Rapid Publication

Ghrelin, an Endogenous Growth Hormone Secretagogue, Is a Novel Orexigenic Peptide That Antagonizes Leptin Action Through the Activation of Hypothalamic Neuropeptide Y/Y1 Receptor Pathway

Mitsuyo Shintani, Yoshihiro Ogawa, Ken Ebihara, Megumi Aizawa-Abe, Fumiko Miyanaga, Kazuhiko Takaya, Tatsuya Hayashi, Gen Inoue, Kiminori Hosoda, Masayasu Kojima, Kenji Kangawa, and Kazuwa Nakao

Ghrelin, an endogenous ligand for growth hormone secretagogue (GHS) receptor originally isolated from the stomach, occurs in the hypothalamic arcuate nucleus and may play a role in energy homeostasis. Synthetic GHSs have activated the hypothalamic arcuate neurons containing neuropeptide Y (NPY), suggesting the involvement of NPY in some of ghrelin actions. This study was designed to elucidate the role of ghrelin in the regulation of food intake. A single intracerebroventricular (ICV) injection of ghrelin (5–5,000 ng/rat) caused a significant and dose-related increase in cumulative food intake in rats. Ghrelin (500 ng/rat) was also effective in growth hormone–deficient spontaneous dwarf rats. Hypothalamic NPY mRNA expression was increased in rats that received a single ICV injection of ghrelin (500 ng/rat) (~160% of that in vehicle-treated groups, $P < 0.05$). The ghrelin's orexigenic effect was abolished dose-dependently by ICV co-injection of NPY Y1 receptor antagonist (10–30 μg/rat). The leptin-induced inhibition of food intake was reversed by ICV co-injection of ghrelin in a dose-dependent manner (5–500 ng/rat). Leptin reduced hypothalamic NPY mRNA expression by 35% ($P < 0.05$), which was abolished by ICV co-injection of ghrelin (500 ng/rat). This study provides evidence that ghrelin is an orexigenic peptide that antagonizes leptin action through the activation of hypothalamic NPY/Y1 receptor pathway.
Diabetes **50:227–232, 2001**

From the Department of Medicine and Clinical Science (M.S., Y.O., K.E., M.A.-A., F.M., K.T., T.H., G.I., K.H., K.N.), Kyoto University Graduate School of Medicine, Kyoto; and the Department of Biochemistry (M.K., K.K.), National Cardiovascular Center Research Institute, Osaka, Japan.

Address correspondence and reprint requests to Yoshihiro Ogawa, Department of Medicine and Clinical Science, Kyoto University Graduate School of Medicine, 54 Shogoin Kawahara-cho, Sakyo-ku, Kyoto 606-8507 Japan. E-mail: ogawa@kuhp.kyoto-u.ac.jp.

Received for publication 16 August 2000 and accepted 9 November 2000. Posted on the World Wide Web at www.diabetes.org/diabetes on <<insert date> 2001.

AGRP, agouti-related protein; GH, growth hormone; GHRH, growth hormone–releasing hormone; GHS, growth hormone secretagogue; GHS-R, GHS receptor; ICV, intracerebroventricular; NPY, neuropeptide Y.

Growth hormone secretagogues (GHSs) are synthetic compounds that cause the release of growth hormone (GH) from the pituitary (1). They act through the GHS receptor (GHS-R) (2), a previously orphaned G-protein–coupled receptor that is expressed in the hypothalamus, pituitary, pancreas, etc. (2–4). GHSs stimulate GH release by a direct pituitary action (5,6), but several lines of evidence have suggested that it does so via a hypothalamic mechanism as well (7,8). Clinically, GHSs alone or in combination with growth hormone–releasing hormone (GHRH) have been used for diagnosis and treatment of various forms of GH deficiency (1,9,10).

Using cells expressing GHS-R as assay systems, Kojima et al. (11) have recently isolated from the rat stomach a novel GH-releasing acylated peptide of 28 amino acids. Termed ghrelin, it can specifically stimulate GH release in vivo and in vitro. Previous studies have demonstrated that central as well as peripheral administration of GHS increases food intake and body weight in rats (12–16). Furthermore, GHS administration induces c-*fos* mRNA expression in the hypothalamic arcuate neurons containing neuropeptide Y (NPY), a potent stimulator of food intake (17). Indeed, GHS-R is colocalized with NPY in the rat hypothalamic arcuate nucleus (18). Expressed in the hypothalamic arcuate nucleus (11), it is likely that ghrelin acts also as an orexigenic peptide that regulates hypothalamic NPY production. However, the effect of ghrelin on feeding behavior has not been tested so far.

Leptin is an adipocyte-derived blood-born satiety factor that acts directly on the hypothalamus, where it regulates a large number of molecules implicated in energy homeostasis (19–21). We and others have demonstrated that the satiety effect of leptin is mediated through the activation of the hypothalamic melanocortin system (22–24). Several lines of evidence have suggested that hypothalamic NPY also mediates some aspects of leptin actions (25–28). Indeed, it has been reported that the leptin receptor is expressed in the majority of the arcuate NPY neurons (29). Currently, there is evidence for the existence of at least six functional NPY receptor sub-

types (Y1–Y6) (30,31). NPY Y1 receptor antagonists suppress endogenous and exogenous NPY-induced feeding, suggesting that Y1 receptor is a major NPY receptor subtype for its orexigenic action (32–34). Furthermore, fasting-induced refeeding is severely affected in Y1-deficient mice (35), suggesting that the hypothalamic NPY/Y1 receptor pathway is activated in response to fasting when plasma leptin concentrations are reduced (36–38). It has been reported that leptin can antagonize the action of exogenously administered NPY (39). Given that leptin reduces the otherwise increased hypothalamic arcuate NPY mRNA expression in fasted rats (28,40) and that fasting-induced refeeding is inhibited by leptin treatment (28,40,41), it is conceivable that the satiety effect of leptin is mediated at least partly through the inhibition of the hypothalamic NPY/Y1 receptor pathway.

