

Hypothalamic expression of NPY mRNA, vasopressin mRNA and CRF mRNA in response to food restriction and central administration of the orexigenic peptide GHRP-6

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Abstract

In this study, we examined the effects of restricted feeding and of central administration of an orexigenic ghrelin agonist GHRP-6 on peptide mRNA expression in the hypothalamus. We compared rats fed *ad libitum* with rats that were allowed food for only 2 h every day, and treated with a continuous chronic i.c.v. infusion of GHRP-6 or vehicle. *Ad libitum* fed rats exposed to GHRP-6 increased their food intake and body weight over 6 days, but, at the end of this period, neuropeptide Y mRNA expression in the arcuate nucleus was not different to that in control rats. By contrast, expression of neuropeptide Y mRNA in the arcuate nucleus was elevated in food-restricted rats, consistent with the interpretation that increased expression reflects increased hunger. However, neuropeptide Y mRNA expression was no greater in food-restricted rats infused with GHRP-6 than in food-restricted rats infused with vehicle; thus if the drive to eat was stronger in rats infused with GHRP-6, this was not reflected by higher levels of neuropeptide Y mRNA expression. Expression of vasopressin mRNA and corticotrophin releasing factor (CRF) mRNA in the paraventricular nucleus (PVN) was not changed by food restriction. GHRP-6 infusion increased CRF mRNA expression in *ad libitum* rats only.

Keywords: *Arcuate nucleus, ghrelin, paraventricular nucleus, hypothalamus*

Introduction

Ghrelin is secreted from the stomach into the circulation in response to food deprivation (Kojima et al. 1999), and is also expressed in a population of neurons on the ventral border of the arcuate nucleus (Cowley et al. 2003). Ghrelin acts within the hypothalamus at “growth-hormone secretagogue (GHS) receptors” that are densely expressed in the arcuate and ventromedial nuclei (Bennett et al. 1997). These receptors were named, before the identification of ghrelin, after a class of synthetic agonists, the best characterised of which is growth hormone-releasing peptide-6 (GHRP-6) (Korbonits & Grossman 1995)

Systemic or central administration of GHS agonists (including ghrelin itself) has two well-characterised consequences. Firstly, GHS agonists stimulate growth hormone (GH) secretion; this reflects hypothalamic

actions as well as pituitary actions, and involves release of GH-releasing-hormone from neurosecretory neurons in the arcuate nucleus (Casanueva & Dieguez 1999). Secondly, GHS agonists stimulate feeding, and this is thought to reflect activation of orexigenic neuropeptide Y (NPY) containing neurons in the arcuate nucleus (Nakazato et al. 2001, Shintani et al. 2001, Seoane et al. 2003). In addition, administration of some GHS agonists results in a small but significant stimulation of ACTH secretion, apparently by a direct hypothalamic action, and presumably via release of corticotrophin releasing factor (CRF) and/or vasopressin from parvocellular neurosecretory neurons of the paraventricular nucleus (PVN) (Thomas et al. 1997).

Although chronic central infusion of GHS leads to a sustained increase in feeding, body weight and adiposity (Okada et al. 1996, Tung et al. 2001), after

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six days of continuous central administration of GHRP-6 we found no evidence of an increase in NPY mRNA expression in the arcuate nucleus (Bailey et al. 1999). A possible explanation is that NPY expression is homeostatically regulated by feedbacks that result from increased feeding, including increased production of leptin, which is inhibitory to NPY neurons (Wang et al. 1997).

Therefore, for this study we reasoned that if rats were to eat voraciously during the limited availability of food, they would be unable to eat more even if stimulated by GHRP-6. The feedback signals from eating would be maintained; therefore any effects of chronic GHRP-6 infusion on NPY mRNA expression would be revealed. In particular, if NPY mediates the effect of ghrelin on appetite, as suggested by recent studies (Shintani et al. 2001, Chen et al. 2004, van den Top et al. 2004), then we would expect to see a sustained increase in NPY mRNA expression. We compared rats fed *ad libitum* with rats that were allowed to eat for only 2 h every day, in which time they consumed approximately 65% of their normal daily intake. Half of the rats in each group were exposed to a continuous chronic i.c.v. infusion of GHRP-6, and half to a similar infusion of vehicle. After 6 days, we measured expression of NPY mRNA in the arcuate nucleus, and expression of vasopressin mRNA and CRF mRNA in the PVN.

