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Agouti-Related Peptide, Neuropeptide Y, and Somatostatin-Producing Neurons Are Targets for Ghrelin Actions in the Rat Hypothalamus

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Ghrelin, the endogenous ligand of the GH secretagogue receptor, acts at central level to elicit GH release and regulate food intake. To elucidate the neural circuit that exerts its effects, we measured the expression of hypothalamic neuropeptides involved in weight regulation and GH secretion after ghrelin administration. Adult male rats, fed or fasted for 72 h, were treated centrally (intracerebroventicularly) with a single dose of ghrelin (5 μg). After 2, 4, and 6 or 8 h, agouti-related peptide, melanin-concentrating hormone, neuropeptide Y, prepro-orexin, GHRH, and somatostatin mRNA levels were measured by *in situ* hybridization. We found that ghrelin increased agouti-related peptide and neuropeptide Y expression in the arcuate nucleus of the hypothalamus of fed and fasted rats. In contrast, no change was demonstrated in the mRNA levels of the other neuropeptides studied at any time evaluated. Finally, we examined the effect of ghrelin on GHRH and somatostatin mRNA levels in GH-deficient (dwarf) rats. Our results show that ghrelin increases somatostatin mRNA levels in the hypothalamus of these rats. This study furthers our understanding of the molecular basis and mechanisms involved in the effect of ghrelin on food intake and GH secretion. (*Endocrinology* 144: 544–551, 2003)

GH SECRETAGOGUES (GHSs) are artificial compounds that release GH in all species tested to date. Until 1999, these molecules mimicked an unknown endogenous factor that activates the GHS receptor (GHS-R) (1). The earlier cloning of GHS-R suggested that an endogenous ligand for this receptor might exist (2). Indeed, after intensive research by different groups, the isolation of an endogenous ligand of the GHS-R, ghrelin (3), was recently reported. The purified ligand was found to be a peptide of 28 amino acids, in which the serine 3 residue was *n*-octanoylated. More recently, a second endogenous ligand for the GHS-R, des-Gln^{14}-ghrelin, whose biological activity and sequence are identical to ghrelin except for one glutamine in position 14, has been purified and characterized (4). These peptides have been shown to exert a very potent and specific GH-releasing activity *in vitro* and *in vivo* as well as to increase the transcription rate of the Pit gene (5, 6). Taking into account that ghrelin is secreted prevalently from the stomach and circulates in normal subjects at considerable plasma concentrations, it has been postulated that this molecule is secreted from the stomach, circulates in the bloodstream, and stimulates GH synthesis and secretion by the somatotrophs (3). Moreover, ghrelin has emerged as a regulatory signal involved in energy homeostasis (7), reproduction (8), and gastrointestinal (9, 10) and cardiovascular (11–13), function among others.

Recent data have led to the recognition that ghrelin plays an important role in energy homeostasis, and the evidence is as follows. Ghrelin administration induces a positive energy balance in rodents by decreasing fat utilization without significantly changing energy expenditure or locomotor activity (14, 15). The effect of ghrelin appears to be exerted at the central level, and its chronic administration is associated with metabolic changes that lead to an efficient metabolic state, resulting in increased body weight and fat mass (14, 15). In keeping with this, an inverse relationship has been reported between plasma ghrelin levels and body mass index in humans (16).

Recent findings have demonstrated the interaction between ghrelin and orexigenic systems in the rat hypothalamus. Therefore, GHS-R mRNA is expressed in neuropeptide Y/agouti-related peptide (NPY/AgRP) neurons in the arcuate nucleus of the hypothalamus (ARC) (17–19), and the central administration of ghrelin increases the mRNA content of NPY and AgRP in the ARC (15, 20, 21). On the other hand, the lack of expression of the GHS-R in the lateral hypothalamus (LHA) (17), where a large number of orexigenic neurons are located (22, 23), suggested that this hypothalamic nucleus was unlikely to be a target of the effects of ghrelin on food intake. However, some of the biological effects of ghrelin might be mediated by as yet unknown receptors, different from the only one cloned until now (24, 25). In keeping with this possibility, it was found that ghrelin administration leads to increased c-Fos expression in neurons of the LHA (26). Taking into consideration that the main central roles of ghrelin appear to be those related to the regulation of body weight and GH secretion, we decided to study the hypothalamic expression of neuropeptides implicated in both processes. Specifically, the functional linkage of ghrelin and body weight homeostasis was carried out by

Abbreviations: AgRP, Agouti-related peptide; ARC, arcuate nucleus of the hypothalamus; GHS, GH secretagogue; GHS-R, GH secretagogue receptor; icv, intracerebroventicularly; LHA, lateral hypothalamus; MCH, melanin-concentrating hormone; NPY, neuropeptide Y; PeN, periventricular nucleus of the hypothalamus; prepro-OX, prepro-orexin; SSC, standard saline citrate; SST, somatostatin.

measuring hypothalamic AgRP, melanin-concentrating hormone (MCH), NPY, and prepro-orexin (prepro-OX) mRNA levels. Furthermore, we examined the effect of ghrelin on AgRP and NPY in the adaptation to fasting, because their responses are frequently dependent on the nutritional status of the animals (27, 28).

Finally, we assessed the effect of ghrelin on GHRH and somatostatin (SST) mRNA levels. These two neuropeptides are the major regulators of GH secretion (29). Furthermore, in view of the fact that GH feeds back to regulate GHRH and SST expression at the hypothalamic level, we also studied the effects of ghrelin in an experimental model of GH deficiency, namely the dwarf Lewis rat (30–32). This model displays an autosomal recessive mutation due to a selective failure in GHRH signaling in the somatotrope cell, which results in almost undetectable plasma GH levels (30, 31). Therefore, these rats provide a unique opportunity to study the regulation of GHRH and SST in the practical absence of endogenous GH.