The aim of this study is to elucidate the role of ghrelin in the regulation of food intake. Here we provide evidence that ghrelin is an orexigenic peptide that antagonizes leptin action through the activation of the hypothalamic NPY/Y1 receptor pathway.

RESEARCH DESIGN AND METHODS

Seven-week-old male Sprague-Dawley rats (200–220 g) and 9- to 11-week-old spontaneous dwarf rats (SDRs) (100–110 g) (42,43) were purchased from Japan SLC (Hamamatsu, Japan). They were housed in a temperature-, humidity-, and light-controlled room (12-h light/12-h dark cycle) and allowed free access to standard rat food (CE-2,352 kcal/100 g; Japan CLEA, Tokyo), unless otherwise indicated. All experimental procedures were approved by the Kyoto University Graduate School of Medicine Committee on Animal Research.

Intracerebroventricular injection experiments. A stainless steel intracerebroventricular (ICV) cannula (outer diameter 1.09 mm) (Becton Dickinson, Sparks, MD) was implanted under anesthesia in the skull of rats 5–7 days before the injection experiments, using coordinates (6.5 mm anterior to the lambdoidal suture; ± 1.4 mm lateral to the midline; 4.5 mm from the dural surface) (24,44). The ICV cannula placement was confirmed in all rats by introducing Evans blue after the experiments. Only the animals that showed the correct ICV cannula placement were included in this study.

ICV injection of ghrelin in Sprague-Dawley rats and SDRs. For the ICV injection of human ghrelin alone (5–5,000 ng/10 µl/rat) (11), cumulative food intake was measured in Sprague-Dawley rats and SDRs during 4 h at the early light phase (10:00–14:00) after the ICV injection. It was reported that a single ICV injection of ghrelin at doses of 10–200 pmol/rat (~33–660 ng/rat) stimulates pituitary GH release dose-dependently (45).

ICV co-injection of ghrelin and NPY Y1 receptor antagonist in Sprague-Dawley rats. To examine the involvement of NPY/Y1 pathway in ghrelin's orexigenic effect, we used an NPY Y1 receptor antagonist (J-115814) (Banyu Pharmaceuticals, Ibaraki, Japan). In vitro binding analysis revealed that J-115814 displaces [^{125}I]peptide YY binding to the cloned human and rat Y1 receptors with inhibitory constant (K_i) values of 1.4 and 1.8 nmol/l, respectively, whereas it shows low affinities for human Y2 (K_i >10,000 nmol/l), Y4 (K_i >620 nmol/l), Y5 receptors (K_i >6,000 nmol/l) (M. Hata, S. Mashiko, A. Ishihara, O. Okamoto, J. Haga, T. Ohe, T. Kanno, N. Murai, Y. Ishii, T. Fukuroda, T. Fukami, M. Ihara, unpublished observations). Feeding induced by NPY (5 µg/rat) was inhibited dose-dependently by ICV co-injection of J-115814 (10–100 µg/rat) (data not shown).

For the ICV co-injection of ghrelin and J-115814, cumulative food intake was measured in Sprague-Dawley rats for 4 h at the early light phase (10:00–14:00) after a single ICV co-injection of ghrelin and J-115814 (500 ng + 10–30 µg/10 µl/rat) or vehicle (10 µl).

ICV co-injection of leptin and ghrelin in Sprague-Dawley rats. For the ICV co-injection of leptin and ghrelin, cumulative food intake was measured in Sprague-Dawley rats for a period of 4 h (19:00–23:00) at the onset of the dark phase after a single ICV co-injection of leptin and ghrelin (2 µg + 5–500 ng/10 µl/rat) or vehicle (10 µl).

Total RNA extraction and RNA analysis. Total RNA was extracted from the whole hypothalami (24) obtained from Sprague-Dawley rats 4 h after a single ICV injection of ghrelin and/or leptin or vehicle. A 232-bp rat NPY cDNA fragment was prepared by reverse transcriptase–polymerase chain reaction using two oligonucleotide primers (sense 5′-CTGTGTGGACTGACCCTCGC-3′ and antisense 5′-CATTTTCTGTGCTTTCTCTC-3′). Northern blot analysis of NPY mRNA was performed (24) using rat NPY cDNA as a probe. A human β-actin genomic probe (Wako Pure Chemical, Osaka, Japan) was used to monitor the amount of RNA in each sample. We confirmed that the intensity of hybridization signals shows a linear relation to the amount of total RNA used in Northern blot analysis (data not shown). The hybridization signal intensity was quantitated using an image analyzer BAS-2500 (Fuji Photo Film, Tokyo) and normalized for the β-actin signal intensity.

Statistical analysis. All values were expressed as means ± SE. Statistical significance of difference in mean values was assessed by Duncan's multiple-range test following one-way analysis of variance.