The physiological implications of the effects of ghrelin on ACTH secretion are unclear. Acute central administration of CRF is anorectic (Morley & Levine 1982), so effects of ghrelin on CRF release appear unlikely to be associated with the effects of GHS on feeding. However, chronic stress is associated with an increase in palatable feeding (Dallman et al. 2003, Pecoraro et al. 2004), and chronic activation of CRF/vasopressin neurons might contribute to the maintained increase in feeding and adiposity observed in rats treated chronically with GHS (Lall et al. 2001, Tung et al. 2004).

Materials and methods

All procedures were performed in accordance with the UK Animals (Scientific Procedures) Act, 1986 and associated guidelines. Adult male Sprague–Dawley rats (mean \pm S.E.M.: 328.8 \pm 7.4 g) were maintained in a controlled environment (21–23°C; 12 h light–dark; lights on at 07:00 h), and rats had free access to standard rat chow and water before surgery. Rats were anaesthetised with halothane (<5% halothane in 50:50 O₂/N₂O) and a stainless steel cannula connected via polythene tube to an osmotic minipump (ALZET 2001: delivery rate 1 μ l/h; ALZET Corporation, USA) was implanted into the right lateral ventricle (co-ordinates: 2 mm lateral, 3 mm posterior to bregma, 4 mm below the skull: (Paxinos & Watson 1996)) and affixed using dental cement and screws.

The minipump itself was placed in the subscapular region and the wound sutured. The minipump contained either GHRP-6 (1 μ g/ μ l; n = 14) or sterile artificial CSF (aCSF; pH7.2, composition in mM: NaCl 138, KCl 3.36, NaHCO₃ 9.52, Na₂HPO₄ 0.49, urea 2.16, CaCl₂ 1.26 and MgCl₂ 1.18; n = 12), which was delivered at 1 μ l/h for 7 days. All rats received *ad libitum* food and water for the first 24 h after surgery, then were randomly assigned to receive either *ad libitum* food or food for only 2 h per day (09:00–11:00 h). All rats had free access to water, and were handled daily to minimise stress related effects whilst measuring body weight and amounts of food and water consumed. After 7 days of central infusion, rats were killed by decapitation at 11:00–12:00 h and the brains immediately removed, frozen on dry ice, and stored at –70°C until processed for *in situ* hybridisation (ISH).

In situ hybridisation

Oligonucleotide probes were synthesised (MWG, Germany) according to previously tested sequences corresponding to rat NPY mRNA, vasopressin mRNA and CRF mRNA. The probe sequences were complementary to: rat NPY (30 mer) coding region: bases 5'-GGA GTA GTA TCT GGC CAT GTC CTC TGC TGG-3', rat vasopressin (45 mer) coding region: bases 5'-GAC CCG GGG CTT GGC AGA ATC CAC GGA CTC TTG TGT CCC AGC CAG-3' and rat CRF (42 mer) coding region: bases 5'-CCT GTT GCT GTG AGC TTG CTG AGC TAA CTG CTC TGC CCT GCC-3'.

Coronal brain sections were cut on a cryostat at 15 μ m thickness through the hypothalamus and were thaw mounted onto RNAase-free gelatinised slides. Every third section was collected and counterstained with toluidine blue to locate areas of interest for ISH. Brain sections containing the arcuate nucleus were processed for NPY mRNA expression and those containing the PVN processed for either vasopressin mRNA or CRF mRNA expression. All sections were fixed in 4% paraformaldehyde, washed in phosphate-buffered saline, acetylated in triethanolamine/acetic anhydride solution, dehydrated in ascending alcohols (70–100%), delipidated in chloroform, and partially rehydrated in 95% ethanol. Each oligonucleotide probe was 3'-labelled with ³⁵S-dATP using terminal deoxynucleotidyl transferase and purified using spin columns (QIAquick nucleotide removal kit, Qiagen, Crawley, UK). Hybridisation, using 100 000 c.p.m. labelled probe per section, was performed overnight at 37°C in humidified chambers. Excess probe was removed by rinsing three times in 1 \times SSC at room temperature, followed by four 15 min washes in 1 \times SSC at 55°C, two 30 min washes in 1 \times SSC at room temperature, and finally two rinses in distilled water before being air dried.

Sections probed for AVP mRNA or CRF mRNA were exposed to autoradiographic film (Hyperfilm- β -max: Amersham, UK) for 10 and 21 days, respectively at room temperature, which was then developed (Kodak D-19: Sigma UK) and fixed (Hypam rapid fixer: Ilford, UK). Sections probed for NPY mRNA were then dipped in liquid autoradiographic emulsion (G5, Ilford, UK), exposed for 21 days at 4°C, developed and fixed as above, and counterstained with haematoxylin and eosin.

