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Central Administration of Ghrelin and Agouti-Related Protein (83–132) Increases Food Intake and Decreases Spontaneous Locomotor Activity in Rats

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Ghrelin was recently identified as an endogenous ligand of the GH secretagogue receptor. The novel peptide hormone is produced by gastric A-like cells, and circulating levels rise before feeding, suggestive of ghrelin as an endogenous hunger factor. ghrelin stimulates food intake and promotes adiposity after peripheral or central administration, likely by activating hypothalamic neurons expressing the orexigenic neuropeptides neuropeptide Y (NPY) and agouti-related protein (AGRP). To examine whether ghrelin-induced feeding resembles NPY and AGRP [AGRP fragment (83–132)] induced orexia, we compared the short- and long-term orexigenic capacity of the three peptides. A single intracerebroventricular injection of ghrelin (0.2, 1.0, and 5.0 μg) increased food intake in a dose-dependent manner. A prolonged and uncompensated increase in feeding was seen after the highest dose of ghrelin. The prolonged effects on feeding (+72 h) closely resembled those of AGRP (83–132) but not NPY. Surprisingly, ghrelin injections reduced overall locomotor activity by 20% during the first 24-h observation period. AGRP (83–132) had similar effects on locomotor behavior, whereas NPY had no effect. In summary, ghrelin causes long-term increases of food intake and, like AGRP, plays a previously unknown role as a suppressor of spontaneous physical activity. Expanding the current model of food intake control to include mechanisms regulating physical activity may promote our understanding of two major etiological factors causing obesity. (*Endocrinology* 145: 4645–4652, 2004)

INCREASED SEDENTARY BEHAVIOR and dramatic changes of dietary habits are thought to be the key environmental factors contributing to the dramatic worldwide rise in the number of obese individuals (1, 2). For the past decade, the focus of the scientific community has been on identifying pathways and signals involved in the regulation of food intake and body weight (3–5). A more recently discovered and particularly promising candidate is the peptide hormone ghrelin. Ghrelin is an acylated 28-amino residue peptide predominantly produced by the A-like cells in the stomach (6, 7). The main role of ghrelin was initially believed to be the stimulation of GH secretion via activation of the GH secretagogue receptor (GHS-R) in the hypothalamus and pituitary. However, it was recently demonstrated that ghrelin also induces a positive energy balance (7–9) via activation of the GHS-R in the hypothalamus. Exogenous administration of ghrelin increases food intake and oscillations in plasma levels of ghrelin in relation to meals suggest a possible role in meal initiation (10, 11). The current consensus of the scientific community is that the orexigenic effect of ghrelin mediated via stimulation of the GHS-R-expressing agouti-related protein (AGRP)/neuropeptide Y (NPY) neurones in the medial portion of the hypothalamic arcuate nucleus (Arc) (12–15). It is well documented that NPY causes an acute but transient increase in feeding, whereas AGRP causes prolonged increases in food intake (16, 17). Thus, we speculated that exogenous administration of ghrelin would result in both an acute as well as chronic effects on feeding. It has not been reported whether exogenous central administration of ghrelin causes alterations in locomotor behavior, and because previous results furthermore indicate that changes in food intake and resting energy expenditure after ghrelin administration cannot fully explain the gain of body fat observed (7), we decided to include this parameter in our experiments. To determine whether ghrelin-related effects on feeding resembled those of NPY and AGRP, we examined the effects of these three orexigenic peptides on food intake, meal patterns, and locomotor activity. In accordance with the literature, we used an AGRP fragment, AGRP (83–132) as a substitute for full-length AGRP.

Abbreviations: AGRP, Agouti-related protein; Arc, arcuate nucleus; CTA, conditioned taste aversion; GHS-R, GH secretagogue receptor; icv, intracerebroventricular; NPY, neuropeptide Y; SPA, spontaneous physical activity; SPD, Sprague Dawley rat.

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Materials and Methods

Animals and surgery

One hundred twenty-six male Sprague Dawley rats (SPDs) weighing between 250 and 300 g were housed under standard light cycle [12-h light phase, 12-h dark phase (lights on at 0400 h)] and at controlled ambient conditions (temperature 22 C; humidity 70%). The animals had

ad libitum access to fresh chow (standard diet 1324; Altromin, Chr. Petersen, Ringsted Denmark; changed every morning) and tap water. All experiments were conducted in accordance with the internationally accepted principles for the care and use of laboratory animals and approved by the Danish Ethical Committee for Animal Research. Under hypnorm/dormicum (1 ml/kg body weight; Merck, Whitehouse Station, NJ) anesthesia, all rats were stereotaxically implanted with a stainless steel 4-mm guide cannula (Holm Finmekanik A/S, Stenløse, Denmark) aimed at the lateral ventricle (1 mm posterior and 1.5 mm lateral, relative to bregma). After the surgical procedure, the animals were housed individually in custom-made cages (Ellegaard Systems A/S, Faaborg, Denmark), allowing precise continuous measurements of food and locomotor behavior. During a 7-d recovery period, the animals were handled daily to habituate them to the injection procedure. Eight rats were not included in experiments due to a poor recovery phase. At the end of the experiments, rats were killed using CO_2 followed by decapitation.