TABLE 1. Antisense oligonucleotides for rat AgRP, rat GHRH, rat MCH, rat NPY, rat prepro-OX, and rat SST

mRNA	GenBank accession no.	Antisense oligonucleotide sequence	5′ position
AgRP	AF206017	5′-CGACGCGGAGAACGAGACTCGCGGTTCTGTGGATCTAGCACCTCTGCC-3′	136
GHRH	U10156	5′-CCGGTAGCTGCTGGTGAAGATGGCGTCTGC-3′	220
MCH	M29712	5′-CCAACAGGGTCGGTAGACTCGTCCCAGCAT-3′	529
NPY	M20373	5′-AGATGAGATGTGGGGGGAAACTAGGAAAAGTCAGGAGAGCAAGTTTCATT-3′	400
Prepro-OX	AF041241	5′-TTCGTAGAGACGGCAGGAACACGTCTTCTGGCGACA-3′	240
SST	NM_012659	5′-GTTCGAGTTGGCAGACCTCTGCAGCTCCAG-3′	339

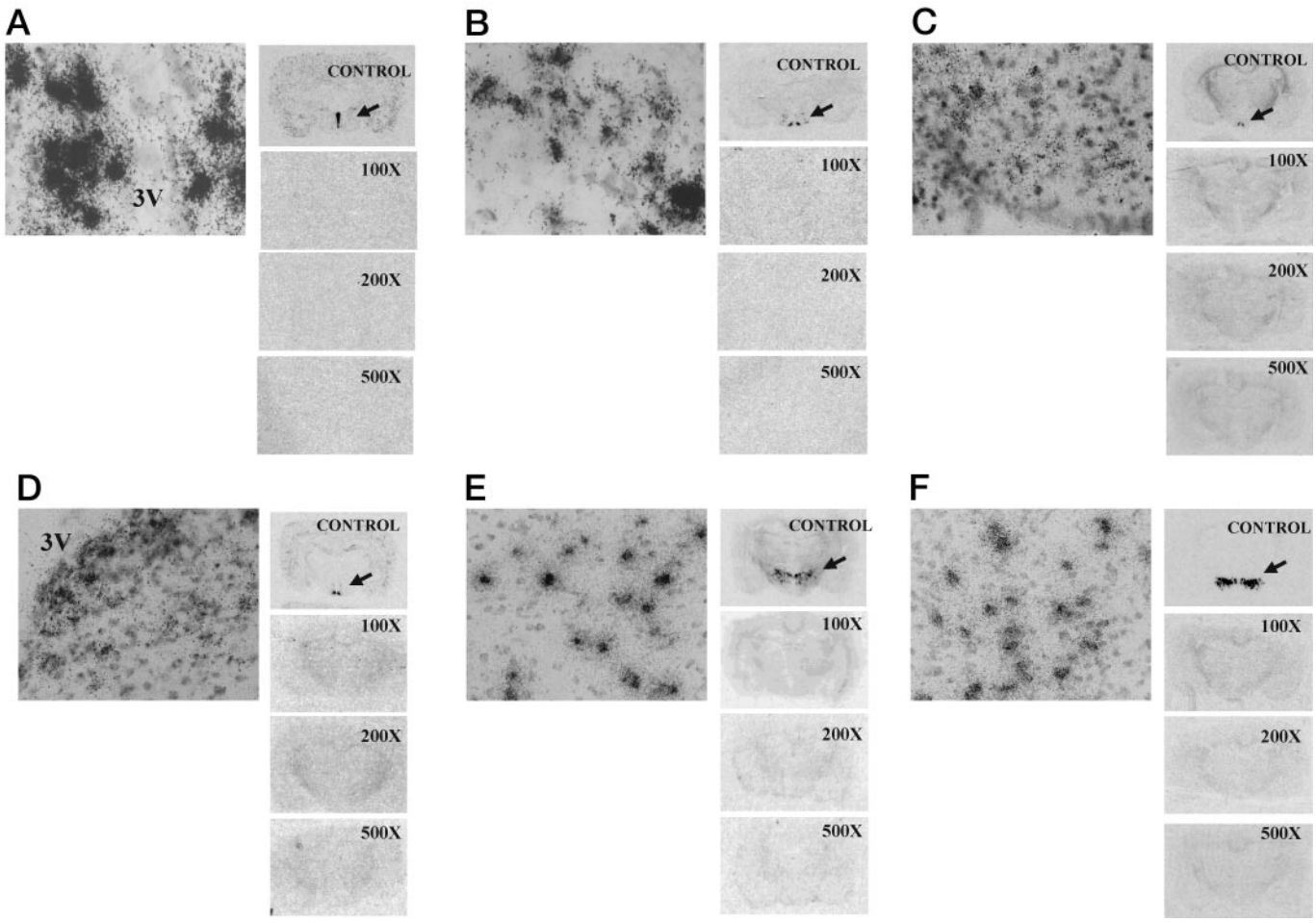

FIG. 1. *Left panels*: A, Brightfield photomicrographs of cells in the PeN that were incubated with a ^{35}S-labeled antisense oligonucleotide SST probe (magnification, ×400). B, Brightfield photomicrographs of cells in the ARC that were incubated with a ^{35}S-labeled antisense oligonucleotide GHRH probe (magnification, ×400). C, Brightfield photomicrographs of cells in the ARC that were incubated with a ^{35}S-labeled antisense oligonucleotide AgRP probe (magnification, ×200). D, Cells in the ARC that were incubated with a ^{35}S-labeled antisense oligonucleotide NPY probe (magnification, ×200). E, Cells in the LHA that were incubated with a ^{35}S-labeled antisense oligonucleotide MCH probe (magnification, ×200). F, Cells in the LHA that were incubated with a ^{35}S-labeled antisense oligonucleotide prepro-OX probe (magnification, ×200). *Right panels*, Autoradiographic images (magnification, ×1) of representative brain coronal sections, incubated with a ^{35}S-labeled antisense oligonucleotide probe in the absence (control) or presence of crescent amounts of nonlabeled (×100, ×200, and ×500) oligonucleotide probes. A, SST; B, GHRH; C, AgRP; D, NPY; E, MCH; F, prepro-OX. 3V, Third ventricle.

