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Acute Central Ghrelin and GH Secretagogues Induce Feeding and Activate Brain Appetite Centers

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Ghrelin was recently identified as the endogenous ligand for the GH secretagogue (GHS) receptor. Like the synthetic GHSs [*e.g.* GH-releasing peptide-6 (GHRP-6)], ghrelin stimulates feeding and increases body weight in rats. The aim of this study was to identify brain regions that are activated by GHSs and determine whether the responses observed were secondary to food intake. In addition, possible mediators of GHS actions were examined. Intracerebroventricular (icv) injection of ghrelin or GHRP-6 into rats significantly stimulated food intake and transiently reduced core body temperature. The effect of both ghrelin and GHRP-6 on food intake was blocked by preadministration of a Y1 NPY receptor antagonist (BIBO3304). Using c-Fos immunohistochemistry, we demonstrated that icv ghrelin or GHRP-6 activated several hypothalamic brain regions, including the arcuate nucleus, paraventricular nucleus, dorsomedial nucleus, lateral hypothalamus, and two regions of the brainstem, the nucleus of the tractus solitarius and the area postrema. The cell activation induced by GHRP-6 was independent of food intake, as the same pattern and extent of c-Fos expression were observed in animals that were denied access to food following treatment. Finally, double immunohistochemistry indicated that orexin-containing, but not melanin-concentrating hormone-containing, neurons in the lateral hypothalamus were activated significantly by central administration of GHRP-6. (*Endocrinology* 143: 155–162, 2002)

GH SECRETAGOGUES (GHS) are small synthetic molecules that stimulate the release of GH from the pituitary (1, 2). The endogenous ligand for the GHS receptor (GHS-R) (3), ghrelin, has recently been isolated from the rat stomach (4), and ghrelin immunoreactivity also has been located in the hypothalamic arcuate nucleus (4). In addition to being expressed in the anterior pituitary, the GHS-R is found in various hypothalamic and thalamic nuclei, the dentate gyrus, substantia nigra, ventral tegmentum, and facial nucleus of the brainstem (5–8), suggesting a central role for ghrelin. Indeed, central administration of ghrelin causes GH release in rats (9, 10), and GHSs have been implicated in the regulation of energy balance. Single central injections of GHSs, including ghrelin, stimulate feeding in rodents (10–18). Likewise, daily injections or infusions of GHSs increase food intake and body weight (17, 19–21). Ghrelin's effect on body weight in rodents is due in part to altered metabolism and energy expenditure (16, 21).

The precise mechanism of the anabolic actions of GHSs has yet to be fully clarified, although they do not appear to act indirectly via the secretion of GH (12, 15, 17, 18, 21). Previous functional mapping studies have shown that systemic or central administration of GHSs in rodents induce the immediate-early gene c-*fos* only in the hypothalamic arcuate nucleus (22–24) despite the relatively wide distribution of the receptor within the brain (5–8). The highest proportion of arcuate neurons activated by systemic GHS contain NPY (25), and many more neurons are activated if the animal is first fasted, a manipulation known to remove inhibitory influences from NPY neurons (26). Moreover, greater than 90% of arcuate NPY neurons possess GHS-R mRNA, suggesting that they are an important target (27). In support, it has been reported that blocking the action of endogenous NPY inhibits GHS-induced feeding in rodents (15–18).

Recent studies have demonstrated that additional brain regions can express c-Fos protein after systemic synthetic GHS (28, 29) or central administration of ghrelin (17, 30). However, no systematic quantification of cell number or identification of neuronal phenotype activated by ghrelin in these additional areas has been reported. Furthermore, it has yet to be determined whether the neuronal activation observed after GHSs is secondary to food intake, because the consumption of a large meal may itself lead to c-Fos expression in the brain. For example, a difference in the c-Fos expression pattern is observed between groups of animals allowed access to food or not after central NPY injection (31, 32).

Thus, in this study we compared firstly the effects of central administration of ghrelin and the synthetic GHS, GH-releasing peptide-6 (GHRP-6), on feeding and core body temperature. Secondly, we examined whether the feeding response to ghrelin or GHRP-6 was affected by preadministration of a Y1 NPY receptor-selective antagonist (BIBO3304). Thirdly, we quantified the induction of c-Fos (as a marker for neuronal activation) in the forebrain and brainstem and determined whether any of the neuronal activity observed was secondary to food intake rather than a consequence of the initial stimulus. Finally, as arcuate NPY-containing neurons are implicated in the feeding response of GHSs, and these neurons project to orexin- and melanin-concentrating hormone (MCH)-containing neurons in the lateral hypothalamus (33, 34), we sought to determine whether these latter cell types are activated by GHS treatment.

Abbreviations: GHRP-6, GH-releasing peptide-6; GHS, GH secretagogue; GHS-R, GH secretagogue receptor; icv, intracerebroventricular; MCH, melanin-concentrating hormone.