Peptides

Recombinant ghrelin was a generous gift from Dr. Mark Heiman (Endocrine Research Laboratories, Eli Lilly & Co., Indianapolis, IN) and was generated as published elsewhere (7). AGRP fragment (83–132) was a generous gift from Prof. Randy Seeley (University of Cincinnati; custom made by American Peptides, Sunnyvale, CA). NPY was purchased from Bachem AG (Bubbendorf, Switzerland). Vehicle and solution medium for the above-mentioned peptides consisted of 0.9% PBS containing 0.05% BSA (Sigma-Aldrich, St. Louis, MO).

Measurement of food intake and locomotor activity

Food intake data were collected using MANI FeedWin, an online computerized feeding system using digital weighing cells. Locomotor activity was registered using photocell beam breaks spanning the home cage. Activity was defined as more than two consecutive beam brakes. All data were analyzed using the FeedWin database software (Ellegaard Systems A/S). This system has been engineered and validated (by N.V. and M.T.-C. from Rheoscience and Soren Ellegaard from Ellegaard Systems A/S).

Experimental design: experiment 1

On the day of the experiment, 32 *ad libitum*-fed rats were randomly assigned to four groups (n = 8). Four hours into the light period, animals were injected intracerebroventricular (icv) with either vehicle or ghrelin in various doses (0.2, 1.0, and 5 μg, all in 5 μl vehicle). Food intake and spontaneous locomotor activity were measured every 5 min for 20 h after the injection. All animals were allowed at least a 5-d drug-free washout period followed by a novel randomization to one of the four groups mentioned above. The maximal number of injections with neuropeptide was limited to three injections.

Experimental design: experiment 2

For these experiments a separate group of 36 *ad libitum*-fed rats were used. On the experimental day, the *ad libitum*-fed rats were randomized in two groups. Four hours into the light period, animals were injected icv with either vehicle or ghrelin (5 μg in 5 μl vehicle). Food intake and activity were measured every 15 min for the next 72 h. Fresh water and chow was provided after 24 and 48 h.

Experimental design: experiment 3

For these experiments a separate group of 32 *ad libitum*-fed rats were used. On the experimental day, the *ad libitum*-fed rats were randomized in four groups. Four hours into the light period, animals were injected icv with vehicle, ghrelin, AGRP (83–132), or NPY (5 μg in 5 μl vehicle for all three compounds). Food intake and activity were measured every 5 min for the next 72 h. Fresh water and chow were provided after 24 and 48 h. After a 6-d washout period, the treatments were changed in a standard cross-over design. The experimental paradigm as described above was followed.

By use of software developed by Ellegaard Systems A/S, the data sampled in experiment 3 were used to assess differences in meal pattern after administration of each of the three neuropeptides. A meal was defined as food intake of 0.5 g or more within two 5-min periods. The termination criteria for a meal were three subsequent 5-min periods with a food intake less that 0.2 g. Meal duration equals the amount of 5-min periods the meal lasted. Meal size was defined as the quantity of food consumed during each meal. Intermeal interval (minutes) was the time between the end of one meal and the initiation of the following meal.

Experiment design: experiment 4

Conditioned taste aversion. A separate group of 18 naive rats were used in this experiment. One week before the experiments, animals were offered two drinking bottles with tap water to accustom them to the experimental procedure. On the day of the experiment, animals were randomized in three groups and either icv injected with vehicle or ghrelin (5 μg in 5 μl vehicle) or ip injected with LiCl (80 mg/kg in 0.15 M solution). After the randomization process, a standard two-bottle taste aversion assay was performed (for a detailed description see Ref. 18). Preference for tap water *vs.* saccharin solution was measured 72 and 96 h after conditioning.

Statistics

All values for food (grams), saccharine (milliliters), water (milliliters), and activity (beam brakes) are expressed as means ± SEM unless otherwise indicated. The data were analyzed at time points 2, 4, 8, 20, 24, 48, and 72 h. The data were compared by a one-way ANOVA with *post hoc* analysis using Fisher's protected least significant difference test using Stat-View software (Abacus Concepts Inc., Berkeley, CA). Values of $P < 0.05$ were considered significant.

Results

Experiment 1

Intracerbroventricular ghrelin dose-dependently increases food intake in rats. A single central administration of ghrelin increased food intake in male SPDs (Fig. 1A). The increase in food intake, compared with the vehicle-treated control group, was statistically significant for all doses used (0.2, 1.0, and 5.0 μg) from 2 to 4 h (*i.e.* at T = 4 h: 0.2 μg *vs.* 1.0 μg *vs.* 5.0 μg *vs.* vehicle; mean + SEM: 7.3 ± 0.9 g; 7.0 ± 0.7 g; 8.4 ± 0.9 g; 4.8 ± 1 g, respectively; see also Fig. 1, n = 8 each group, $P < 0.05$ for ghrelin *vs.* control), whereas no difference was found at these time points between the groups treated with various doses of ghrelin. Eight hours into the observation period, only the 1.0- and 5.0-μg dose groups differed significantly from the vehicle-treated group (Fig. 1A). At the end of the experimental period (T = 20 h), food intake in both groups receiving the highest doses of ghrelin icv (1.0 and 5.0 μg) differed significantly from food intake in the vehicle-treated group [vehicle *vs.* ghrelin (1.0 μg) *vs.* ghrelin (5.0 μg): mean + SEM: 25.8 ± 0.9 g *vs.* 28.5 ± 1.0 g *vs.* 29.6 ± 1.0 g *vs.* 24.0 ± 1.2 g, respectively, Fig. 1A, n = 8 each group]. There was no statistically significant difference in food intake between the 1.0 μg- and 5.0 μg-treated group.