RESULTS

ICV injection of ghrelin in Sprague-Dawley rats. A single ICV injection of ghrelin (5–5,000 ng/rat) resulted in a significant and dose-related increase in cumulative food intake in 8-week-old Sprague Dawley rats compared with vehicle-treated groups during 4 h at the early light phase (Fig. 1*A*). Treatment with 5,000 ng ghrelin increased cumulative food intake approximately fourfold relative to vehicle-treated groups (5.32 ± 1.28 vs. 1.25 ± 0.32 g, n = 10 each, P < 0.01). In this study, no significant difference in cumulative food

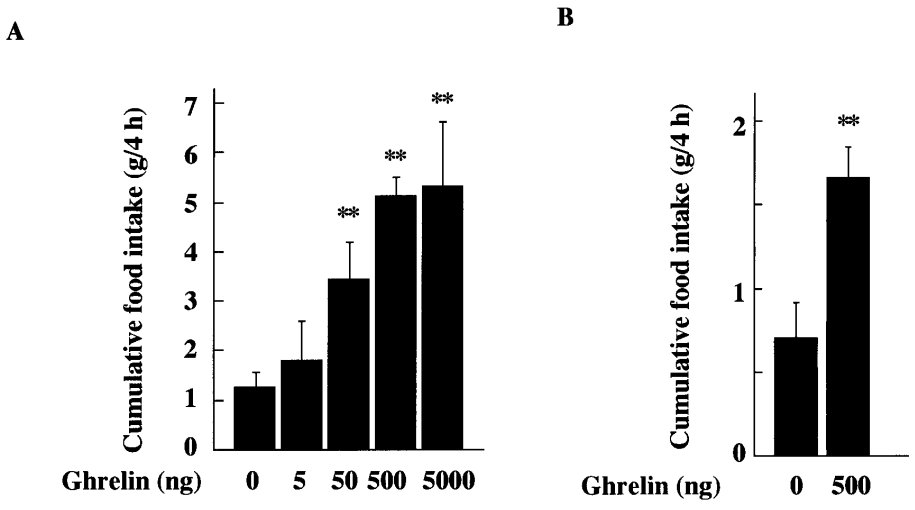

FIG. 1. *A*: Effect of a single ICV injection of ghrelin (5–5,000 ng/rat) on cumulative food intake in 8-week-old Sprague-Dawley rats. *B*: Effect of a single ICV infection of ghrelin (500 ng/rat) on cumulative food intake in 9- to 11-week-old SDRs. **P < 0.01 vs. vehicle-treated groups.

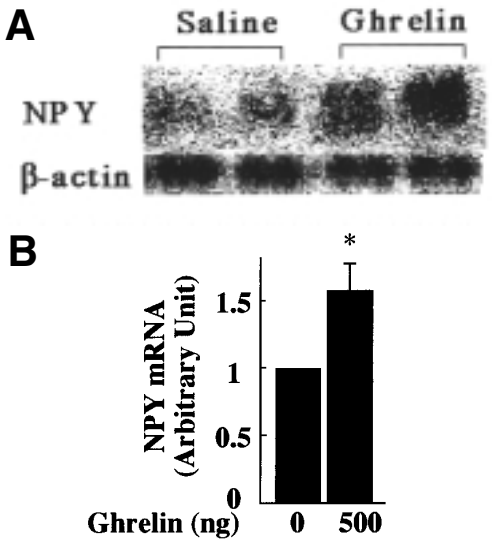

FIG. 2. Northern blot analysis of hypothalamic NPY mRNA expression in 8-week-old Sprague-Dawley rats 4 h after the ICV injection of ghrelin (500 ng/rat). *A*: Representative blots are shown. *B*: Graph shows the quantification of NPY mRNA levels relative to β-actin mRNA levels. Data are presented as relative ratios ± SE (n = 12 each). *P < 0.05 vs. vehicle-treated groups.

intake was noted between ghrelin- and vehicle-treated animals 24 h after the injection (data not shown).

ICV injection of ghrelin in SDRs. To explore whether ghrelin's orexigenic effect is mediated by increased release of GH, we examined the effect of a single ICV injection of ghrelin on food intake in GH-deficient SDRs. A single ICV injection of ghrelin at a dose of 500 ng/rat caused a significant increase in cumulative food intake in SDRs compared with vehicle-treated groups during 4 h after the injection (1.66 ± 0.08 vs. 0.70 ± 0.19 g, n = 8–10 each, P < 0.01) (Fig. 1*B*).

Effects of ghrelin on hypothalamic NPY mRNA expression. Northern blot analysis revealed that NPY mRNA expression is increased significantly in the hypothalamus obtained from rats that received a single ICV injection of 500 ng ghrelin compared with vehicle-treated rats 4 h after the injection (~1.6-fold, n = 12 each, P < 0.05) (Fig. 2*A* and *B*).

ICV co-injection of ghrelin and Y1 receptor antagonist. To assess whether the orexigenic effect of ghrelin is mediated by NPY and, if so, to assess whether Y1 receptor is involved, we examined the effect of ICV co-injection of ghrelin and Y1 receptor antagonist in rats. After a single ICV injection of 500 ng ghrelin, cumulative food intake was increased by ~400% relative to vehicle-treated groups (5.12 ± 0.38 vs. 1.25 ± 0.32 g, n = 10 each, P < 0.01) (Fig. 3). By co-injection of J-115814 at doses of 10 and 30 µg/rat, increased food intake by ghrelin was reversed in a dose-dependent manner. Treatment with 30 µg J-115814 reversed completely the ghrelin-induced increase in cumulative food intake relative to vehicle-treated groups (1.22 ± 0.71 vs. 1.25 ± 0.32 g, n = 10 each, P > 0.1). In this study, treatment with 30 µg J-115814 alone did not affect food intake in rats (Fig. 3).