Materials and Methods

Animals and surgery

Male Sprague Dawley rats (Charles River Laboratories, Inc., Sandwich, UK), weighing 250–300 g (8–10 wk old), were used in all studies and were housed at a constant ambient temperature of 21 ± 2 C on a 12-h light, 12-h dark cycle (lights on at 0800 h). Rat chow (Beekay International, Hull, UK) and tap water were provided *ad libitum*, except in Exp 4. All procedures conformed to the requirements of the United Kingdom Animals (Scientific Procedures) Act, 1986. To allow for intracerebroventricular (icv) injections, rats were anesthetized with 2.5% halothane (AstraZeneca, Macclesfield, UK) and stereotaxically implanted with guide cannulas into the lateral ventricle [posterior, 0.8 mm from bregma; lateral, 1.5 mm from bregma; according to the atlas of Paxinos and Watson (35)]. The tip of the guide cannula was positioned 1 mm above the injection site (ventral, 3.5 mm from the surface of the skull). In some experiments core body temperature was monitored, remotely in undisturbed animals, by radiotransmitters (TA10TA-F40, Data Sciences, Minneapolis, MN) that were implanted into the peritoneum at the same time as cannulation. All animals were allowed to recover from surgery for a minimum of 5–7 d and then were housed individually. Icv injections were carried out in conscious, unrestrained animals commencing 2 h after lights on (1000 h).

Exp 1: food intake and core body temperature

Animals were injected icv with either vehicle (2 μl saline; n = 8) or GHRP-6 (2 μg in 2 μl; n = 8; Bachem, Saffron Walden, UK). In a separate experiment, groups of animals (n = 6/group) were injected icv with either vehicle (2 μl saline) or ghrelin (0.01, 0.1, or 1 μg in 2 μl; Phoenix Pharmaceuticals, Inc., Belmont, CA). Immediately after injections animals were presented with a preweighed amount of chow, and food consumption was measured 1, 2, 3, and 4 h later. Core body temperature was monitored continuously throughout the experimental procedure.

Exp 2: NPY antagonist pretreatment

In two separate experiments animals (n = 5–13/group) were injected icv with either vehicle (4 μl water) or BIBO3304 (30 μg in 4 μl; gift from Boehringer-IngelheimPharma KG, Biberach, Germany). Fifteen minutes later the animals were given vehicle (1 or 2 μl saline), GHRP-6 (2 μg in 2 μl), or ghrelin (1 μg in 1 μl). Food consumption was measured 1, 2, and 3 h later.

Exp 3: c-Fos immunohistochemistry

Rats (n = 5/group) were given icv injections of GHRP-6 (2 μg), ghrelin (0.1 μg), or the equivalent volume of vehicle (2 μl saline), followed by a preweighed amount of food. Ninety minutes after peptide administration food intake was measured, and the rats were anesthetized with sodium pentobarbitone (90 mg/kg, ip; Sagatal, Rhône-Mérieux, Harlow, UK) and perfused transcardially with fixative (4% paraformaldehyde). Equivalent 30-μm forebrain or brainstem sections from each brain, 90 μm apart, were cut on a sledge microtome. Free-floating sections were incubated with a rabbit polyclonal anti-c-Fos antibody (1:1000; Oncogene Research Products, Cambridge, MA) and then with a peroxidase-labeled goat antirabbit IgG antibody (1:500; Vector Laboratories, Inc., Burlingame, CA). Nuclear c-Fos was visualized using a nickel-intensified diaminobenzidine reaction to produce a black precipitate. The number of neurons expressing c-Fos was counted bilaterally in nuclei defined by the atlas of Paxinos and Watson (35). The number of immunopositive neurons per section was assessed for each animal. These values were then averaged to determine a group mean for each area of the brain.

Exp 4: effect of food intake on c-Fos immunohistochemistry

Rats (n = 5/6/group) were given an icv injection of either vehicle (2 μl saline) or GHRP-6 (2 μg). After injections animals were either allowed access to food or food was withheld. Ninety minutes later food intake was measured (where appropriate), and transcardial perfusion followed by immunohistochemistry for c-Fos protein was carried out as described above (see Exp 3).

Exp 5: c-Fos and orexin or MCH immunohistochemistry

Rats were injected icv with either vehicle (2 μl saline; n = 6) or GHRP-6 (2 μg; n = 6). After injections animals were allowed access to food, which was measured 90 min later, and transcardial perfusion was performed. Two sets of forebrain sections were taken through the level of the lateral hypothalamus (−2.12 to −4.52 mm to bregma) (35), and immunohistochemistry for c-Fos protein was carried out as described above (see Exp 3). Sections then were incubated sequentially in either a rabbit polyclonal antibody raised against orexin A (1:100; Oncogene Research Products), or MCH (1:1000; Phoenix Pharmaceuticals, Inc.), biotinylated antirabbit IgG (1: 200; Vector Laboratories, Inc.), and then a streptavidin-biotin-peroxidase complex (1:200; Amersham Pharmacia Biotech, Little Chalfont, UK). Cytoplasmic orexin or MCH staining was visualized by a normal diaminobenzidine reaction to yield a brown precipitate. The number of neurons expressing c-Fos and/or orexin or MCH was counted bilaterally in the lateral hypothalamus as defined by the atlas of Paxinos and Watson (35).