Using the $\times 5$ objective to view NPY mRNA expression in emulsion-dipped sections, a cell was considered to be positive when it contained more than three times the background tissue density of silver grains. The number of NPY mRNA positive cells was counted in five arcuate nuclei per arcuate area, on both sides, from the region 2.8–3.3 mm posterior to bregma (Paxinos & Watson 1996), for each rat, to generate rat means and group mean \pm S.E.M. The silver grain area per cell was measured using a computerised image analysis system ($\times 10$ objective; NIH Image, version 1.62, as developed by National Institute of Health: <http://rsb.info.nih.gov/nih-image>). The silver-grain area per cell, the cell area and a corresponding background silver-grain area were measured using the $\times 10$ objective, for five randomly-selected NPY mRNA-containing cells in each arcuate nucleus (region 2.8–3.3 mm posterior to bregma). After subtraction of the background silver-grain area, the mean value of silver grain area per unit cell area was obtained for each arcuate nucleus and subsequently for each rat, and finally group mean \pm S.E.M was calculated.

The film autoradiograms of CRF mRNA and AVP mRNA expression were quantified using the computerised image analysis system, $\times 5$ objective. The optical grain density was measured over six parvocellular PVN (pPVN) and six magnocellular PVN (MPVN) profiles from each rat, and background measurements for adjacent areas of tissue were made for each profile and subtracted. For each area, average values for each rat were used to calculate group means. Using the $\times 10$ objective, silver grain density was measured in emulsion dipped, haematoxylin and eosin counterstained sections, over six magnocellular and six parvocellular neurons per PVN region per side for four sections per rat. Rat means followed by group mean \pm S.E.M were then calculated. Group means for all areas and groups were compared statistically using Two-way ANOVA followed by Student-Newman Keuls tests.

Results

Effect of chronic infusion of GHRP-6 on food and water intake in ad libitum fed and restricted fed rats

On the day after surgery, all rats received vehicle i.c.v., and on this day, rats given restricted food (for 2 h only)

ate little, as they were naïve to the restricted availability of food. On the following day, the food intake of food-restricted rats increased to 6–8 g/rat. The effects of GHRP-6 on food and water intake and body weight of rats were analysed and illustrated from this day (day 2) of the experiment.

Rats fed *ad libitum* showed no significant variation in food intake throughout the six days of aCSF infusion. However, rats infused with GHRP-6 ate significantly more than rats infused with aCSF (Figure 1A) as measured by cumulative food intake over 6 days of infusion, and *post hoc* tests revealed significant differences in food intake on days 5 and 6 of the experiment (one-way repeated measures ANOVA; $P = 0.05$, Figure 1A). Over six days, rats infused with GHRP-6 ate 17.7 g more than rats infused with aCSF- (*t*-test, $*P < 0.05$; Figure 1C).

In food-restricted rats, food intake was similar in aCSF- and GHRP-6 infused rats throughout the period of infusion (Figure 1B and D) with no significant differences between these groups ($P > 0.05$; two-way ANOVA).

Like food intake, the water intake of food-restricted rats was almost completely absent on the day after surgery, and returned to the average level on the following day at about 33 ml of water intake in rats on both feeding regimes (Figure 1E and F). In rats fed *ad libitum* the cumulative water intake over five days was 14% greater in rats infused with GHRP-6 than in rats infused with aCSF (*t*-test, $P < 0.05$; Figure 1E). In food-restricted rats cumulative water intake was lower than in rats fed *ad libitum*, but there were no significant differences between aCSF- and GHRP-6-infused rats (Figure 1F).

Ad libitum fed rats infused with GHRP-6 gained more weight than rats treated with aCSF (Figure 1G; *t*-test, $*P < 0.05$). For food-restricted rats, both aCSF and GHRP-6 groups showed a progressive decrease in body weight beginning on day 2 (Figure 1H), but there was no significant difference in weight loss between rats infused with aCSF and with GHRP-6.

Effects of food restriction and GHRP-6 on expression of NPY mRNA in the arcuate nucleus

In *ad libitum* fed rats infused with aCSF, the arcuate nucleus showed a relatively even distribution of NPY mRNA expression throughout the mediobasal arcuate nucleus. The distribution of NPY mRNA in the arcuate nucleus was not different in *ad libitum* fed rats infused with GHRP-6; also the number of cells that expressed NPY mRNA and the amount of NPY mRNA expressed per cell were not significantly different ($P > 0.05$, one-way ANOVA: Figure 2).