Intracerebroventricular ghrelin dose-dependently decreases locomotor activity. In the experiment described above, we measured locomotor activity after central administration of ghrelin. A single dose of ghrelin decreased spontaneous locomotor activity as assessed by cumulative beam breaks over the entire 20-h observation period [(T, 20 h: vehicle *vs.* ghrelin, 0.2 μg *vs.* 1.0 μg *vs.* 5.0 μg; mean + SEM, 5313 ± 417 beam breaks *vs.* 4571 ± 287 beam breaks and 4570 ± 350 and. 4385 ± 243 beam breaks, respectively, n = 8 in each group,

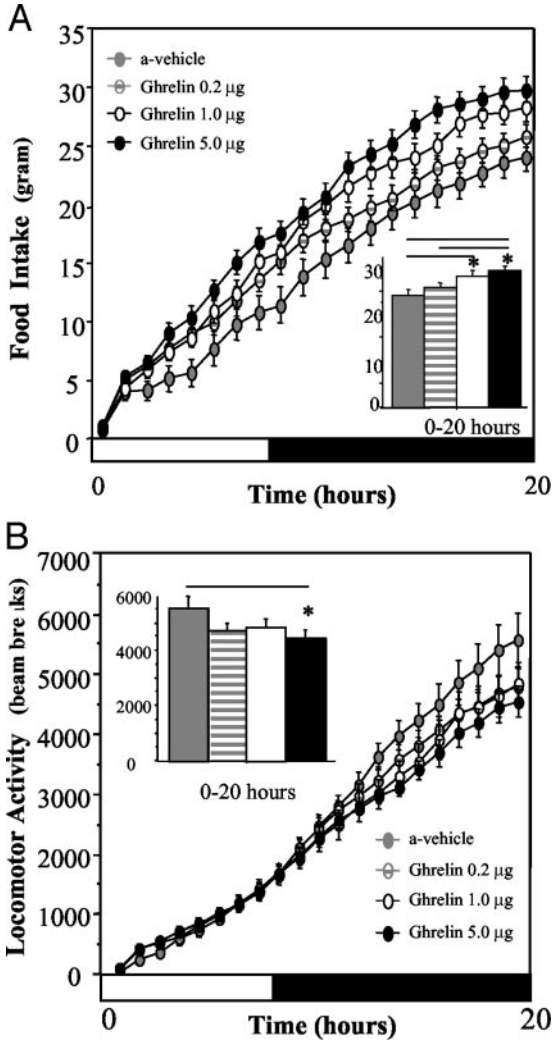

FIG. 1. Ghrelin has opposite effects on feeding and locomotor behavior. A, Central administration of ghrelin increases feeding in SPD rats. B, The increase in feeding is accompanied by a decrease in spontaneous locomotor activity. All data (grams or consecutive beam breaks) are expressed as mean + SEM, with n = 8–16. *, $P < 0.05$ *vs.* corresponding vehicle group as determined by ANOVA, followed by Fisher's *post hoc* analysis. Vehicle, *Gray circle*; 0.2 μg ghrelin, *striped circle*; 1.0 μg ghrelin, *white circle*; 5.0 μg ghrelin, *black circle*.

$P < 0.05$) (Fig. 1B)]. The decrease in locomotor activity was detectable until the end of the experiment in the two groups with the highest doses (1.0 and 5.0 μg), whereas the 0.2-μg ghrelin dose did not result in a decrease locomotor activity.

Experiment 2

A single icv injection of ghrelin causes an uncompensated increase in food intake. The long-term effects of a single dose of ghrelin (5 μg) on food intake were studied 24, 48, and 72 h after administration. As seen in experiment 1, there was a robust increase in food intake after the first 24-h period (T: 24 h, mean + SEM; vehicle *vs.* ghrelin 5.0 μg: 24.0 ± 1.6 g *vs.* 31.2 ± 1.0 g, $P < 0.05$, n = 13 each group, Fig. 2, A and B). During the second 24-h period (24–48 h after peptide administration), there was a tendency to increased feeding in the ghrelin-treated animals. During the third 24-h period, we