Statistical analyses

All data are presented as the mean ± SEM. Core body temperatures were plotted as the mean change from the point of injection (time zero). Statistical analyses were carried out using two-tailed *t* tests for two group experiments and ANOVA followed by *post-hoc* Tukey multiple comparisons test for experiments containing three or more groups. For the analysis of c-Fos expression, when SD values were not equal between groups, these parametric tests were replaced by nonparametric Mann-Whitney *U* tests or Kruskal-Wallis ANOVA, followed by Dunn's multiple comparisons test.

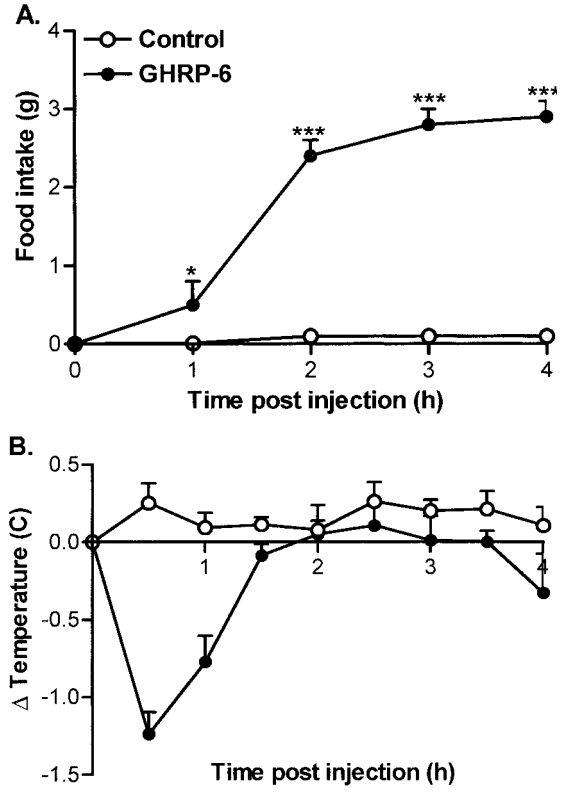

FIG. 1. Effect of icv injection of 2 μg GHRP-6 (●) or 2 μl saline (control; ○) on cumulative food intake (A) and change in core body temperature (B) in satiated rats (1000 h injection). All data are the mean ± SEM (n = 8 in each group). *, $P < 0.05$; ***, $P < 0.001$ (*vs.* control).

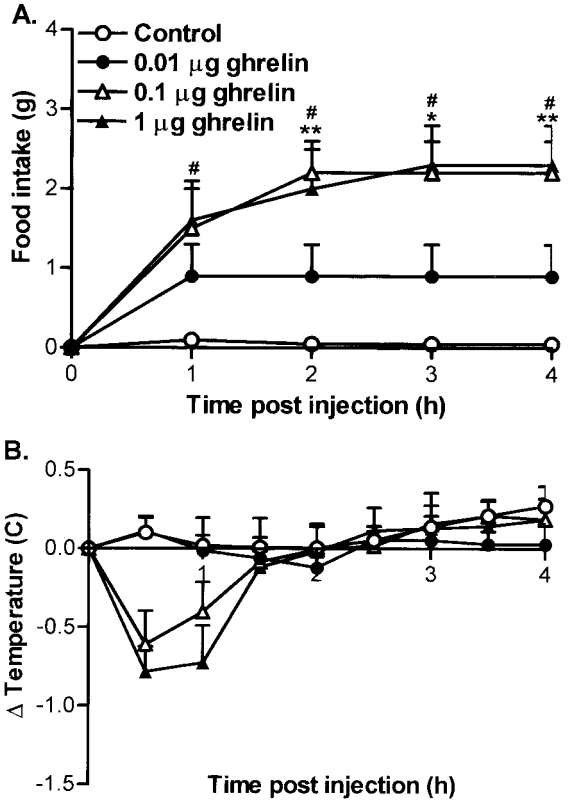

FIG. 2. Effect of icv injection of 0.01 μg (●), 0.1 μg (△), or 1 μg (▲) ghrelin or 2 μl saline (control; ○) on cumulative food intake (A) and change in core body temperature (B) in satiated rats (1000 h injection). All data are the mean ± SEM (n = 6 in each group). *, $P < 0.05$; **, $P < 0.01$ (*vs.* control for 0.1 μg). #, $P < 0.05$ (*vs.* control for 1 μg).