Many intensely labelled neurons were detected in the arcuate nucleus of food-restricted rats (Figure 2C and D). Significantly, more cells expressed NPY mRNA in food restricted rats than in *ad-libitum* fed rats, but

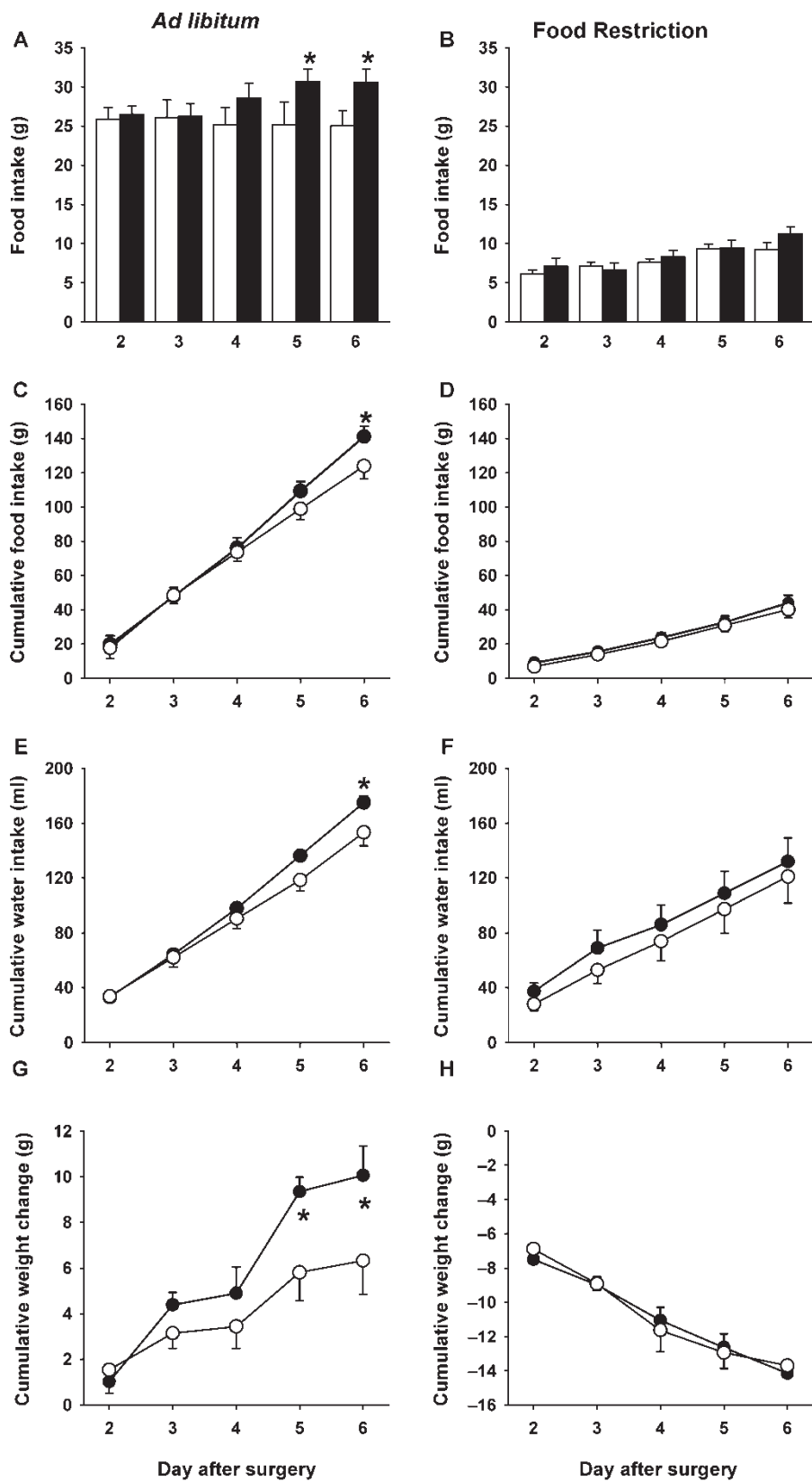


Figure 1. Effect of i.c.v. infusion of GHRP-6 or aCSF on food intake and body weight. On the day of surgery rats were implanted with chronic infusion minipumps containing either GHRP-6 ($1 \mu\text{g}/\mu\text{l}$; $n = 7$ per group; black bars and circles) or aCSF ($n = 6$ per group, white bars and circles). On the following day rats were subsequently assigned to two groups which received food either *ad libitum* (left column) or for 2 h only per day (09:00–11:00 h; food-restricted; right column). Individual rat weight, food and water intake were measured daily at 08:00 h. Data are expressed as group mean \pm S.E.M. for (A, B) food intake (g), (C, D) cumulative food intake (g), (E, F) cumulative water intake (ml) and (G, H) cumulative body weight change. * $P < 0.05$: two-way repeated measures ANOVA, followed by Student-Neuman-Keuls Test.