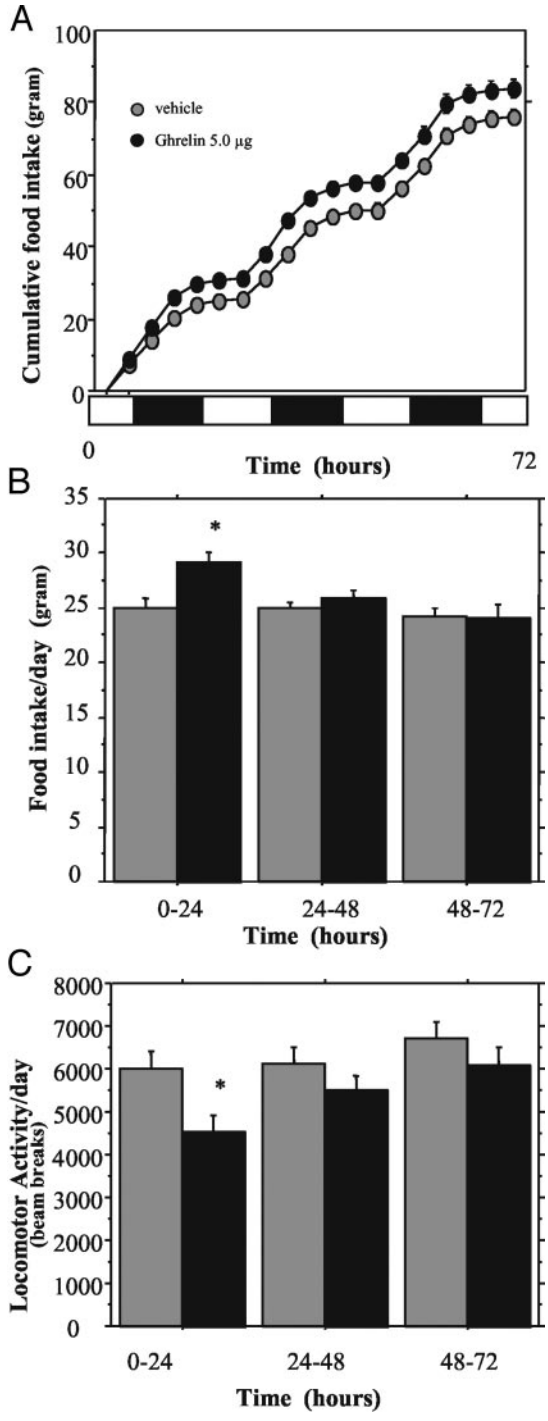

FIG. 2. Central administration of ghrelin causes prolonged changes in feeding and locomotor behavior. A, Central administration of ghrelin (5.0 μg, *black circles*) causes an acute robust increase in feeding when compared with vehicle (*gray circles*). The increase in feeding is uncompensated for the following two 24-h periods. B, The orexigenic effect of ghrelin (*black bar*) on feeding is most pronounced within the first 24-h period (0–24 h), whereas there is a tendency to increased feeding in the second 24-h interval (24–48) when compared with vehicle (*white bars*). C, The increased feeding after ghrelin (*black bars*) administration is accompanied by a decrease in activity when compared with vehicle (*white* bars). All data (grams or consecutive beam breaks) are expressed as mean + SEM, with n = 8–16. *, $P < 0.05$ *vs.* corresponding vehicle group as determined by ANOVA, followed by Fisher's *post hoc* analysis.

found no difference in food intake between vehicle and ghrelin-treated animals. When analyzing cumulative changes in food intake, we found an increased cumulative food intake after 48 as well as after 72 h after the single icv injection of ghrelin (T: 72 h, vehicle *vs.* ghrelin; mean ± SEM, 74.8 ± 2.2 g *vs.* 83.9 ± 1.3 g, n = 13 in both groups, $P < 0.05$, Fig. 2B).

A single injection of icv ghrelin decreases spontaneous locomotor activity. A single dose of ghrelin decreased 24-h activity [T, 24-h: vehicle *vs.* ghrelin (5 μg), mean ± SEM, 5986 ± 446 beam breaks *vs.* 4754 ± 398 beam breaks; n = 7–8 in both groups, Fig. 2C]. In the two following 24-h periods, ghrelin-treated animals did not show any decreased spontaneous locomotor activity.

Experiment 3

The long-term effects of icv ghrelin on feeding resembles that of icv AGRP (83–132). The ghrelin receptor-positive neurones within the Arc also express the orexigenic neuropeptides NPY and AGRP. Given that the three compounds work on different receptor systems and with different efficacy, we chose to administer equal doses (micrograms) of ghrelin, AGRP (83–132), and NPY icv and examine whether the feeding pattern resembled each other. All three neuropeptides had a marked effect on feeding (Fig. 3A). The neuropeptide with the fastest (after the injection) effect on acute feeding behavior was NPY, which increased feeding as early as 15 min after access to food. The orexigenic effect lasted for the