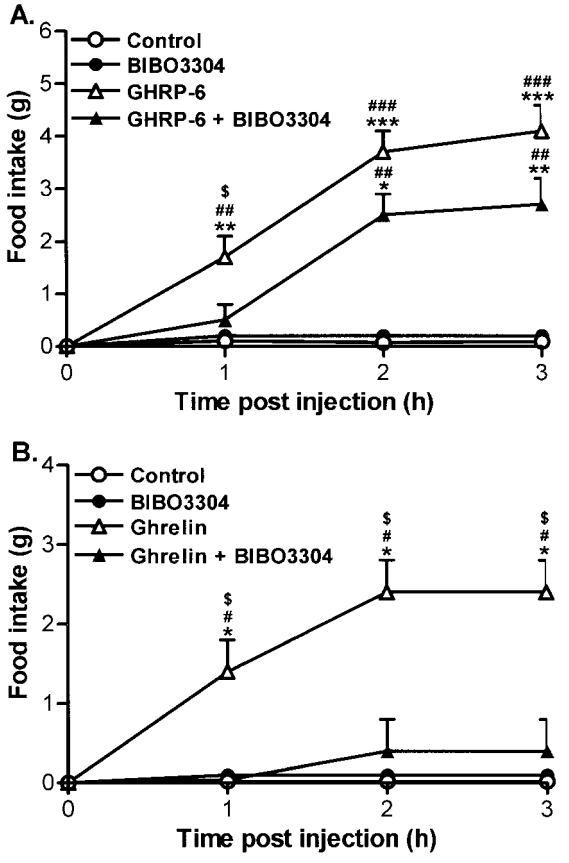

FIG. 3. Effect of the Y1 NPY receptor antagonist, BIBO3304 on GHRP-6-induced (A) or ghrelin-induced (B) food intake. Thirty micrograms of BIBO3304 or control (water) were injected icv 15 min before central administration of 2 μg GHRP-6, 1 μg ghrelin, or control (saline). Injections were performed in satiated rats at 1000 h. All data are the mean ± SEM (n = 5–13/group). *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$ (*vs.* control). #, $P < 0.05$; ##, $P < 0.01$; ###, $P < 0.001$ (*vs.* BIBO3304). $, $P < 0.05$ (*vs.* GHRP-6 plus BIBO3304 or ghrelin plus BIBO3304).

Results

Exp 1: effect of GHRP-6 and ghrelin on food intake and core body temperature

Icv injection of 2 μg GHRP-6 significantly increased food intake in satiated rats over the 4-h test period (Fig. 1A). The effect on food intake began within the first hour postinjection, was almost complete at the 2 h point, and was increased 30-fold at 4 h relative to the control. Intracerebroventricular injection of GHRP-6 also caused a rapid reduction in core body temperature, which reached a nadir 30 min after injection (change in temperature at 30 min: control, +0.3 ± 0.1 C; GHRP-6, −1.2 ± 0.1 C; $P < 0.001$) and had returned to control values by 120 min (Fig. 1B).

Icv injection of ghrelin (0.01–1 μg) caused a dose-dependent increase in food intake over 4 h (Fig. 2A). However, results of ANOVA and *post-hoc* tests showed significant increases in food intake only after 1 μg ghrelin at 1 h and 0.1 and 1 μg at 2, 3, and 4 h after injection. Ghrelin (0.1 and 1 μg) also caused a rapid hypothermic response that was temporally similar to that observed after GHRP-6 injection, but the magnitude of the change was smaller (change in temperature at 30 min: control, +0.1 ± 0.1 C; 0.01 μg, +0.1 ± 0.1 C; 0.1 μg, −0.6 ± 0.2 C; 1 μg, −0.8 ± 0.2 C; $P < 0.05$ and $P < 0.01$ *vs.* control for 0.1 and 1 μg, respectively).

Exp 2: effect of a NPY antagonist on GHRP-6- or ghrelin-induced food intake

Icv injection of GHRP-6 (2 μg) in satiated animals significantly stimulated feeding over the 3-h test period compared with that in control animals. Prior administration of BIBO3304 (30 μg) significantly inhibited the effect of GHRP-6 on feeding in the first hour postinjection (Fig. 3A). Similarly, ghrelin (1 μg)-induced food intake was significantly inhibited at 1, 2, and 3 h postinjection by BIBO3304 (30 μg; Fig. 3B). Central administration of vehicle or BIBO3304 alone in either experiment had no effect on feeding.

Exp 3: c-Fos immunohistochemistry

Icv injection of ghrelin or GHRP-6 significantly increased feeding over the 90-min test period (vehicle, 0.2 ± 0.2 g; ghrelin, 2.7 ± 0.5 g; GHRP-6, 3.1 ± 0.3 g; $P < 0.01$ and $P < 0.001$ *vs.* control for ghrelin and GHRP-6, respectively). There was no significant difference in the amount of food intake between animals receiving GHRP-6 and those given ghrelin ($P > 0.05$).

A distinctive pattern of neuronal activation, as assessed by immunohistochemistry for the protein product of the immediate-early gene, c-*fos*, was noted after the injection of ghrelin or GHRP-6 compared with the control. Significant increases in the number of c-Fos-positive neuronal profiles were recorded in the hypothalamic arcuate nucleus, the hypothalamic paraventricular nucleus, the dorsomedial hypothalamus, the lateral hypothalamus, and the nucleus of the tractus solitarius and the area postrema of the brainstem (Figs. 4 and 5). The induction of c-Fos in the hypothalamic arcuate nucleus was mostly in the medial regions and was relatively strong compared with staining in other regions.