ICV co-injection of leptin and ghrelin. A single ICV injection of 2 µg leptin resulted in a significant reduction of cumulative food intake in rats relative to vehicle-treated groups (1.03 ± 0.21 vs. 3.11 ± 0.24 g, n = 12 each, P < 0.01) (Fig. 4). This is consistent with our previous report (24). Co-injection of ghrelin (5–500 ng/rat) reversed dose-dependently the inhibition of food intake by leptin. Treatment with 500 ng ghrelin reversed the leptin-induced inhibition of food intake (2.19 ± 0.23 vs. 1.03 ± 0.21 g, n = 12 each, P < 0.01). Co-injection of 500 ng ghrelin and 2 µg leptin decreased significantly food intake relative to that in vehicle-treated groups (2.19 ± 0.23 vs. 3.11 ± 0.24 g, n = 12 each, P < 0.05). In this study, a single ICV injection of 500 ng ghrelin increased cumulative food intake (5.07 ± 0.37 vs. 3.11 ± 0.24 g, n = 12 each, P < 0.01) during 4 h at the onset of the dark phase.

Effects of ICV co-injection of leptin and ghrelin on hypothalamic NPY mRNA expression. Northern blot analysis revealed a significant reduction of hypothalamic NPY mRNA expression in rats that received a single ICV injection of leptin (2 µg/rat) relative to vehicle-treated groups 4 h after the injection (~65%, n = 12 each, P < 0.05) (Fig. 5*A* and *B*). The leptin-induced decrease in NPY mRNA expression was reversed by ICV co-injection of ghrelin at a dose of

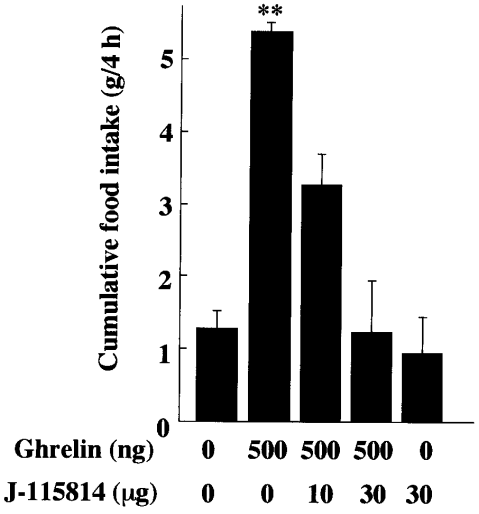

FIG. 3. Effect of a single ICV co-injection of ghrelin and NPY Y1 receptor antagonist on cumulative food intake in 8-week-old Sprague-Dawley rats. **P < 0.01 vs. vehicle-treated groups.

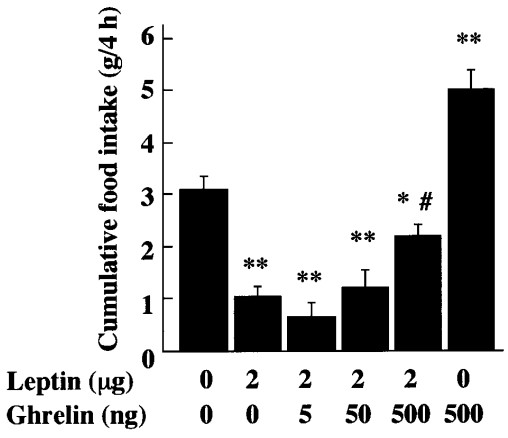

FIG. 4. Effect of a single ICV co-injection of leptin and ghrelin on cumulative food intake in 8-week-old Sprague-Dawley rats. *P < 0.05 and **P < 0.01 vs. vehicle-treated groups; #P < 0.01 vs. leptin-treated groups.

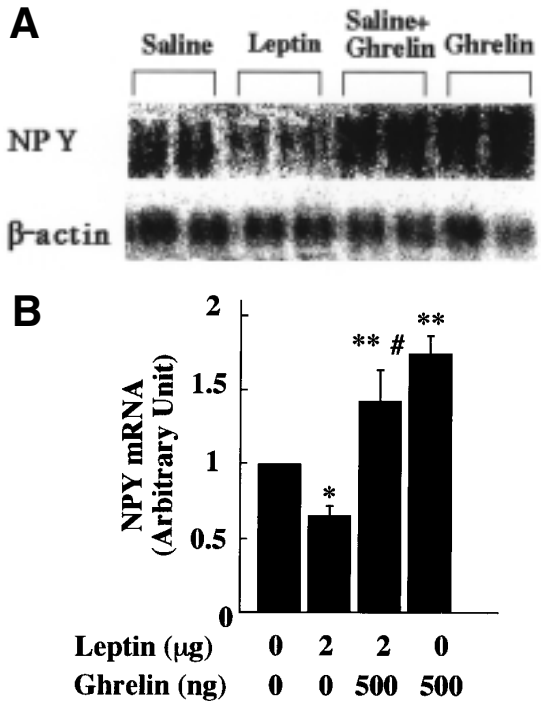

FIG. 5. Northern blot analysis of hypothalamic NPY mRNA expression in 8-week-old Sprague-Dawley rats 4 h after the ICV co-injection of leptin (2 μg/rat) and ghrelin (500 ng/rat). *A*: Representative blots are shown. *B*: Graph shows the quantification of NPY mRNA levels relative to β-actin mRNA levels. Data are presented as relative ratios ± SE (n = 12 each). *P < 0.05 and **P < 0.01 vs. vehicle-treated groups; $^{\#}P$ < 0.01 vs. leptin-treated groups.