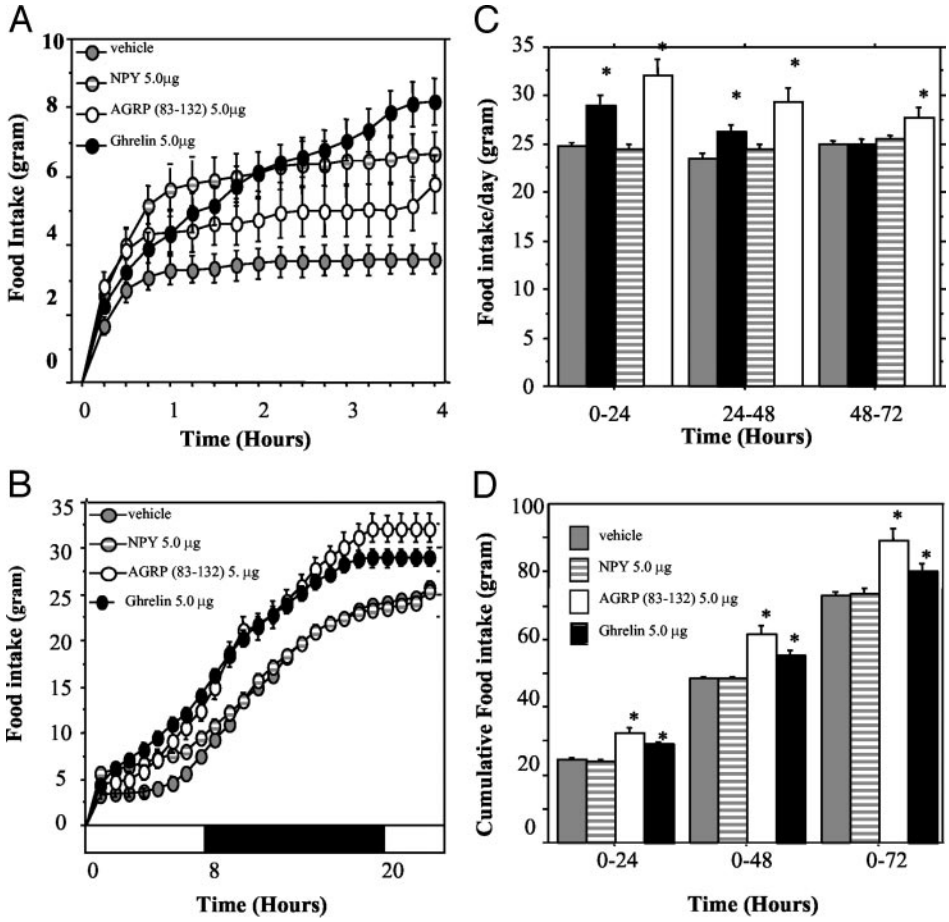

FIG. 3. The feeding and locomotor pattern after a single central dose of ghrelin resembles the effect of both NPY and AGRP (83–132). A, The orexigenic response to ghrelin resembles that of NPY during the first early phase after icv administration, whereas AGRP (83–132) has a slightly slower increase in feeding during the first 4 h when compared with vehicle. B, The orexigenic effect of NPY is transient and returns to the trajectory of the food intake curve of vehicle-treated animals. In contrast, the orexigenic effect of both AGRP (83–132) and ghrelin is visible throughout the whole 24-h observation period. B and C, The orexigenic effect of ghrelin on feeding is most pronounced within the first 24-h period (0–24 h), but there is also a significant increase during the second 24-h interval (24–48 h). AGRP (83–132) also has the strongest effect during the first 24-h interval, but food intake is increased in all three consecutive observation intervals. In contrast, food intake after a single injection of NPY was similar to that of vehicle. D, Cumulative food intake after a single dose of ghrelin, AGRP (83–132), NPY, or vehicle. A single dose of ghrelin or AGRP (83–132) results in a robust noncompensated increase in food intake throughout the observation period. E, The 24-h activity measurements demonstrated that a single dose of ghrelin reduces overall activity by approximately 20% when compared with vehicle. In contrast, NPY does not inhibit SPA, whereas the effect of AGRP (83–132) is similar to that of ghrelin. F, In the following 24-h observation period, only AGRP (83–132) significantly decreased activity when compared with vehicle. No effect was seen after NPY administration. G, Cumulative locomotor activity monitored for 72 h demonstrates a prolonged and uncompensated decrease in activity after ghrelin and AGRP (83–132) when compared with vehicle. No effect on locomotor activity was seen after NPY administration. All data (grams or consecutive beam breaks) are expressed as mean + SEM, with n = 8–12. *, $P < 0.05$ *vs.* corresponding vehicle group as determined by ANOVA, followed by Fisher's *post hoc* analysis. Vehicle, *Gray circle or bars*; ghrelin, *black circle or bars*; NPY, *striped circle or bars*; AGRP (83–132), *white circle or bars*.

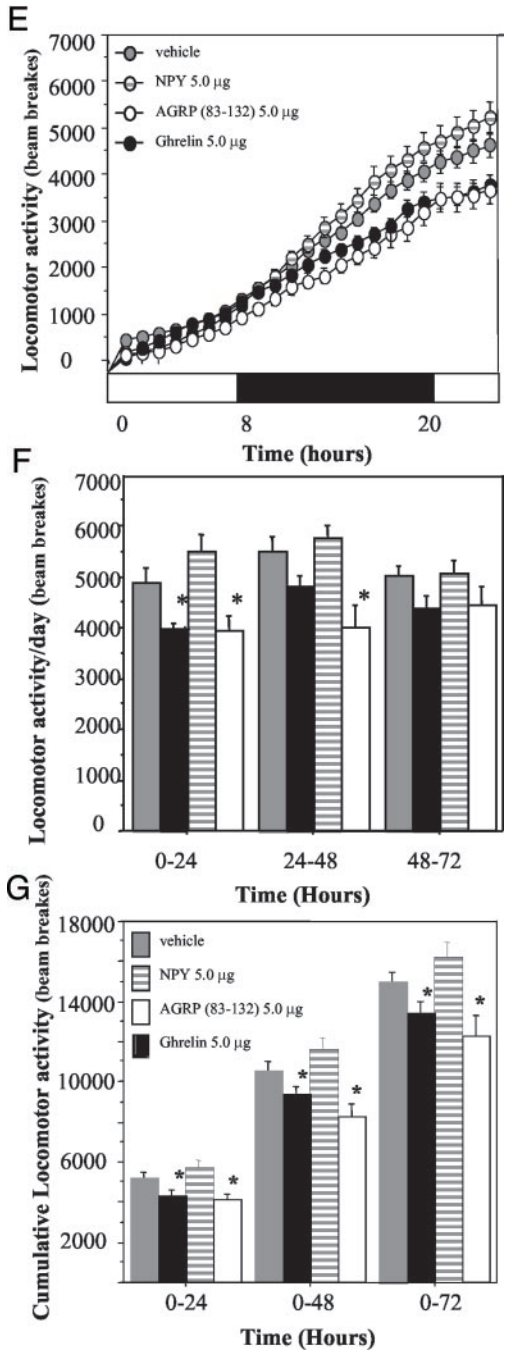

FIG. 3. Continued.