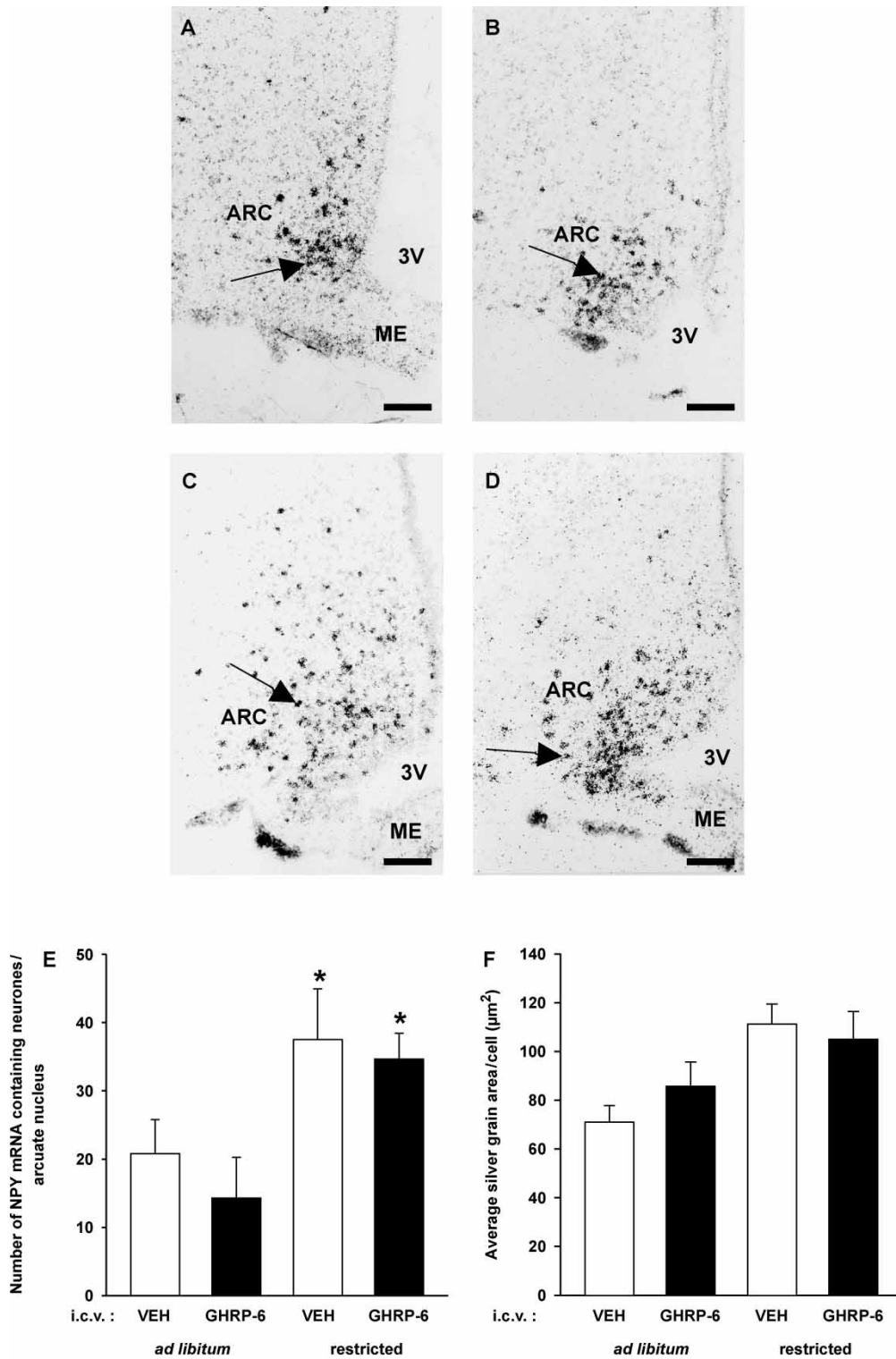


Figure 2. Representative *in situ* hybridization autoradiographs of NPY mRNA expression in the arcuate nucleus from *ad libitum* fed rats (A, B) or food-restricted rats (C, D), also given a continuous i.c.v. infusion of either aCSF (A, C; $n = 6$) or GHRP-6 ($1 \mu\text{g}/\mu\text{l}$; B, D, $n = 7$) for 7 days. Emulsion dipped sections, counterstained with haematoxylin and eosin showing cellular location of NPY mRNA as black silver grains (arrow). 3V: third ventricle, ME: median eminence, ARC: arcuate nucleus. Scale bar: $50 \mu\text{m}$. (E, F). Quantification of the expression of NPY mRNA in the arcuate nucleus of rats treated with chronic i.c.v. infusion of aCSF: (VEH, $n = 6$) or GHRP-6 ($1 \mu\text{g}/\mu\text{l}$, $n = 7$) under an *ad libitum* or food-restricted diet. The number of cells that contained more than $3 \times$ background levels of silver grains was counted for each arcuate nucleus per rat and the group mean \pm S.E.M. calculated for each treatment group (E). The average silver grain area per cell was measured (μm^2) and the mean \pm S.E.M. calculated for each group (F). * $P < 0.05$, Student-Neuman-Keuls Test.

chronic administration of GHRP-6 had no significant effect on NPY mRNA expression in either food-restricted rats or *ad libitum* fed rats (two-way ANOVA, $P < 0.05$, Student-Newman-Keuls Test: Figure 2E).

We analysed five rostro-caudal divisions of the arcuate nucleus; the effects of food restriction were similar and significant in all regions except for the most caudal part (3.60–4.16 mm posterior to bregma), where food-restricted rats infused with aCSF or GHRP-6 contained 39 ± 10 and 29 ± 5.9 NPY mRNA neurons/section respectively, not significantly different from *ad libitum* fed rats infused with aCSF (26 ± 11 neurons/section), or GHRP-6 (30 ± 21 neurons/section; $P > 0.05$, one-way ANOVA).