Materials and Methods

Experimental animals

We used four different rats models: 1) adult male Sprague Dawley rats (250–300 g, 9–11 wk old; Animalario General University of Santiago de Compostela, Santiago de Compostela, Spain), 2) dwarf (HsdOla: dw-4) Lewis rats (150–175 g, 10–12 wk old; Harlan Ibérica, Barcelona, Spain), 3) Lewis rats age-matched (250–300 g, 10–12 wk old) with dwarf Lewis rats (Charles River, Lyon, France), and 4) Lewis rats weight-matched (150–175 g, 6–8 wk old) with dwarf Lewis rats. The animals were allowed free access to standard laboratory pellets of rat chow and tap water. The protocols were approved by the ethics committee of the University of Santiago de Compostela, and experiments were performed in accordance to the rules of laboratory animal care and international law on animal experimen-tation.

Implantation of intracerebroventricular (icv) cannulas and ghrelin treatment

Chronic icv cannulas were implanted under ketamine-xylazine anesthesia (50 mg/kg, ip), as described previously (33, 34) and were demonstrated to be located in the lateral ventricle by methylene blue staining. The animals were caged individually and used for the experiments 1 wk later. During this postoperative recovery period the rats became accustomed to the handling procedure under nonstressful conditions. After this time one group of rats continued to have food available *ad libitum* (fed rats), and the other group was deprived of food for 72 h (fast 72 h). To confirm the efficiency of the food deprivation, plasma leptin levels were measured in the described experimental groups of animals, using a commercial kit (Linco Research, Inc., St. Charles, MO) (35). Food deprivation for 72 h significantly decreased plasma leptin levels (fed rats, 5.1 ± 0.7 ng/ml; fast 72 h rats, 0.1 ± 0.04 ng/ml; $P < 0.001$). Thereafter, rats received either a single administration of ghrelin (Bachem, Bubendorf, Switzerland; 5 μg/rat dissolved in 5 μl distilled water saturated with argon) or vehicle (control rats). Two, 4, 6, or 8 h after the treatment, vehicle- and ghrelin-treated animals were quickly decapitated in an independent room, and their brains were removed rapidly. For *in situ* hybridization, the whole brains were maintained at −80 C until processed. All treatments started in the lights on phase (0900 h). Six to eight animals were used in each experimental group.

In situ hybridization

Coronal hypothalamic sections (16 μm) were cut on a cryostat and immediately stored at −80 C until hybridization. For AgRP, MCH, NPY, prepro-OX, GHRH, and SST mRNA detection we employed the specific antisense oligodeoxynucleotides (Table 1). These probes were 3′-end labeled with [α-^{35}S]deoxy-ATP using terminal deoxynucleotidyl transferase. The specificity of the probes was confirmed by incubating the sections with an excess of the unlabeled probes (Fig. 1). *In situ* hybridizations were performed as previously reported (34, 36). The frozen sections were fixed with 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4) at room temperature for 30 min. They were then dehydrated using 70%, 80%, 90%, 95%, and absolute ethanol (5 min each). The