first 8 h, but at the end of the first 24-h period, no difference in cumulative food intake between vehicle- and NPY-treated animals was detectable (T24: vehicle *vs.* NPY 5.0; mean + SEM: 24.7 ± 0.4 g *vs.* 24.4 ± 0.6 g, n = 18–22 in each group, $P < 0.05$) (Fig 3, A and B). The orexigenic response of ghrelin was almost as rapid as NPY, whereas AGRP (83–132) was not and as such did not reach statistical significance before 2 h after injection when compared with vehicle (Fig. 3, A and B). The increase in food intake for both the ghrelin- and AGRP (83–132)-treated rats was statistically significant within the first 24-h observation period [T0–24 vehicle *vs.* ghrelin 5.0 *vs.* AGRP (83–132) 5.0; mean + SEM: 24.7 ± 0.4 g *vs.* 29.9 ± 0.9 g *vs.* 32.0 ± 1.5 g, n = 11–25 in each group, $P < 0.05$] (Fig. 3, A and B). In contrast to experiment 1, animals in the ghrelin-treated group also had an increased food intake in the second 24-h period; this was also the case for AGRP (83–132) (T24–48: vehicle *vs.* ghrelin 5.0 *vs.* AGRP (83–132) 5.0; mean ± SEM: 23.5 ± 0.5 g *vs.* 26.2 ± 0.7 g *vs.* 29.2 ± 1.4 g, n = 11–25 in each group, $P < 0.05$) (Fig. 3C). As previously reported (17), AGRP (83–132) also had an orexigenic effect within the third 24-h period (T48–72: vehicle *vs.* AGRP (83–132) 5.0; mean ± SEM: 25.1 ± 0.4 g *vs.* 27.7 ± 1.1 g, n = 11–25 in each group, $P < 0.05$). Cumulative food intake was significantly increased after the 72 h for both ghrelin and AGRP (83–132) (T0–72: vehicle *vs.* ghrelin 5.0 and AGRP (83–132) 5.0; mean ± SEM: 73.3 ± 0.6 g *vs.* 80.2 ± 0.7 g and 88.9 ± 1.4 g, n = 11–25 in each group, $P < 0.05$) (Fig. 3D).

The effect of icv ghrelin treatment on locomotor behavior resembles the action of AGRP (83–132). As in experiments 1 and 2, feeding and activity are measured simultaneously in the tested animals. As seen in Fig. 3E, the observed robust reduction in ambulatory movement of ghrelin-treated animals was similar to our prior experiments (experiment 2). The curve for locomotor activity of ghrelin-treated animals differed from the trajectory of the curve of vehicle-injected controls in the beginning of the dark phase (Fig. 3E) and stayed significantly lower for the first 24-h period (T0–24 vehicle *vs.* ghrelin 5.0; mean ± SEM: 4904 ± 255 beam breaks *vs.* 3962 ± 125 beam breaks, n = 7–14 in each group, $P < 0.05$) (Fig. 3E). The effect of AGRP (83–132) on locomotor activity closely resembled the effect observed in the ghrelin-treated animals, and as such we observed a pronounced decrease in locomotor behavior in both the first and the second 24-h observation period [T24–48 vehicle *vs.* AGRP (83–132) 5.0; mean ± SEM: 5505 ± 304 beam breaks *vs.* 4002 ± 450 beam breaks, n = 8–14 in each group, $P < 0.05$, Fig. 3, E and F]. In contrast, we found no acute or prolonged effect of NPY on locomotor behavior when compared with vehicle (Fig. 3, E–G). Cumulative spontaneous locomotor activity was significantly decreased for more than 72 h after both ghrelin and AGRP (83–132) administration [T0–72: vehicle *vs.* ghrelin 5.0 and AGRP (83–132) 5.0; mean ± SEM: 15447 ± 523 beam breaks *vs.* 13144 ± 503 beam breaks and 12383 ± 993 beam breaks, n = 11–25 in each group, $P < 0.05$] (Fig. 3G).

Meal pattern during the first 24-h period. Within the first 24-h period, none of the analyzed meal pattern parameters differed between the NPY and vehicle-injected group. Ghrelin and AGRP (83–132) increased 24-h food intake. For ghrelin the increase in total intake was not due to increased meal size but primarily caused by an increased number of meals (T0–24; vehicle *vs.* ghrelin, mean ± SEM, 9.3 ± 0.6 meals *vs.* 10.1 ± 0.6 meals, n = 20 and 11 for vehicle and ghrelin, respectively, $P = 0.08$, Table 1). In contrast, the increase in 24-h food intake after AGRP (83–132) could be ascribed to an increase in meal size [T0–24 total intake; vehicle *vs.* AGRP (83–132), mean ± SEM, 2.8 ± 0.2 g *vs.* 3.7 ± 0.3 g, n = 20 and 8 for AGRP (83–132) vehicle and AGRP (83–132), respectively, $P < 0.05$].