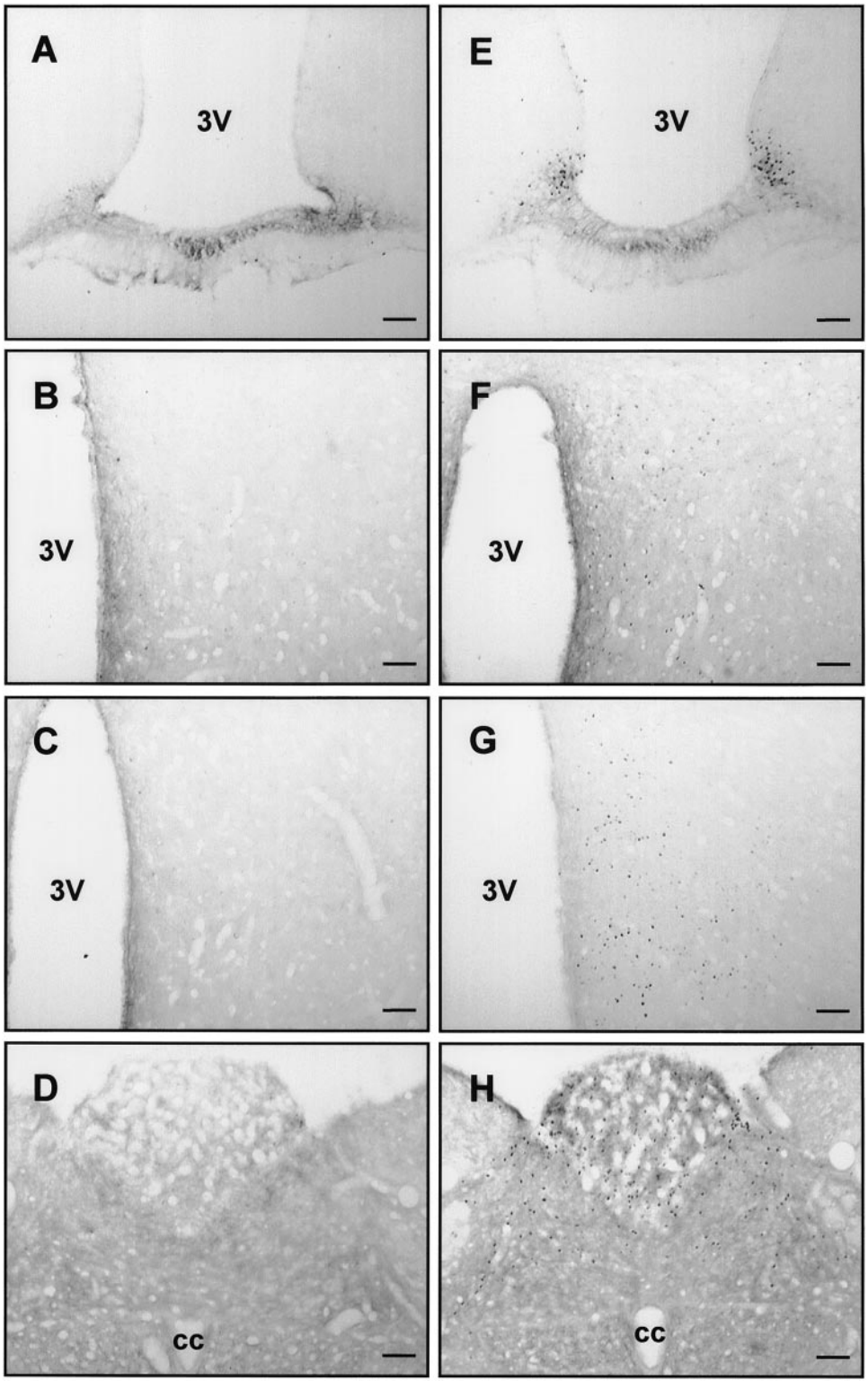

FIG. 4. Representative photomicrographs illustrating c-Fos immunohistochemistry in the hypothalamus and brainstem 90 min after icv injection of control (2 μl saline; A–D) or GHRP-6 (2 μg; E–H). *Nota bene*, icv injection of ghrelin (0.1 μg) resulted in similar patterns of c-Fos compared with injection of GHRP-6. Significant increases in the number of c-Fos-positive neurons were seen in the arcuate nucleus (A and E), paraventricular hypothalamic nucleus (B and F), dorsomedial hypothalamic nucleus (C and G), and area postrema and nucleus tractus solitarius of the brainstem (D and H). See Fig. 5. for quantification. cc, Central canal; 3V, third ventricle. *Scale bars*, 100 μm.