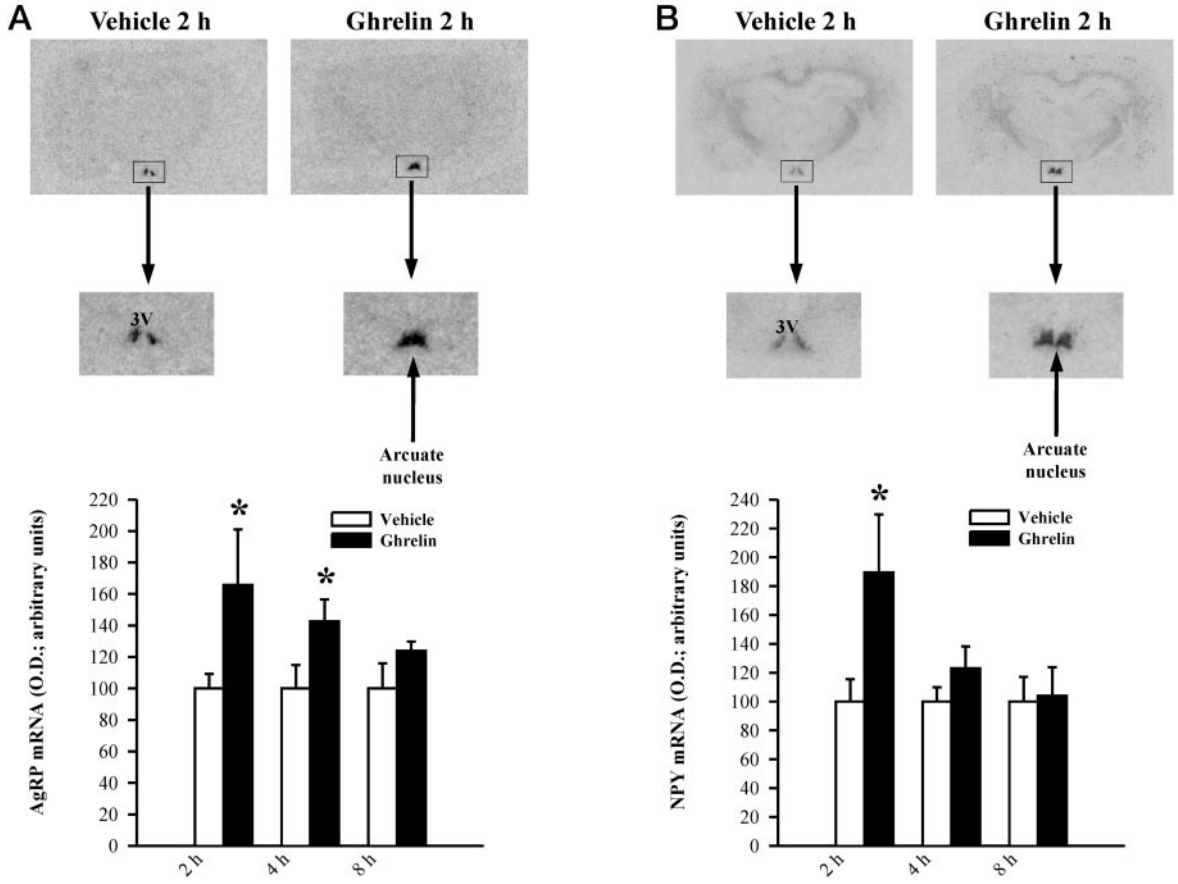

FIG. 2. A, *Upper panel*, Autoradiographic images (magnification, ×5) of representative brain coronal sections, incubated with a ^{35}S-labeled antisense oligonucleotide AgRP probe, in the vehicle- and ghrelin-treated groups for 2 h. Areas delineated in both sections are shown at higher magnification in the *bottom* (magnification, ×60). *Lower panel*, AgRP mRNA levels in the ARC of the described experimental groups. *, $P < 0.05$ *vs.* vehicle treated for 2 and 4 h, respectively. 3V, Third ventricle. B, *Upper panel*, Autoradiographic images (magnification, ×5) of representative brain coronal sections, incubated with a ^{35}S-labeled antisense olignucleotide NPY probe, in the vehicle-treated group and the ghrelin-treated group for 2 h. Areas delineated in both sections are shown at higher magnification in the *bottom* (magnification, ×60). 3V, Third venticle. *Lower panel*, NPY mRNA levels in the ARC of the described experimental groups. *, $P < 0.05$ *vs.* vehicle treated for 2 h.

hybridization was carried out overnight at 37 C in a moist chamber. Hybridization solution contained 5×10^5 (AgRP, prepro-OX, SST, and GHRH) or 1×10^6 cpm (MCH and NPY) per slide of the labeled probe, 4× standard saline citrate (SSC), 50% deionized formamide, 1× Denhardt's solution, 10% dextran sulfate, and 10 μg/ml sheared, single-stranded salmon sperm DNA. Afterward, the hybridization sections were sequentially washed in 1× SSC at room temperature, four times in 1× SSC at 42 C (30 min/wash), and once in 1× SSC at room temperature (1 h), and then rinsed in water and ethanol. Finally, the sections were air-dried and exposed to Hyperfilm β-Max (Amersham International, Little Chalfont, UK) at room temperature for 4–6 d for AgRP, MCH, NPY, SST, and prepro-OX and for 14 d for GHRH. After the films were developed, sections were dipped in Ilford K5 autoradiographic emulsion (Ilford, UK) and exposed for 2 wk (AgRP, MCH, NPY, SST, and prepro-OX) or 6 wk (GHRH) at 4 C. The slides were then developed in Kodak D-19 developer (Eastman Kodak Co., Rochester, NY), fixed (Kodak fixer), and counterstained with methylene blue.

To compare anatomically similar regions, the slides were matched according to the rat brain atlas of Paxinos and Watson (37). The slides from control and treated animals at each treatment time were always exposed to the same autoradiographic film. All sections were scanned, and the specific hybridization signal was quantified by densitometry using a digital imaging system (Molecular Analyst, Bio-Rad Laboratories, Inc., Richmond, CA) (34, 38). The OD of the hybridization signal was determined and subsequently corrected by the OD of its adjacent background value. For this reason a rectangle, with the same dimensions in each case, was drawn enclosing the hybridization signal over each nucleus and over adjacent brain areas of each section (background). We used 16–20 sections for each animal (4–5 slides, 4 sections/slide). The mean of these 16–20 values was used as the densitometry value for each animal. The coefficients of intraindividual variation were 5.8% for AgRP, 6.3% for MCH, 6.9% for NPY, 5.4% for prepro-OX, 6.6% for GHRH, and 6.0% for SST.

Statistical analysis and data presentation

In experiments with wild-type normal rats, the data were analyzed using a nonparametric Mann-Whitney test. We compared control with treated animals. To analyze the time dependence effect of the treatment, we performed an ordinary ANOVA followed by a *post hoc* Bonferroni test for the treated groups at the different time points. For each test, significance was set at $P < 0.05$.

In experiments with dwarf Lewis rats treated with ghrelin, the data were analyzed using ANOVA. Therefore, we compared Lewis rats (age- and weight-matched) treated with vehicle, dwarf rats treated with vehicle, and dwarf rats treated with ghrelin. The data (mean ± SEM) were expressed as the percentage of change in relation to Lewis rats age-matched and treated with vehicle (100%).