TABLE 1. Twenty-four-hour meal pattern analysis

	Meals	Mean meal size (g)	Total intake (g)	Intermeal interval (min)	Meal duration (min)
Vehicle	9.2 ± 0.5	2.8 ± 0.2	24.1 ± 0.7	103.5 ± 7.6	27.6 ± 1.8
NPY (5 μg)	9.3 ± 0.6	2.8 ± 0.2	23.8 ± 0.6	99.1 ± 5.0	26.4 ± 2.6
Ghrelin (5 μg)	10.1 ± 0.6[b]	3.0 ± 0.2	28.9 ± 1.1[a]	73.8 ± 2.8[a]	26.2 ± 1.8
AGRP (5 μg)	8.5 ± 0.8	3.7 ± 0.3[a]	30.5 ± 2.1[a]	113.0 ± 16.5	30.0 ± 3.1

Ghrelin and AGRP (83–132) both increased 24-h food intake when compared with vehicle. For ghrelin, the increase in total intake was primarily caused by an increased number of meals, whereas the increased food intake following AGRP (83–132) could be ascribed to an increase in meal size. No changes were seen after NPY administration. All data are presented as mean + SEM, with n = 6–8 for all groups.

[a] $P < 0.05$ *vs.* corresponding vehicle group as determined by analysis of variance, followed by Fisher's *post hoc* analysis.

Experiment 4

Intracerebroventricular ghrelin administration does not result in the formation of a conditioned taste aversion. To investigate whether part of the hyperphagic and/or sedative effect following central administration of ghrelin could be due to toxic or nonspecific actions of ghrelin, a taste aversion experiment was performed. As seen in Fig. 4, ghrelin was unable to produce a conditioned taste aversion (CTA), whereas the positive control, LiCl, elicited a robust taste aversion. Ghrelin did not cause taste aversion.

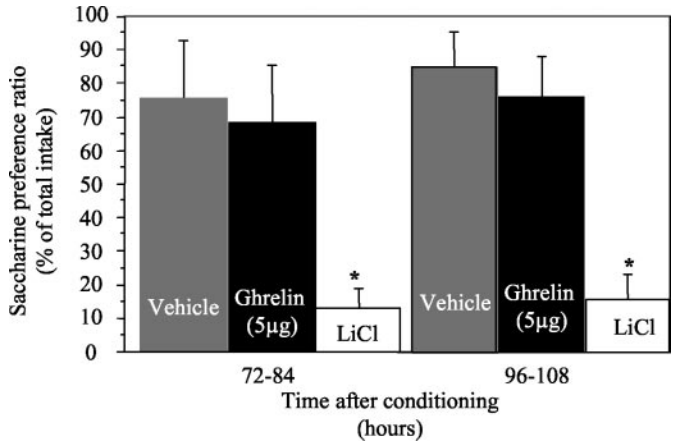

FIG. 4. Ghrelin does not trigger CTA in a two-bottle CTA paradigm. Central administration of 5.0 μg ghrelin (*black bars*) or vehicle (*white bars*) does not cause CTA 72 or 96 h after conditioning. As a positive control, ip injection of 80 mg/kg LiCl (*gray bars*) resulted in a robust CTA at 72 and 96 h. All data [saccharin preference ratio = (saccharin intake, milliliters)/total fluid intake] are expressed as mean + SEM, with n = 6–8 for all groups. *, $P < 0.05$ *vs.* corresponding vehicle group as determined by ANOVA, followed by Fisher's *post hoc* analysis.

Discussion

Obesity represents one of the most urgent global health threats as well as one of the leading causes of death throughout industrialized nations (19). Efficacious and safe therapies remain at large. Attempts to decrease fat mass via pharmacological reduction of caloric intake have had limited potency or intolerable side effects. Increasingly widespread sedentary lifestyle is often cited as a major contributor to the increasing prevalence of obesity (20). Moreover, low levels of spontaneous physical activity (SPA) are a major predictor of fat mass accumulation during overfeeding in humans, pointing to a substantial role for SPA in the control of energy balance. Despite this, very little is known about the molecular mechanisms by which SPA is regulated.

We show here for the first time that central effects of ghrelin on energy balance include a sustained increase in food intake and a sustained decrease in spontaneous locomotor activity. The effect on feeding remains uncompensated for at least 72 h after injection. Furthermore, we have demonstrated a novel inhibitory effect of AGRP (83–132) on spontaneous locomotor behavior.

The orexigenic effect of ghrelin is most apparent within the initial 8 h (injected 4 h into the light phase) but remains elevated by the end of the first 24-h period. Interestingly, there was no sign of a compensatory decrease in food intake during the following two 24-h feeding periods. Our data confirm and extend previous studies of ghrelin induced feeding (7–9, 21–23) because our new data show that ghrelin not only causes an acute increase in feeding but also results in a prolonged, uncompensated increase in food intake. Furthermore, we extended earlier findings on orexigenic effects after central administration of NPY and AGRP (83–132) (16, 17, 24), showing that NPY causes a robust and acute, but transient, increase in feeding, whereas AGRP (83–132) administration results in a less acute but prolonged and uncompensated increase in feeding.