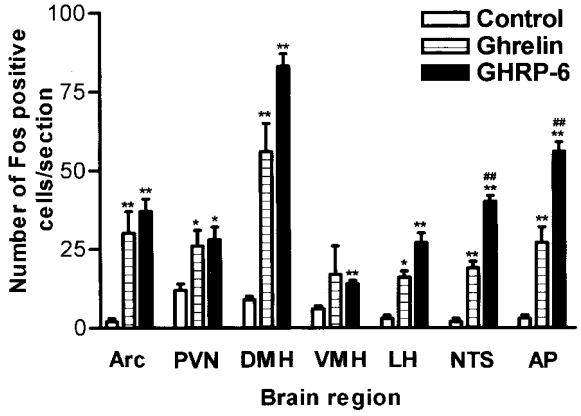

FIG. 5. Bar graph illustrating the number of c-Fos-positive nuclei per section in several brain regions 90 min after icv injection of ghrelin (0.1 μg), GHRP-6 (2 μg), or control (2 μl saline). All data are the mean ± SEM (n = 5 in each group). *, $P < 0.05$; **, $P < 0.01$ (*vs.* control). ##, $P < 0.01$ (*vs.* ghrelin). Arc, Arcuate nucleus; AP, area postrema; DMH, dorsomedial hypothalamic nucleus; LH, lateral hypothalamus; NTS, nucleus of the tractus solitarius; PVN, paraventricular hypothalamic nucleus; VMH, ventromedial hypothalamic nucleus.

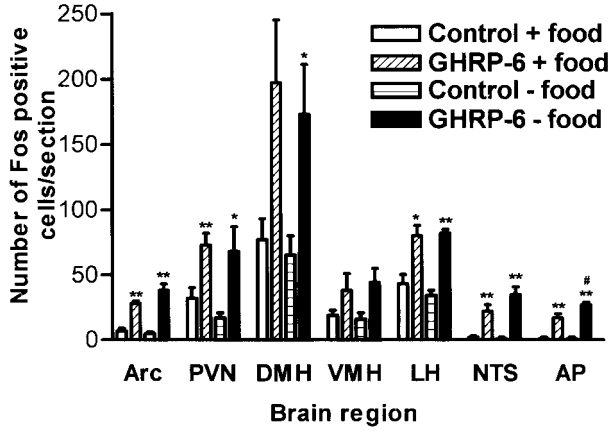

FIG. 6. Bar graph illustrating the number of c-Fos-positive nuclei per section in several brain regions 90 min after icv injection of GHRP-6 (2 μg) or in controls (2 μl saline) in animals allowed or denied access to food. All data are the mean ± SEM (n = 5–6/group). *, $P < 0.05$; **, $P < 0.01$ (*vs.* appropriate control). #, $P < 0.05$ (*vs.* GHRP-6 plus food). Arc, Arcuate nucleus; AP, area postrema; DMH, dorsomedial hypothalamic nucleus; LH, lateral hypothalamus; NTS, nucleus of the tractus solitarius; PVN, paraventricular hypothalamic nucleus; VMH, ventromedial hypothalamic nucleus.

There was also a small increase in the average number of c-Fos-positive profiles in the ventromedial hypothalamus, which was statistically significant only in the GHRP-6-treated animals. No c-Fos staining above background was recorded in the hypothalamic supraoptic nucleus or the brainstem dorsal motor nucleus of the vagus nerve (results not shown). Generally, there were no major differences noted between the pattern of response between injection of ghrelin and GHRP-6, although there were significantly more c-Fos-positive cells in the nucleus of the tractus solitarius and the area postrema after GHRP-6 injection.

Exp 4: effect of food intake on c-Fos immunohistochemistry

In the two groups of animals that were allowed access to food, icv injection of GHRP-6 stimulated food intake over 90 min compared with that of control animals (control, 0.1 ± 0.1 g; GHRP-6, 2.3 ± 0.6 g; $P < 0.01$).

The pattern of c-Fos-positive profiles induced by icv injection of GHRP-6 in animals allowed access to food was comparable to that reported in Exp 3. Furthermore, equivalent pattern and number of cells expressing c-Fos were observed in GHRP-6-treated animals that had food withheld (Fig. 6). Significant increases in the induction of c-Fos were observed in both groups of animals given GHRP-6 (plus or minus food) compared with their appropriate control in the hypothalamic arcuate nucleus, hypothalamic paraventricular nucleus, dorsomedial hypothalamus (only in the minus food group), lateral hypothalamus, and nucleus of the tractus solitarius and the area postrema of the brainstem.

Exp 5: effect of GHRP-6 on orexin and MCH neurons

Animals that were treated icv with GHRP-6 (2 μg) ate significantly more food than controls over the 90-min test period (control, 0.7 ± 0.3 g; GHRP-6, 3.2 ± 0.7 g; $P < 0.05$). Double immunohistochemistry revealed that in the lateral hypothalamus, GHRP-6 significantly increased the percentage of orexin neurons expressing c-Fos (control, 13 ± 2%; GHRP-6, 56 ± 4%; $P < 0.01$; Fig. 7, A and B). However there was no significant difference in the percentage of MCH neurons expressing c-Fos between control and GHRP-6-treated animals (control, 6 ± 1%; GHRP-6, 5 ± 1%; $P > 0.05$; Fig. 7, C and D).

Discussion

Central injection of ghrelin or GHRP-6 stimulated feeding in satiated rats. These findings are in agreement with several previous studies reporting the orexigenic actions of centrally administered GHSs (10–18, 20, 21). In addition, doses of ghrelin or GHRP-6 that caused feeding produced a transient drop in core body temperature. The mechanism of this hypothermic response is unclear, but may be related to the changes in metabolism observed after icv injection of ghrelin in rodents (16, 21), in that the anabolic effects of ghrelin may include both an increase in food intake and a decrease in energy expenditure.