Because of the presence of circadian changes in the hypothalamic mRNA content of the neuropeptides evaluated in this study (39–44), the results were normalized by making comparisons with control animals killed the same day and at the same hour. The coefficients of interindividual variation were 20.7% for AgRP, 12.7% for MCH, 20.9% for NPY, 10.9% for prepro-OX, 13.1% (wild-type rats) and 14.4% (dwarf rats) for GHRH, and 10.8% (wild-type rats) and 12.7% (dwarf rats) for SST.

In each case the data (mean ± SEM) were expressed as the percentage

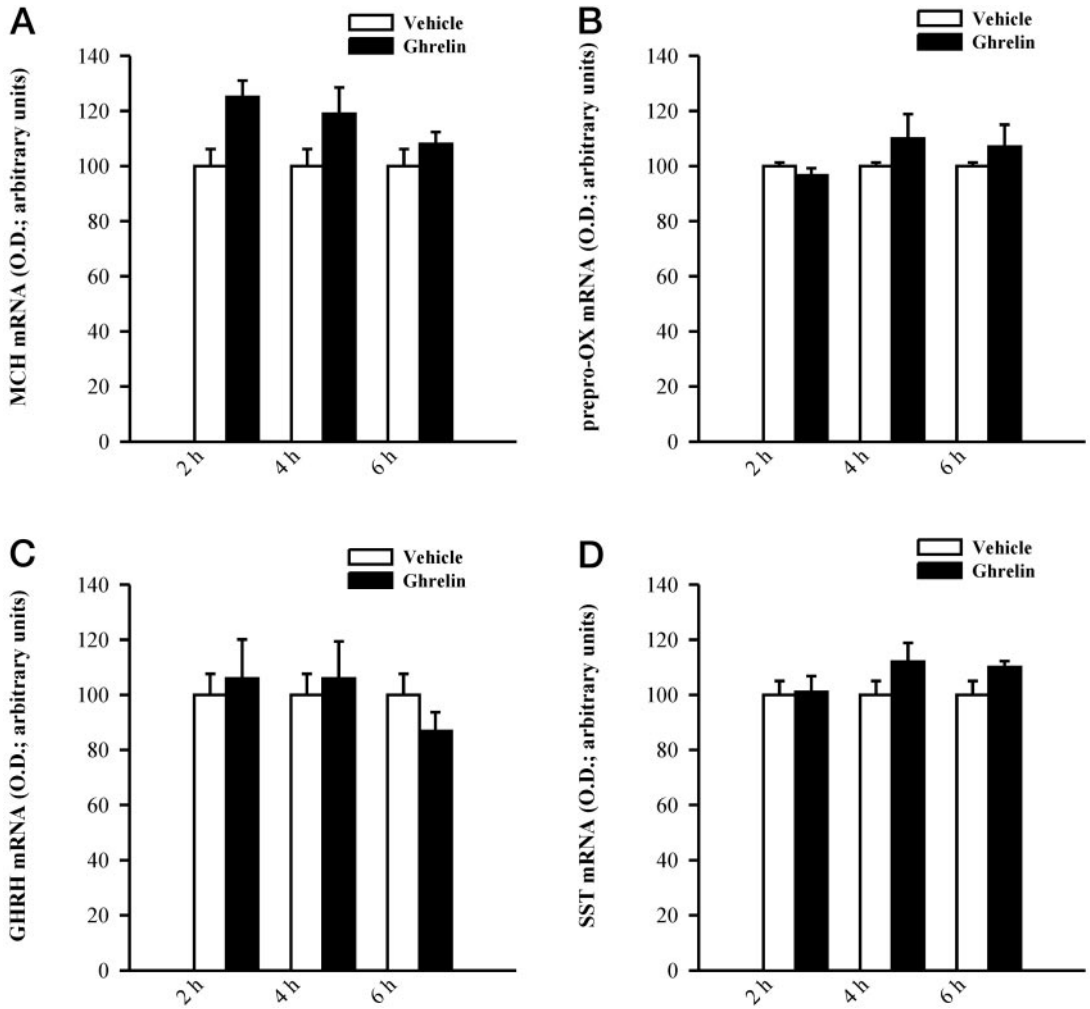

FIG. 3. Effect of ghrelin on mRNA levels of MCH in the LHA (A), prepro-OX in the LHA (B), GHRH in the ARC (C), and SST in the PeN (D).

of change in relation to control values (vehicle-treated animals = 100%). All statistical analyses and graphical representations presented in this work have been performed using 1 data point/animal, obtained from the mean of the 16–20 sections for each animal.

Results

In agreement with previous reports (36, 45), our *in situ* hybridization histochemistry showed SST mRNA-positive cells distributed widely throughout the hypothalamus. The highest density of positive cells was located in the periventricular nucleus of the hypothalamus (PeN) (Fig. 1A). *In situ* hybridization with an oligoprobe for GHRH mRNA demonstrated labeling in numerous cells in the ventrolateral part of the ARC (36, 45) (Fig. 1B).

In agreement with previous reports (46) our *in situ* hybridization histochemistry showed AgRP (Fig. 1C) and NPY (Fig. 1D) mRNA-positive cells located in the ARC. *In situ* hybridization with oligoprobes for MCH (Fig. 1E) and prepro-OX (Fig. 1F) mRNA demonstrated labeling of numerous cells in the LHA, as previously reported (46, 47). In all cases, incubation of the sections with an excess (100-, 200-, and 500-fold) of the unlabeled probes displaced the positive signal (Fig. 1, *right panels*).