500 ng/rat (n = 12 each, P < 0.01) (Fig. 5*A* and *B*). Co-injection of 500 ng ghrelin and 2 μg leptin increased NPY mRNA expression relative to vehicle-treated groups (~130%, n = 12 each, P < 0.01). Treatment with 500 ng ghrelin alone increased NPY mRNA expression by 160% relative to vehicle-treated groups (Fig. 5*A* and *B*).

DISCUSSION

This study demonstrates that a single ICV injection of ghrelin, a newly discovered naturally occurring GHS, increases food intake in rats. This is consistent with previous reports that central administration of GHRP-6 and KP-102, both of which are synthetic GHSs, increases food intake and body weight in rats (12,13). Although originally isolated from the stomach, ghrelin also occurs in the hypothalamic arcuate nucleus (11). Furthermore, GHS-R is expressed in several hypothalamic nuclei critical for energy homeostasis, such as the arcuate nucleus, ventromedial hypothalamic nucleus, and paraventricular nucleus (3,18). These findings, taken together, suggest that ghrelin acts centrally as an orexigenic peptide. It has been reported that peripheral administration of GHSs can increase food intake and body weight in rats (15). Expression of GHS-R is also detected in peripheral tissues, such as the pituitary, pancreas, and renal pelvis (2–4). Currently, it is unknown that ghrelin, which is released from the stomach into the circulation, can affect feeding behavior centrally, peripherally, or both.

In this study, ghrelin can increase food intake in GH-deficient SDRs, indicating that ghrelin does not require GH for its orexigenic effect. This is consistent with the notion that the orexigenic effect of hexarelin analogs when injected centrally and peripherally does not involve GH release (15). It was reported that systemic administration of GHS can induce c-*fos* mRNA expression in the hypothalamic arcuate GHRH neurons (14,17), where it can stimulate the release of GHRH (46). Given that GHRH is a potent orexigenic peptide (47), it is interesting to speculate that ghrelin increases food intake via GHRH. However, the orexigenic effect of KP-102 is not blocked by GHRH antagonist (13). Thus, it is likely that ghrelin stimulates feeding behavior via mechanisms different from those of GHRH.

A previous study showed that GHS-R mRNA is expressed in a large population of hypothalamic arcuate neurons containing NPY (18). Indeed, systemic administration of GHS can induce c-*fos* mRNA expression in some of hypothalamic arcuate NPY neurons (17). These findings suggest that GHS can act through GHS-R that is expressed in hypothalamic arcuate neurons containing NPY. In this study, we demonstrate that a single ICV injection of ghrelin can increase hypothalamic NPY mRNA expression. Furthermore, the orexigenic effect of ghrelin is abolished by ICV co-injection of Y1 receptor antagonist. These observations suggest that ghrelin increases food intake at least partly through the activation of the hypothalamic NPY/Y1 pathway. It is interesting to speculate that other actions of ghrelin might be mediated by the hypothalamic NPY/Y1 pathway.

Evidence has accumulated indicating that NPY plays a role in leptin action (25–28). Hypothalamic NPY production is increased in leptin-deficient *ob/ob* mice or *db/db* mice or *fa/fa* rats with leptin receptor mutation (25,48,49), and Y1 antagonist partially reverses the spontaneous feeding of *fa/fa* rats (32,34). It has been shown that leptin treatment can reduce the otherwise increased hypothalamic arcuate NPY mRNA expression during fasting (28,40), when it inhibits significantly fasting-induced refeeding (28,40,41). Furthermore, fasting-induced refeeding is severely affected in Y1-deficient mice (35). Thus, NPY, when induced in response to leptin signaling deficiency, may activate Y1 receptor, thus leading to a marked induction of feeding. It is also conceivable that the satiety effect of leptin is mediated at least partly through the inhibition of hypothalamic NPY/Y1 pathway. In this study, we demonstrate that the satiety effect of leptin is abolished by ICV co-injection of ghrelin, which indicates the antagonism of the satiety effect of leptin by ghrelin. It has been reported that the biologically active longest isoform of leptin receptor mRNA is expressed in ~50% of hypothalamic arcuate NPY neurons (50), where GHS-R mRNA is mostly expressed (>90% of hypothalamic arcuate NPY neurons) (18). It is likely that ghrelin and leptin act on the same NPY neurons to regulate hypothalamic NPY production. Therefore, we postulate that ghrelin and leptin share the hypothalamic NPY/Y1 pathway as one of the downstream mechanisms; the antagonism of the satiety effect of leptin by ghrelin may be due to the ghrelin-induced activation of the hypothalamic NPY/Y1 pathway. Evidence has suggested that the Y5 receptor is also involved in the control of food intake (51,52). Further studies are needed to elucidate the role of the hypothalamic NPY/Y5 receptor pathway in orexigenic action of ghrelin.

In this study, although the leptin-induced decrease in hypothalamic NPY mRNA expression is completely abolished by ICV co-injection of ghrelin, the satiety effect of leptin is only partially reversed by ghrelin. These observations suggest the

involvement of other orexigenic or anorexigenic systems in the antagonism of leptin action by ghrelin. Because Agouti-related protein (AGRP), an endogenous antagonist of the hypothalamic melanocortin system (24), is expressed in the arcuate NPY neurons (53), it is interesting to see whether ghrelin can increase hypothalamic AGRP mRNA expression. Indeed, we have observed that hypothalamic AGRP mRNA expression is increased significantly in rats that received a single ICV injection of ghrelin compared with vehicle-treated rats (M.S., Y.O., K.N, unpublished data). These observations suggest that the orexigenic effect of ghrelin is mediated at least partly by increased production of AGRP, which should contribute to the inhibition of the hypothalamic melanocortin system. In this regard, it is interesting to know the role of the hypothalamic α-MSH/MC4-R/AGRP pathway in the orexigenic action of ghrelin.