The current consensus of the scientific community is that the orexigenic effect of ghrelin is mediated via an activation of AGRP/NPY neurones located in the medial portion of the Arc (15, 25). Functional anatomical and *in vitro* studies have documented that administration of ghrelin and ghrelin mimetics induces c-Fos in NPY/AGRP positive neurones expressing the GHS-R (14, 26–28), subchronic treatment with ghrelin or ghrelin mimetics up-regulates NPY as well as AGRP mRNA in the Arc (29), and ghrelin applied to hypothalamic slices selectively activates NPY/AGRP neurons (21). Most importantly, ghrelin-induced effects on energy balance do not occur in mice with a genetic disruption of the NPY and AGRP gene (15). Our *in vivo* data support this hypothesis because the initial phase of ghrelin's orexigenic response is very similar to that of exogenously administered NPY, whereas the more prolonged effect of ghrelin closely resembles the one of AGRP (83–132).

We show here for the first time that the orexigenic peptides ghrelin and AGRP (83–132) decrease spontaneous locomotor activity. Central administration of AGRP (83–132) resembles the inhibitory action of ghrelin on activity as central administration resulted in a robust an uncompensated decrease in spontaneous locomotor activity. This indicates a role for ghrelin-induced AGRP release in ghrelin's effects on energy

balance. Our data, however, do not support previous reports on NPY as an inhibitor of spontaneous locomotor activity (30–32). If anything, NPY increased spontaneous locomotor activity in our experiments, which is in line with earlier data by Pedrazzini *et al.* (33). The long-lasting and uncompensated difference of cumulative locomotor activity following a single injection of ghrelin and AGRP (83–132) in comparison with vehicle-injected controls intriguingly suggests the activation of common pathways to powerfully and chronically promote a positive energy balance via multiple physiological mechanisms.

The novel combined effects on feeding and locomotor behavior by ghrelin and AGRP (83–132) could principally be due to ghrelin/AGRP (83–132)-treated animals spending most of their time feeding, and as a consequence we would record a decrease in activity. However, the time course of ghrelin [and AGRP (83–132)]-induced effects on food intake and locomotor activity clearly indicates that decreased locomotor activity is independent from increased food intake. Increased food intake occurs within the first 8 h after ghrelin injection, whereas decreased locomotor activity is observed with a delay and does not occur before 10 h into the experiment. Furthermore, the decrease in activity is not due to nausea or malaise because ghrelin administration does not result in a CTA. More importantly, NPY-treated animals, which show an even more impressive feeding response within the first hours after administration, exhibit increased rather than decreased locomotor activity, further suggesting an independent central regulation of food intake and SPA via overlapping, but not identical hypothalamic circuits. Other explanations for ghrelin-induced suppression of locomotor are possible. The decrease in activity could also be due to an increased gastric load (9 g over a 24-h period), and as such the mere food content in the stomach would prone the rat to decrease activity due to constipation and/or nausea. That would, however, result in a compensatory decrease in food intake in the following feeding period. This is, however, not the case because ghrelin-treated animals eat about 1 g more than vehicle-treated in the following 10-h feeding period.

If ghrelin/AGRP's effect on feeding and activity can be dissociated, one must look for differential neural circuits that ghrelin/AGRP could independently modulate. AGRP could modulate activity be interaction with melanocortin-4 receptor located in the lateral hypothalamus (34). The lateral hypothalamus harbors a population of orexin (hypocretin) and melanin-concentrating hormone neurons and thus is tempting to speculate that the reduction in activity caused by AGRP (and thus also ghrelin) could be due to interaction with one of the two (or both) populations of neurons (35). These hypotheses are so far speculative and need further experimental validation.

Extrapolation of data from rodent obesity research studies to human physiology has been successful in an impressively high number of studies; however, in some instances rodent findings cannot be correlated to humans. Surprisingly, ghrelin plasma levels in obese people are low; therefore, circulating ghrelin levels in morbid diet-induced obesity are not likely to be involved in the pathogenesis of the associated chronically positive energy balance. In patients with Prader-Willi syndrome, the most frequent form of genetically caused obesity, ghrelin levels are high and even exceed levels of anorectic or cachectic individuals (36–38). The patients suffer from a unique syndrome of ravenous hunger and decreased SPA (39, 40). Whether the results of our study showing ghrelin-induced suppression of spontaneous motor activity in rodents is of relevance for Prader-Willi syndrome depends on when an effective ghrelin receptor antagonist becomes available.

Increased sedentary behavior represents one reason for the increasing prevalence of obesity and its devastating consequences. Low physical activity levels are also a major determinant of body fat gain during overfeeding (41). We here show for the first time that within the established regulatory system controlling food intake and body weight, ghrelin and its main putative downstream neuropeptide mediator, AGRP, not only increase food intake but also cause a sustained suppression of SPA. The putative regulation of SPA by several other players in the same neuroendocrine networks, all of which are known to regulate food intake, has not previously been investigated in a systematic manner. We therefore propose that expanding the current model of food intake control to include a detailed characterization of the neuroendocrine mechanisms regulating physical activity may promote our understanding of one of the major etiological factors causing obesity. Equally important could be a more complete blueprint of a neuroendocrine network that simultaneously regulates food intake, resting thermogenesis, and SPA and allow for the development of efficacious pharmacological strategies to prevent and/or treat obesity in the future.

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