Treatment with ghrelin increases AgRP and NPY mRNA levels in the ARC of fed rats

Using *in situ* hybridization analysis, a significant increase in AgRP (Fig. 2A) and NPY (Fig. 2B) mRNA levels was observed in the ARC after treatment with ghrelin. This increase was transient, being evident 2 and 4 h after treatment in the case of AgRP and 2 h after treatment in the case of NPY. On the other hand, we did not find any change in the mRNA content of MCH, prepro-OX (in the LHA), GHRH (in the

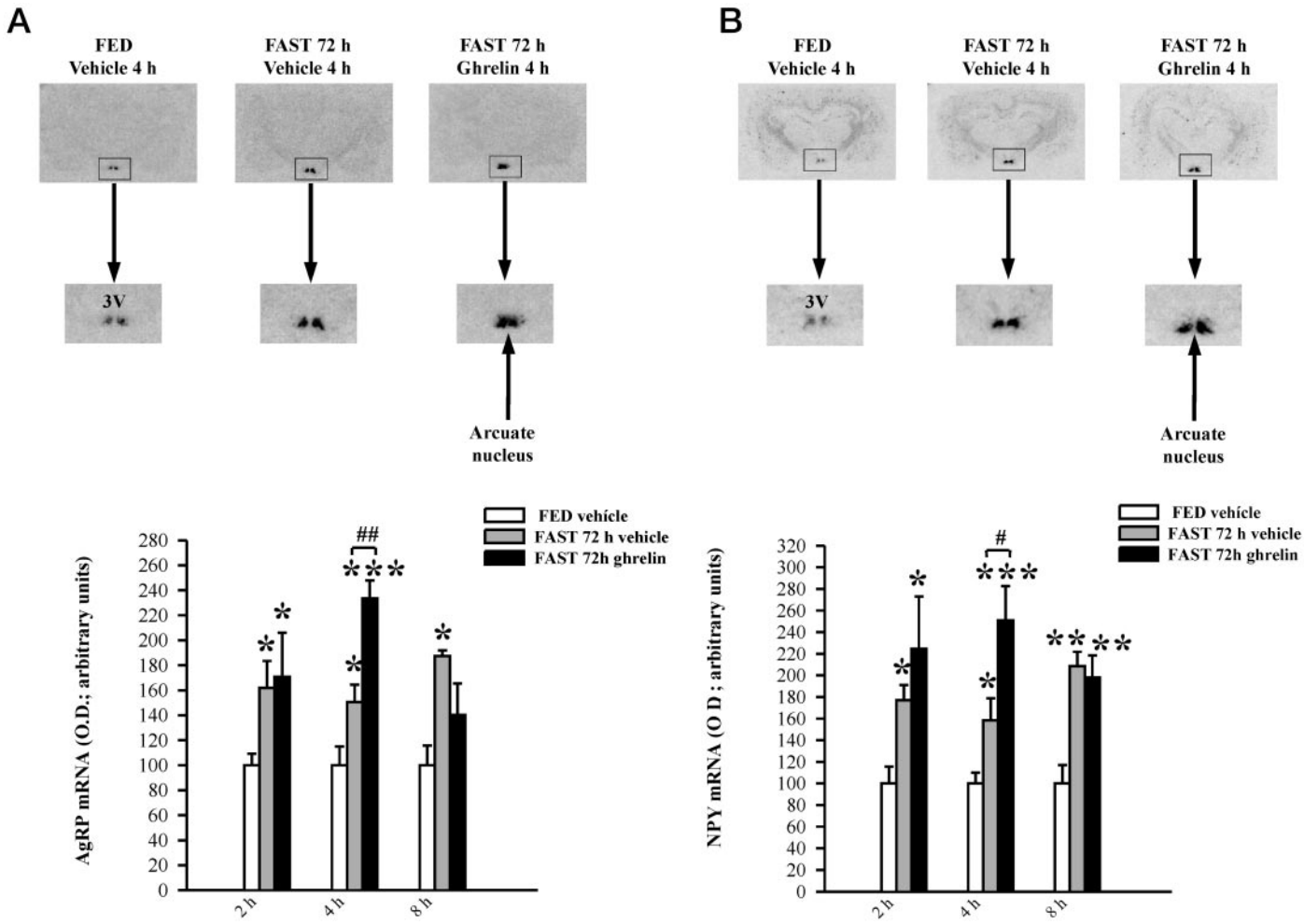

FIG. 4. A, *Upper panel*, Autoradiographic images (magnification, ×1.6) of representative brain coronal sections, incubated with a ^{35}S-labeled antisense oligonucleotide AgRP probe, in the vehicle-treated group for 4 h, the 72-h fasted group treated with vehicle for 4 h, and the 72-h fasted group treated with ghrelin for 4 h. Areas delineated in the sections are shown at higher magnification in the *bottom* (magnification, ×15). 3V, Third ventricle. *Lower panel*, AgRP mRNA levels in the ARC of the described experimental groups. *, $P < 0.05$ *vs.* vehicle-treated for 2, 4, and 8 h, respectively; ***, $P < 0.001$ *vs.* vehicle-treated for 4 h; ##, $P < 0.01$, 72-h fasted treated with vehicle for 4 h *vs.* 72-h fasted treated with ghrelin for 4 h. B, *Upper panel*, Autoradiographic images of representative brain coronal sections, incubated with a ^{35}S-labeled antisense oligonucleotide NPY probe, in the vehicle-treated group for 4 h, the 72-h fasted group treated with vehicle for 4 h, and the 72-h fasted group treated with ghrelin for 4 h. Areas delineated in the sections are shown at higher magnification in the *bottom.* 3V, Third ventricle. *Lower panel*, NPY mRNA levels in the ARC of the described experimental groups. *, $P < 0.05$ *vs*. vehicle treated for 2 h and 4 h, respectively; **, $P < 0.01$ *vs*. vehicle treated for 8 h; ***, $P < 0.001$ *vs*. vehicle for 4 h; #, $P < 0.05$, 72-h fasted treated with vehicle for 4 h *vs*. 72-h fasted treated with ghrelin for 4 h.