Expression was also analysed as silver grain area per unit cell area (Figure 2F). Neither food restriction nor chronic infusion of GHRP-6 induced significant changes in average NPY mRNA content per cell in the arcuate nucleus ($P > 0.05$, Student Newman Keuls test; Figure 2F). It seems that restricted feeding induced an increase in NPY mRNA expression in the arcuate nucleus by increasing the number of cells that express NPY mRNA to a level above background, and that this masked the increase in expression within cells

that is apparent from the increased number of intensely stained cells.

CRF and vasopressin mRNA expression in the PVN

CRF mRNA expression, estimated by the density of the film autoradiograph, was greatest in the *ad libitum* fed rats infused with GHRP-6, but did not differ significantly between groups (data not shown). We also analysed expression by cell counts on emulsion-dipped sections, and by measuring grain density per cell. No treatment had a significant effect on the number of cells that expressed CRF mRNA in the PVN. Two-way ANOVA determined that overall, there was no significant effect of GHRP-6 or food restriction on CRF mRNA expression, but there was a significant interaction between these treatments ($P = 0.026$). *Post hoc* pairwise analyses indicated that CRH mRNA expression in the pPVN was significantly greater in *ad libitum* fed rats infused with GHRP-6 than in *ad libitum* fed rats infused with aCSF ($P < 0.05$, Student Newman Keuls, Figure 3A), but GHRP-6 had no significant effect in food-restricted rats ($P > 0.05$).