In 2000, Tschop and colleagues (21) reported that the orexigenic effects of ghrelin are present in NPY-deficient mice, thus questioning the role of NPY neurons in this response. However, this knockout mouse may not be an ideal model. It does not have the expected lean phenotype (36) and can respond to other stimuli thought to be mediated by these neurons (37). Other transmitters, present in NPYergic neurons (*e.g.* agouti-related protein and γ-aminobutyric acid) and other parallel pathways (*e.g.* αMSH in POMC neurons), may not be affected. The results presented here and previously (15–18) using NPY receptor antagonists are more supportive of a key role for central NPY pathways in mediating the effects of GHSs on food intake. Additional evidence includes the location of GHS-R mRNA in arcuate NPY neurons (27) as well as the activation of these neurons by systemic or central injections of GHSs (17, 25). Two caveats concerning the hypothesis that the major target for GHSs is arcuate NPY

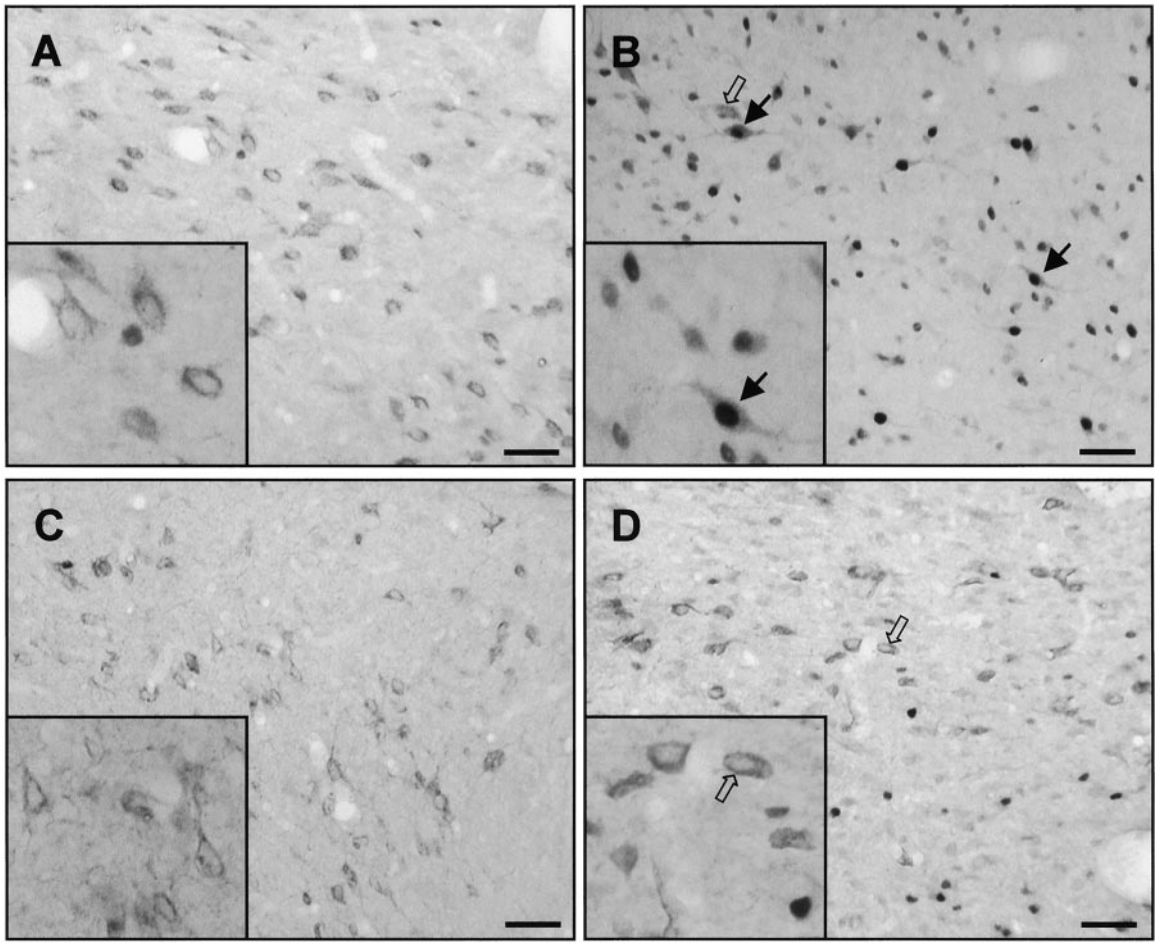

FIG. 7. Effect of GHRP-6 on the activity of orexin-containing (A and B) or MCH-containing (C and D) neurons in the lateral hypothalamus. Photomicrographs represent equivalent sections of the lateral hypothalamus after icv injection of vehicle (2 μl saline, A and C) or 2 μg GHRP-6 (B and D). Note the increase in the number of c-Fos-positive nuclear profiles (*dark black spots*) colocalized in orexin (but not MCH) neurons after GHRP-6 treatment (*closed arrows*). *Open arrows* indicate examples of nonactivated orexin or MCH neurons. *Insets* represent portions of the main pictures at ×2 magnification. *Scale bars*, 50 μm.

neurons need mention. First, the use of receptor antagonists does not distinguish between NPY derived from neurons in the arcuate nucleus and those in the dorsomedial hypothalamic nucleus that may also form part of the pathway. Second, antagonism of signaling through Y1 NPY receptors may cause anxiety in rodents (for review, see Ref. 38); thus, the specificity of receptor antagonism should be viewed cautiously.