ARC), and SST (in the PeN) at any time point (2, 4, and 6 h) as assessed by *in situ* hybridization (Fig. 3).

Treatment with ghrelin increases NPY and AgRP mRNA levels in the ARC of fasted rats

Using *in situ* hybridization analysis, a significant increase in AgRP (Fig. 4A) and NPY (Fig. 4B) mRNA levels was observed in the ARC after 72 h of food deprivation. In fasted rats treated with ghrelin, we observed a further increase in AgRP and NPY mRNA content in the ARC 4 h after the treatment compared with fasted vehicle-treated rats (Fig. 4).

Ghrelin increases SST mRNA levels in the PeN of dwarf Lewis rats

Using *in situ* hybridization analysis, we detected a significant decrease in SST mRNA content in the PeN of dwarf Lewis rats compared with vehicle-treated, wild-type (age- and weight-matched) Lewis rats (Fig. 5A). Treatment with ghrelin increased SST mRNA levels in the PeN of dwarf Lewis rats 6 h after treatment, reaching levels observed in vehicle-treated (age- and weight-matched) Lewis rats (Fig. 5A). The GHRH mRNA content in the ARC was increased in dwarf Lewis rats compared with vehicle-treated wild-type (age- and weight-matched) Lewis rats. Ghrelin did not change GHRH mRNA levels in dwarf Lewis rats 6 h after treatment (Fig. 5B).

Discussion

In agreement with previous studies (15, 20, 21) we found that *in vivo* ghrelin administration to *ad libitum* fed rats led to a clear-cut increase in AgRP and NPY mRNA contents in the medial ARC, thus indicating that AgRP/NPY neurons