In conclusion, we demonstrate that ghrelin is a novel orexigenic peptide that antagonizes leptin action through the activation of hypothalamic NPY/Y1 receptor pathway. This study suggests that ghrelin may play an important role in the regulation of energy homeostasis.

ACKNOWLEDGMENTS

This work is supported in part by research grants from the Japanese Ministry of Education, Science, Sports, and Culture; the Japanese Ministry of Health and Welfare; the Yamanouchi Foundation for Research on Metabolic Disorders; the Foundation for Total Health Promotion; the Kato Memorial Trust for Nanbyo Research, the Inamori Foundation; the Naito Foundation; the Uehara Memorial Foundation; and "Research for the Future" (RFTF) of the Japanese Society for the Promotion of Science (JSPS-RFTF 96100204 and 98L00801).

We would like to thank S. Yura for discussions and M. Ihara and A. Kanatani for providing J-115814. The authors also acknowledge M. Nagamoto for technical assistance and Y. Isa and Y. Nakajima for secretarial assistance.

REFERENCES

1. Casanueva FF, Dieguez C: Growth hormone secretagogues: physiological role and clinical utility. *Trends Endocrinol Metab* 10:30–38, 1999
2. Howard AD, Feighner SD, Cully DF, Arena JP, Liberator PA, Rosenblum CI, Hamelin M, Hreniuk DL, Palyha OC, Anderson J, Paress PS, Diaz C, Chou M, Lui KK, McKee KK, Pong S-S, Chaung L-Y, Elbrecht A, Dashkevicz M, Heavens R, Rigby M, Sirinathsinghji DJS, Dean DC, Melillo DG, Patchett AA, Nargund R, Griffin PR, DeMartino JA, Gupta SK, Acheffer JM, Smith RG, Van der Pleog LHT: A receptor in pituitary and hypothalamus that functions in growth hormone release. *Science* 273:974–977, 1996
3. Guan XM, Yu H, Palyha OC, McKee KK, Feighner SD, Sirinathsinghji DJ, Smith RG, Van der Ploeg LH, Howard AD: Distribution of mRNA encoding the growth hormone secretagogue receptor in brain and peripheral tissues. *Mol Brain Res* 48:23–29, 1997
4. Yokote R, Sato M, Matsubara S, Ohye H, Niimi M, Murao K, Takahara J: Molecular cloning and gene expression of growth hormone-releasing peptide receptor in rat tissues. *Peptides* 19:15–20, 1998
5. Cheng K, Chan W-S, Butler B, Wei L, Schoen WR, Wyvratt MJ Jr, Fisher MH, Smith RG: Stimulation of growth hormone release from rat pituitary cells by L-692, 429, a novel no-peptidyl GH secretagogue. *Endocrinology* 132:2729–2731, 1993
6. Ong H, McNicoll N, Escher E, Collu R, Deghenghi R, Locatelli V, Ghigo E, Muccioli G, Boghen M, Nilsson M: Identification of a pituitary growth hormone-releasing peptide (GHRP) receptor subtype by photoaffinity labeling. *Endocrinology* 139:432–435, 1998
7. Prombo M, Barreiro J, Penalva A, Peino R, Dieguez C, Casaneuva FF: Absence of growth hormone (GH) secretion after the administration of either GH-releasing hormone (GHRH), GH-releasing peptide (GHRP-6), or GHRH plus PHRP-6 in children with neonatal pituitary stalk transection. *J Clin Endocrinol Metab* 80:3180–3184, 1995
8. Popovic V, Damjanovic S, Minic D, Djurovic M, Dieguez C, Vasanueva FF: Blocked growth hormone-releasing peptide (GHRP-6)-induced GH secretion and absence of the synergic action of GHRP-6 plus GH-releasing hormone in patients with hypothalamopituitary disconnection: evidence that GHRP-6 main action is exerted at the hypothalamic level. *J Clin Endocrinol Metab* 80:942–947, 1995
9. Svensson J, Lonn L, Jansson J-O, Murphy G, Wyss D, Krupa D, Cerchio K, Polvino W, Gertz B, Boseaus I, Sjpstrpm L, Bengtsson B-A: Two-month treatment of obese subjects with the oral growth hormone (GH) secretagogue MK-677 increases GH secretion, fat-free mass, and energy expenditure. *J Clin Endocrinol Metab* 83:362–369, 1998
10. Micic D, Casabiell X, Gualillo O, Pombo M, Dieguez C, Casanueva FF: Growth hormone secretagogues: the clinical future. *Horm Res* 51 (Suppl. 3):29–31, 1999