Using immunohistochemistry for the protein product of the immediate-early gene, c-*fos*, central administration of GHRP-6 was reported previously to activate only cells of the arcuate nucleus (23). However, more recently, central administration of ghrelin has been shown to activate other regions in both the forebrain (17) and the brainstem (30), although no quantification was carried out. Here, we demonstrate a more extensive neuronal activation by icv injection of GHSs (ghrelin and GHRP-6), with significant increases in the expression of c-Fos observed in the arcuate, paraventricular, dorsomedial, and lateral hypothalamic nuclei, and in two regions of the brainstem, the nucleus of the tractus solitarius and the area postrema. The pattern and magnitude of c-Fos induction described above were similar in animals injected with doses of ghrelin or GHRP-6 that caused comparable food consumption. An exception was that more Fos-positive cells were noted in the nucleus of the tractus solitarius and the area postrema in animals treated with GHRP-6 compared with ghrelin injection.

The differences between the present and a previous study using central administration of GHRP-6 (23) could be due to the relative weakness of c-Fos protein immunostaining in the other brain regions compared with that in the arcuate nucleus. Whether the weaker c-Fos staining was due to these neurons being synaptically, rather than directly, activated cannot be determined from our results, and further anatomical studies, involving tract tracing, and electrophysiology will be required. GHS-R mRNA is located in several brain regions where c-Fos expression was observed, including the arcuate and paraventricular hypothalamic nuclei and the brainstem (including the area postrema) (5–7), suggesting that these neurons may be directly activated. Furthermore, a more recent publication has detected low levels of GHS-R mRNA in the lateral and dorsomedial hypothalamic areas (8). c-Fos is useful only as a marker for electrically activated neurons, and it is clear that GHSs are equally capable of

directly inhibiting hypothalamic neurons (39), which may include anorexigenic mediators. This fact may explain some of the discrepancies between levels of c-Fos induction and the presence of GHS-R, for example in the ventromedial nucleus.

Satiety signals from the gastrointestinal tract are detected by vagal nerve afferents that project to the brainstem nucleus of the tractus solitarius (40, 41). Central processing of these signals can be detected as the induction of c-Fos in brainstem structures (42), suggesting that some of the c-Fos detected may be secondary to the rats consuming a large meal. However, the present study illustrates that the GHS, GHRP-6, activated neurons independently of food intake, as the pattern and extent of c-Fos expression were similar in animals that had food withheld. Thus, the current findings demonstrate that cell activation in the brain after GHS is not due to satiety signaling from the gastrointestinal tract. Instead, some of the activity may be related to efferent signaling from the brain back down to the gut, although we found no evidence of activity in the dorsal motor nucleus of the vagus nerve.

The mediators of the actions of GHS on feeding, perhaps downstream of arcuate NPY neurons, remain to be clarified. In the present study GHSs activated additional hypothalamic nuclei, for example the lateral hypothalamus, an area that contains other orexigenic peptides, including orexin and MCH. As NPY-containing neurons of the arcuate hypothalamic nucleus project directly to lateral hypothalamic orexin- and MCH-containing neurons (33, 34), we sought to determine the phenotype of the cells activated by GHS in the lateral hypothalamus. Our data clearly show that central GHSs activated orexin, but not MCH, neurons. It is possible, therefore, that orexin-containing neurons of the lateral hypothalamus are activated indirectly via arcuate NPY neurons and that GHSs may selectively activate certain orexigenic pathways.

The major source of ghrelin is the stomach (4), and the level of circulating ghrelin fluctuates through the day according to food intake, with starvation increasing ghrelin and meals suppressing it (21). There is only one published report on the presence of ghrelin in the hypothalamic arcuate nucleus determined by immunohistochemistry (4). Until this is confirmed, one might question whether the fluctuations of ghrelin measured in plasma are sufficient to stimulate a feeding response under physiological conditions. It may be more likely that circulating ghrelin can access the brain at the arcuate nucleus to modulate anabolic and catabolic pathways in an opposing way to other hormones, such as leptin and insulin. However, pharmacological activation of GHS-R by central administration of GHS is a useful method for the study of orexigenic pathways.

In summary, we have shown that ghrelin and a synthetic GHS, GHRP-6, are potent inducers of food intake when injected centrally. We hypothesize that NPY neurons of the arcuate nucleus are a primary target for this action of GHSs, and that the stimulation of central NPYergic pathways may lead to further neuronal activation of orexigenic circuits. An additional opposing effect on anorexigenic pathways is likely.

Acknowledgments

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