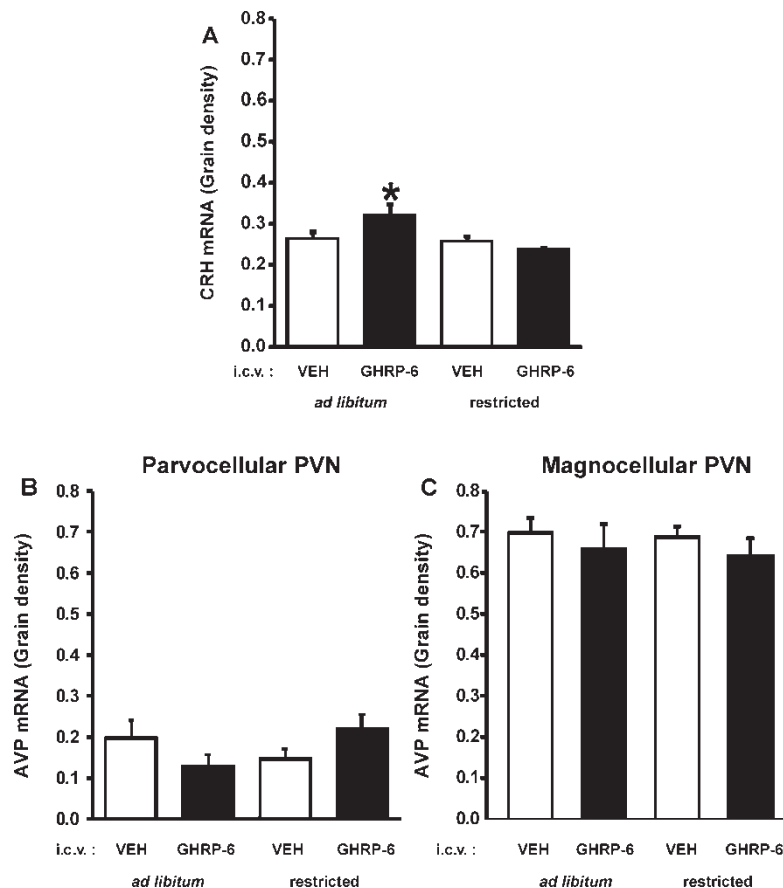


Figure 3. The silver grain density in *in situ* hybridization autoradiographs of CRF mRNA (A) in the PVN and vasopressin (AVP) mRNA (B, C) expression in the parvocellular (B) and magnocellular (C) PVN was measured in emulsion dipped, haematoxylin and eosin counterstained sections. Tissue was from *ad libitum* fed rats and food-restricted rats treated with chronic i.c.v. aCSF (VEH; $n = 6$) or GHRP-6 ($1 \mu\text{g}/\mu\text{l}$; $n = 7$). The bars are group means \pm S.E.M., expressed as silver grain density per cell. * $P < 0.05$, two-way ANOVA, Student-Newman-Keuls Test.

We also measured vasopressin mRNA expression in the PVN by film density, and by cell counts and grain density per cell for both parvocellular (Figure 3B) and magnocellular (Figure 3C) regions. There was no significant effect of either food restriction, or of GHRP-6, nor was there any significant interactions between treatments (Figure 3B and C).

Discussion

In the present study, *ad libitum* fed rats given a chronic i.c.v. infusion of GHRP-6 increased their food intake and body weight over 6 days, as expected, confirming the orexigenic effectiveness of i.c.v. GHRP-6. However, at the end of this period, consistent with previous findings (Bailey et al. 1999), NPY mRNA expression in the arcuate nucleus was not different from that in control rats. By contrast, food-restricted rats showed elevated expression of NPY mRNA in the arcuate nucleus, consistent with the interpretation that increased expression reflects increased hunger. Food-restricted rats infused with GHRP-6 ate not more than food-restricted rats infused with aCSF, indicating that they were eating as much as possible in the available time, as intended by the experimental design so that feedback signals from eating would be similar between the vehicle and GHRP-6 groups. However, NPY mRNA expression in the arcuate nucleus was no greater in food-restricted rats infused with GHRP-6 than in food-restricted rats infused with aCSF. Thus if the drive to eat was stronger in rats infused with GHRP-6, it was not reflected by higher levels of NPY mRNA expression.

Chronic infusions of GHS agonists lead to maintained increases in GH secretion and growth rate through actions both at the pituitary and on GHRH neurons (Casanueva & Dieguez 1999). However chronic GHS infusions do not produce a sustained increase in GHRH mRNA expression, but produce a sustained increase in somatostatin mRNA expression in the periventricular nucleus (Bailey et al. 1999). The likely explanation is that enhanced GH secretion feeds back at the hypothalamus to enhance the activity of somatostatin neurons which inhibit GHRH neurons, opposing the excitatory actions of GHS. In this study we anticipated that an analogous explanation would underlie the failure to observe a sustained increase in NPY mRNA expression in response to chronic GHRP-6, i.e. that the chronic orexigenic drive to NPY neurons from GHRP-6 is offset by signals arising from increased energy intake, including leptin (Hewson et al. 2002).