11. Kojima M, Hosoda H, Date Y, Nakazato M, Matsuo H, Kangawa K: Ghrelin is a growth-hormone-releasing acylated peptide from stomach. *Nature* 402:656–660, 1999
12. Locke W, Kirgis HD, Bowers CY, Abdoh AA: Intracerebroventricular growth hormone-releasing peptide-6 stimulates eating without affecting plasma growth hormone responses in rats. *Life Sci* 56:1347–1352, 1995
13. Okada K, Ishii S, Minami S, Sugihara H, Shibasaki T, Wakabayashi I: Intracerebroventricular administration of the growth hormone-releasing peptide KP-102 increases food intake in free-feeding rats. *Endocrinology* 137:5155–5159, 1996
14. Kamegai J, Hasegawa O, Minami S, Sugihara H, Wakabayashi I: The growth hormone-releasing peptide KP-102 induces *c-fos* expression in the arcuate nucleus. *Mol Brain Res* 39:153–159, 1996
15. Torsello A, Luoni M, Schweiger F, Grilli R, Guiri M, Bresciani E, Deghenghi R, Müller EE, Locatelli V: Novel hexarelin analogs stimulate feeding in the rat through a mechanism not involving growth hormone release. *Eur J Pharmacol* 360:123–129, 1998
16. Shibasaki T, Yamauchi N, Takeuchi K, Ishii S, Sugihara H, Wakabayashi I: The growth hormone secretagogue KP-102-induced stimulation of food intake is modified by fasting, restraint stress, and somatostatin in rats. *Neurosci Lett* 255:9–12, 1998
17. Dickson SL, Luckman SM: Induction of *c-fos* messenger ribonucleic acid in neuropeptide Y and growth hormone (GH)-releasing factor neurons in the rat arcuate nucleus following systemic injection of the GH secretagogue, GH-releasing peptide-6. *Endocrinology* 138:771–777, 1997
18. Willensen MG, Kristensen P, Romer J: Co-localization of growth hormone secretagogue receptor and NPY mRNA in the arcuate nucleus of the rat. *Neuroendocrinology* 70:306–316, 1999
19. Friedman JM, Halaas JL: Leptin and the regulation of body weight in mammals. *Nature* 395:763–770, 1998
20. Elmquist JK, Maratos-Flier E, Saper CB, Flier JS: Unraveling the central nervous system pathway underlying responses to leptin. *Nat Neurosci* 1:445–450, 1998
21. Schwartz MW, Woods SC, Porte D Jr, Seeley RJ, Baskin DG: Central nervous system control of food intake. *Nature* 404:661–671, 2000
22. Seeley RJ, Yagaloff KA, Fisher SL, Burn P, Thiele TE, van Dijk G, Baskin DG, Schwartz MW: Melanocortin receptors in leptin effects (Letter). *Nature* 390: 349, 1997
23. Satoh N, Ogawa Y, Katsuura G, Numata Y, Masuzaki H, Yoshimasa Y, Nakao K: Satiety effect and sympathetic activation of leptin are mediated by hypothalamic melanocortin system. *Neurosci Lett* 249:107–110, 1998
24. Ebihara K, Ogawa Y, Katsuura G, Numata Y, Masuzaki H, Satoh N, Tamaki M, Yoshioka T, Hayase M, Matsuoka N, Aizawa-Abe M, Yoshimasa Y, Nakao K: Involvement of Agouti-related protein, an endogenous antagonist of hypothalamic melanocortin receptor, in leptin action. *Diabetes* 48:2028–2033, 1999
25. Stephens TW, Basinski M, Bristow PK, Bue-Valleskey JM, Burgett SG, Craft L, Hale J, Hoffman J, Hsiung HM, Kriauciunas A, MacKeller W, Rosteck PR Jr, Schoner B, Smith D, Tinsley FC, Zhang X-Y, Heiman M: The role of neuropeptide Y in the antiobesity action of the obese gene product. *Nature* 377:530–532, 1995
26. Erickson JC, Clegg KE, Palmiter RD: Sensitivity to leptin and susceptibility to seizures of mice lacking neuropeptide Y. *Nature* 381:415–418, 1996
27. Erickson JC, Hollopeter G, Palmiter RD: Alteration of the obesity syndrome of *ob/ob* mice by the loss of neuropeptide Y. *Science* 274:1704–1707, 1996
28. Schwartz MW, Seeley RJ, Campfield LA, Burn P, Baskin DG: Identification of target of leptin action in rat hypothalamus. *J Clin Invest* 98:1101–1106, 1996
29. Mercer JG, Hoggard N, Williams LM, Lawrence CB, Hannah LT, Morgan PJ, Trayhurn P: Coexpression of leptin receptor and preproneuropeptide Y mRNA in arcuate nucleus of mouse hypothalamus. *J Neuroendocrinol* 8:733–735, 1996
30. Inui A: Transgenic approach to the study of body weight regulation. *Pharmacol Rev* 52:35–61, 2000