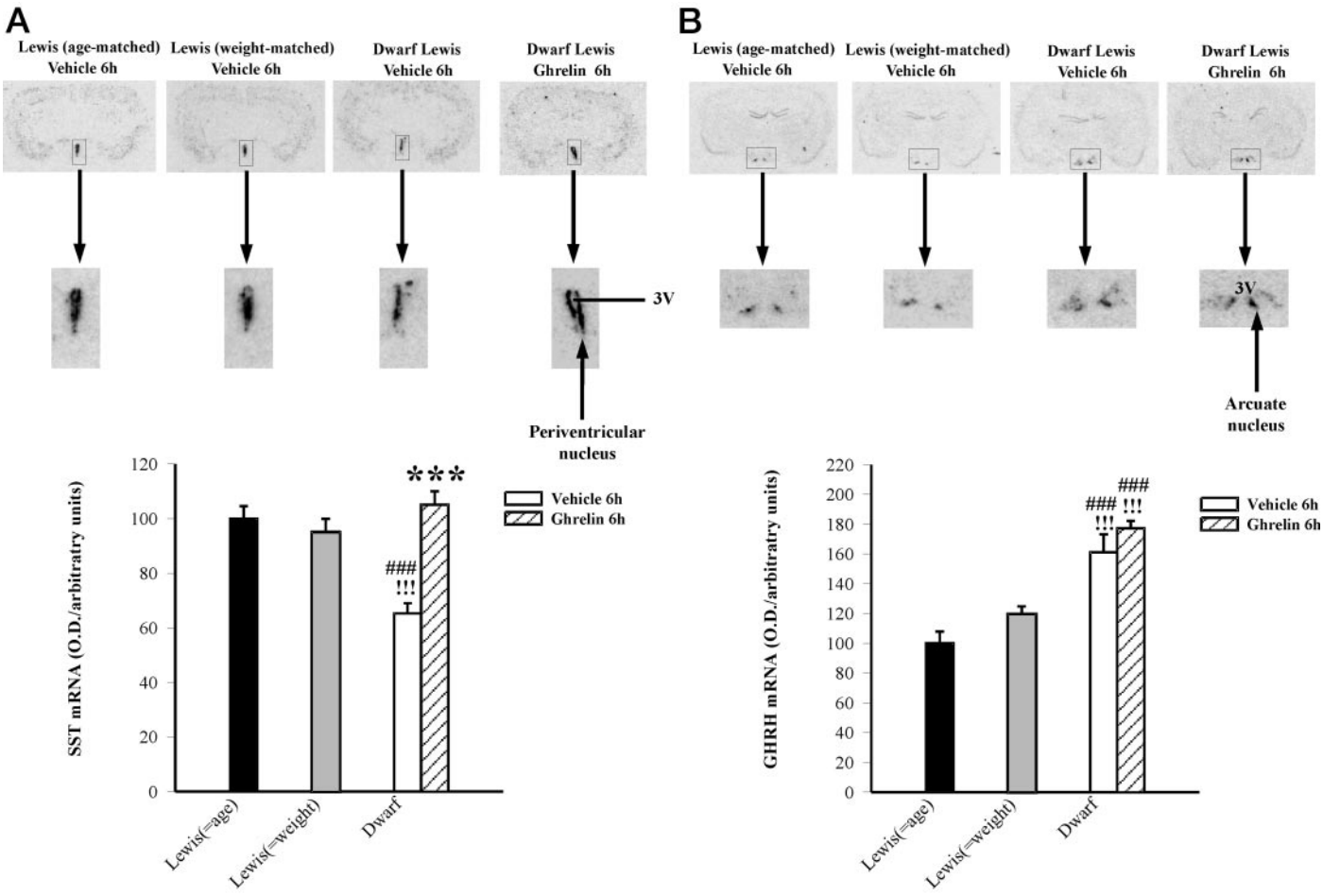

FIG. 5. A, *Upper panel*, Autoradiographic images (magnification, ×1) of representative brain coronal sections, incubated with a ^{35}S-labeled antisense oligonucleotide SST probe, in age-matched Lewis rats treated with vehicle for 6 h, weight-matched Lewis rats treated with vehicle for 6 h, dwarf Lewis rats treated with vehicle for 6 h, and dwarf Lewis rats treated with ghrelin for 6 h. Areas delineated in the sections are shown at higher magnification in the *bottom* (magnification, ×10). 3V, Third ventricle. *Lower panel*, SST mRNA levels in the PeN of the described experimental groups. ***, $P < 0.001$ *vs.* dwarf Lewis vehicle treated for 6 h; ###, $P < 0.001$ *vs.* age-matched Lewis rats treated with vehicle for 6 h; !!!, $P < 0.001$ *vs.* weight-matched Lewis rats treated with vehicle for 6 h. B, *Upper panel*, Autoradiographic images (×1) of representative brain coronal sections, incubated with a ^{35}S-labeled antisense oligonucleotide GHRH probe, in age-matched Lewis rats treated with vehicle for 6 h, weight-matched Lewis rats treated with vehicle for 6 h, dwarf Lewis rats treated with vehicle for 6 h, and dwarf Lewis rats treated with ghrelin for 6 h. Areas delineated in the sections are shown at higher magnification in the *bottom* (×6). 3V, Third ventricle. *Lower panel*, GHRH mRNA levels in the ARC of the described experimental groups. ###, $P < 0.001$ *vs.* age-matched Lewis rats treated with vehicle for 6 h; !!!, $P < 0.001$ *vs.* weight-matched Lewis rats treated with vehicle for 6 h.

are the primary targets of ghrelin orexigenic actions in the hypothalamus. The effect appears to be quite specific, as MCH and prepro-OX mRNA contents in the LHA were unchanged after treatment with ghrelin, thus indicating that these two orexigenic neuropeptides are not involved in the induction of food intake elicited by ghrelin. This was somewhat surprising, because it was recently shown that icv treatment with GHRP-6 (a synthetic GHS) induces c-Fos immunoreactivity in orexin-containing neurons in the LHA (26). It is therefore possible that other neuropeptides synthesizing neurons in this area, such as dynorphin (48) or galanin (49), are targets of ghrelin. Alternatively, it is possible that the effect of GHRP-6 at this level is mediated through a receptor not activated by ghrelin. The finding that the cloned GHS-R does not appear to be expressed in the LHA supports this latter possibility (17). Moreover, similar discrepancies have been shown between the effects of ghrelin and synthetic GHS in other peripheral cell types (50–52).

To further characterize the role of ghrelin on the AgRP/NPY neurons we studied its effects in food-deprived animals. It is known that the nutritional status of the animals markedly influences the hypothalamic expression of neuropeptides involved in the regulation of food intake as well as their set-point and responsiveness to a vast array of stimuli (22, 23, 27, 28). As expected, we found that AgRP and NPY mRNA levels in the ARC were increased after 72 h of food deprivation. Taking into account that fasting increases ghrelin-circulating levels (53, 54), it is possible that the elevation of AgRP and NPY mRNA content could be mediated at least in part by ghrelin. This suggestion is reinforced by recent data showing that starvation-induced feeding was suppressed after central administration of antighrelin antiserum (15). It is noteworthy that even in this situation of increased circulating ghrelin levels, the administration of this peptide to food-deprived rats led to a further increase in AgRP and NPY mRNA contents in the ARC. This suggests that the dose-response characteristics of ghrelin on these hypothalamic target neurons at the level of mRNA expression are maintained across a broad range and that there is an apparent lack of desensitization in this experimental setting.

To fully understand the possible functional linkage between ghrelin and the somatotropic axis, we determined the effects of ghrelin administration (icv) on hypothalamic SST and GHRH mRNA levels. In agreement with previous data (20), we failed to find any meaningful effect of ghrelin in intact normal rats. Nevertheless, these data were somewhat surprising because there is indirect evidence indicating that, at least from a functional point of view, SST mediates some of the effects of ghrelin on GH secretion (55, 56). Taking into account that GH has been found to markedly affect SST and GHRH mRNA levels, and that SST and GHRH neurons express GH receptors (29, 57), it was possible that the ghrelin-induced increase in circulating GH levels could be masking their effects on SST and GHRH mRNA levels. Using dwarf rats we could study GHRH and SST expression in the absence of the feedback exerted by GH, which could be masking the effects of ghrelin at this level. Our data showed, as found in other GH-deficient models (32, 58, 59), that in these animals there is a decrease in SST mRNA content in the PeN and an increase in GHRH mRNA levels in the ARC compared with wild-type (age- and weight-matched) Lewis rats.

We found that, similar to intact rats, ghrelin failed to influence GHRH mRNA content. In contrast, we found a clear-cut stimulatory effect of SST mRNA content in the PeN after ghrelin administration to dwarf rats. These data indicate that SST neurons in the PeN are influenced by ghrelin through a non-GH-dependent mechanism.

In summary, our data confirm that ghrelin administration to *ad libitum*-fed rats increased NPY and AgRP mRNA contents in the ARC. Furthermore, our study provides the first evidence that this effect is independent of the nutritional status in fasted rats. Contrary to previous hypotheses, we demonstrate that ghrelin does not appear to regulate MCH and prepro-OX mRNA levels in the LHA. Finally, we showed that ghrelin administration does not modify mRNA levels in the ARC, but increases the SST mRNA content in the PeN of dwarf Lewis rats. This study furthers our understanding of the mechanism involved in the effects of ghrelin on food intake and GH secretion.

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