The lack of an increase in NPY mRNA expression in food-restricted rats infused with GHRP-6 was thus not as predicted. There is little doubt that GHS agonists activate NPY neurons in the arcuate nucleus. Direct evidence that the arcuate nucleus is a site of action of GHS agonists first came from electrophysiological

studies (Dickson et al. 1995). Evidence that these effects were specific came from studies of Fos expression: in the hypothalamus, the only region in which GHRP-6 evoked significant Fos expression was the arcuate nucleus. Identification by double immunohistochemistry (Dickson et al. 1996) or double *in-situ* hybridisation (Dickson & Luckman 1997) established that, while GHRP-6 activated many arcuate GHRH neurons, most of the activated cells expressed NPY mRNA. This conclusion was supported by studies showing co-localisation of GHS receptors in arcuate NPY neurons (Willesen et al. 1999).

Arcuate NPY cells are involved in the regulation of food intake, and a sub-population of these are glucose sensitive (Muroya et al. 1999). The role of NPY in the control of food intake has been studied extensively, and, for example, the concentration of NPY in the arcuate nucleus of obese Zucker rats is much greater than in lean normophagic rats (Beck et al. 1990). Conversely, NPY mRNA expression in the arcuate nucleus is reduced in rats fed with high-fat diet (Stricker-Krongrad et al. 1998). The effects of NPY on food intake are believed to be exerted by a direct projection from arcuate NPY cells to the PVN.

In the arcuate nucleus, NPY mRNA expression is increased after a single i.c.v. injection of ghrelin (Shintani et al. 2001, Seoane et al. 2003), and is elevated after central administration of ghrelin for 3 days (Kamegai et al. 2001). Feeding induced by acute administration of ghrelin (Tschop et al. 2000) can be prevented by prior i.c.v. administration of anti-NPY IgG (Nakazato et al. 2001). If acute effects of ghrelin on feeding are associated with activation of NPY neurons, why don't we see sustained increases in NPY mRNA expression in response to sustained infusions of GHRP-6, despite the maintained effects of such infusions on feeding? There is no desensitisation apparent in the effects of GHRP-6 infusion on feeding in the *ad-libitum* fed rats, and as acute fasting potentiates the Fos expression in response to GHS in the arcuate nucleus (Hewson & Dickson 2000), we have no reason to expect occlusion of responsiveness by high levels of endogenous ghrelin.

One possibility lies in the functional diversity of the arcuate NPY neurons; chronic activation of a subpopulation specifically involved in feeding might be masked by down-regulation of responsiveness to GHRP-6 in subpopulations with different physiological roles; the regionally specific effect of food-restriction on NPY expression shown here supports the suggestion that the arcuate NPY population is functionally as well as anatomically diverse.

Another possibility that remains to be investigated is that expression of agouti related peptide (AGRP) is upregulated in response to chronic GHS infusions

(Kamegai et al. 2001). AGRP is co-localised with NPY in the arcuate nucleus, and stimulates food intake by antagonistic action at melanocortin MC4 receptors in the hypothalamus (Rossi et al. 1998). Acute fasting increases both AGRP and NPY expression, but although chronic food restriction also increases NPY expression, the acute increase in AGRP mRNA expression is not sustained (Harrold et al. 1999, Bi et al. 2003).

Recently, Pinto et al. (2004) demonstrated that acute exposure to ghrelin induced a decrease in excitatory and an increase in inhibitory inputs to the pro-opiomelanocortin neurones of the arcuate nucleus in *ob/ob* mice. The chronic GHS treatment employed here may have induced similar changes in inputs to the pro-opiomelanocortin neurones, which would result in a decrease in satiety drive and hence increased appetite.

The expression of AVP mRNA was not altered by any treatment, neither by food regime nor by chronic GHS treatment in either the magnocellular or parvocellular PVN. This was surprising as we would have expected an increase in our food restricted rats because acute ghrelin administration induces vasopressin release into the plasma in a NPY dependent manner (Ishizaki et al. 2002). The expression of CRF mRNA in the PVN was not significantly changed in food-restricted rats, indicating that this regime was not conspicuously stressful, and verifying the specificity of the observed change in NPY mRNA expression. Expression of CRF mRNA was slightly increased by GHRP-6 administration in *ad libitum* fed rats, consistent with effects of GHS and ghrelin on ACTH secretion (Wren et al. 2002, Tung et al. 2004); CRF is anorectic, so it is unlikely that increased CRF expression drives feeding behaviour in response to GHS. The lack of an effect of GHRP-6 on CRF mRNA expression in food-restricted rats was unexpected, and raises the possibility that the change in expression in *ad libitum* fed rats is a response to overeating rather than a specific effect of GHS.

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