31. Michel MC, Beck-Sickinger A, Doods HC, Herzog H, Larhammar D, Quirion R, Schwartz T, Westfall T: XVI International Union of Pharmacology recommendations for the nomenclature of neuropeptide Y, peptide YY, and pancreatic polypeptide receptors. *Pharmacol Rev* 50:143–150, 1998
32. Ishihara A, Tanaka T, Kanatani A, Fukami T, Ihara M, Fukuroda T: A potent neuropeptide Y antagonist, 1229U91, suppressed spontaneous food intake in Zucker fatty rats. *Am J Physiol* 274:R1500–R1504, 1998
33. Wieland HA, Engel W, Eberlein W, Rudolf K, Doods HN: Subtype selectivity of the novel nonpeptide neuropeptide Y Y1 receptor antagonist BIBO3304 and its effect on feeding in rodents. *Br J Pharmacol* 125:549–555, 1998
34. Kanatani A, Kanno T, Ishihara A, Hata M, Sakuraba A, Tanaka T, Tsuchiya Y, Mase T, Fukuroda T, Fukami T, Ihara M: The novel neuropeptide Y Y1 receptor antagonist J-104870: a potent feeding suppressant with oral bioavailability. *Biochem Biophys Res Commun* 266:88–91, 1999
35. Pedrazzini T, Seydoux J, Kunstner P, Aubert J-F, Grouzmann E, Beermann F, Brunner H-R: Cardiovascular response, feeding behavior and locomotor activity in mice lacking the NPY Y1 receptor. *Nat Med* 4:722–726, 1998
36. Frederich RC, Hamann A, Anderson S, Lollmann B, Lowell BB, Flier JS: Leptin levels reflect body lipid content in mice: evidence for diet-induced resistance to leptin action. *Nat Med* 1:1311–1314, 1995
37. Maffei M, Halaas J, Ravussin E, Pratley RE, Lee GH, Zhang Y, Fei H, Kim S, Lallone R, Ranganathan S, Kern PA, Friedman JM: Leptin levels in human and rodent: measurement of plasma leptin and *ob* RNA in obese and weight-reduced subjects. *Nat Med* 1:1155–1161, 1995
38. Considine RV, Sinha MK, Heiman ML, Kriauciunas A, Stephens TW, Nyce MR, Ohannesian JP, Marco CC, Mckee LJ, Bauer TL, Caro JF: Serum immunoreactive-leptin concentrations in normal-weight and obese humans. *N Engl J Med* 334:292–295, 1996
39. Smith FJ, Campfield LA, Moschera JA, Bailon PS, Burn P: Feeding inhibition by neuropeptide Y. *Nature* 382:307, 1996
40. Wang Q, Bing C, Al-Barazanji K, Mossakowaska DE, Wang X-M, McBay DL, Neville WA, Taddayon M, Pickavance L, Dryden S, Thomas MEA, McHale MT, Gloyer IS, Wilson S, Buckingham R, Arch JRS, Trayhurn P, Williams G: Interactions between leptin and hypothalamic neuropeptide Y neurons in the control of food intake and energy homeostasis in the rat. *Diabetes* 46:335–341, 1997
41. Cusin I, Rohner-Jeanrenaud F, Stricker-Krongrad A, Jeanrenaud B: The weight-reducing effect of an intracerebroventricular bolus injection of leptin in genetically obese *fa/fa* rats: reduced sensitivity compared with lean animals. *Diabetes* 45:1446–1450, 1996
42. Okuma S, Kawashima S: Spontaneous dwarf rat. *Exp Anim* 29:301–304, 1980
43. Takeuchi T, Suzuki H, Sakurai S, Nogami H, Okumura S, Ishikawa H: Molecular mechanism of growth hormone (GH) deficiency in the spontaneous dwarf rat: detection of abnormal splicing of GH messenger ribonucleic acid by the polymerase chain reaction. *Endocrinology* 126:31–38, 1990
44. Paxions G, Watson C: *The Rat Brain In Stereotaxic Coordinates*. 2nd ed. Sydney, Academic, 1986
45. Date Y, Murakami N, Kojima M, Kuroiwa T, Matsukura S, Kangawa K, Nakazato M: Central effect of a novel acylated peptide ghrelin on growth hormone release in rats. *Biochem Biophys Res Commun* 275:477–480, 2000
46. Guillaume V, Magnan E, Cataldi M, Dutour A, Sauze N, Renard M, Razafindraibe H, Conte-Devolx B, Deghenghi R, Lenaerts V, Oliver C: Growth hormone (GH)-releasing hormone secretion is stimulated by a new GH-releasing hexapeptide in sheep. *Endocrinology* 135:1073–1076, 1994
47. Vaccarino FJ, Bloom FE, Rivier J, Vale W, Koob GF: Stimulation of food intake in rats by centrally administered hypothalamic growth hormone-releasing factor. *Nature* 314:167–168, 1985
48. Wilding JP, Gilbey SG, Bailey CJ, Batt RA, Williams G, Ghatei MA, Bloom SR: Increased neuropeptide-Y messenger ribonucleic acid (mRNA) and decreased neurotensin mRNA in the hypothalamus of the obese (*ob/ob*) mouse. *Endocrinology* 132:1939–1944, 1993
49. Sanacora G, Kershaw M, Finkelstein JA, White JD: Increased hypothalamic content of preproneuropeptide Y messenger ribonucleic acid in genetically obese Zucker rats and its regulation by food deprivation. *Endocrinology* 127:730–737, 1990
50. Baskin DG, Breininger JF, Schwartz MW: Leptin receptor mRNA identifies a subpopulation of neuropeptide Y neurons activated by fasting in rat hypothalamus. *Diabetes* 48:828–833, 1999
51. Gerald C, Qalker MW, Criscione L, Gustafson EL, Hartmann CB, Smith KE, Vaysse P, Durkin MM, Laz TM, Linemeyer Dl, Schaffhauser AO, Whitevread S, Hoffbauer KG, Taber RI, Brancheck TA, Weinshank RL: A receptor subtype involved in neuropeptide-Y-induced food intake. *Nature* 382:168–171, 1996
52. Marsh DJ, Hollopeter G, Kafer KE, Palmiter RD: Role of the Y5 neuropeptide Y receptor in feeding and obesity. *Nat Med* 4:718–721, 1998
53. Hahn TM, Breininger JF, Baskin DG, Schwartz MW: Coexpression of Agrp and NPY in fasting-activated hypothalamic neurons. *Nat Neurosci* 1:271–272, 1998