelin on gastric emptying of saline when emptying was decreased by coinfusion of GLP-1 or PYY(3-36). These results suggest that ghrelin can interact with GLP-1 and PYY(3-36) to produce additive, opposing effects on gastric emptying. Whether the endogenous peptides interact in this manner to affect gastric emptying remains to be determined.

The ability of ghrelin administration alone to accelerate gastric emptying and increase food intake has been well documented (2,7,8). The current work extends these studies by showing that similar intravenous doses of ghrelin attenuate the inhibitory effects of GLP-1 and PYY(3-36) on gastric emptying and food intake. Taken together, these results suggest that ghrelin may stimulate food intake in part by attenuating the inhibitory effects of GLP-1 and PYY(3-36) on gastric emptying and food intake. There are several proposed mechanisms for the effects of each of these peptides alone on food intake and gastrointestinal functions, including endocrine, paracrine, and neurocrine mechanisms with sites of action at the level of gastrointestinal vagal sensory nerves and brain, including hypothalamus and brain stem. It remains to be determined how ghrelin interacts with GLP-1 and PYY(3-36) to affect food intake and gastric emptying.

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