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Divergent Leptin Signaling in Proglucagon Neurons of the Nucleus of the Solitary Tract in Mice and Rats

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The central targets mediating the anorectic and other actions of leptin have yet to be fully identified. Although previous studies focused on the hypothalamus, leptin also acts on neurons in extrahypothalamic sites, including the nucleus of the solitary tract (NTS). Moreover, injection of leptin into the NTS of rats suppresses food intake. Within the central nervous system, glucagon-like peptide (GLP-1), a product of proglucagon, is synthesized almost exclusively in neurons of the NTS. Intracerebroventricular administration of GLP-1 inhibits energy intake, and GLP-1 receptor antagonists attenuate the anorexic effects of leptin in rats. To examine whether NTS proglucagon neurons are directly regulated by leptin, we performed double GLP-1 and phosphorylation of signal transducer and activator of transcription-3 immunohistochemistry on brain sections from ip leptin-treated mice and rats. Leptin induced phosphorylation of signal transducer and activator of transcription-3 in 100% of GLP-1 cells in the caudal brainstem of mice. In striking contrast, 0% of GLP-1-positive neurons in rats responded to leptin. We then measured regulation of NTS proglucagon mRNA using real-time RT-PCR in mice and rats fed *ad libitum*, fasted, or fasted and treated ip with leptin. In mice, proglucagon mRNA fell by fasting, and this was prevented by leptin administration. In rats, by contrast, proglucagon mRNA was unaffected by either fasting or leptin. Taken together, our studies reveal direct regulation of proglucagon neurons by leptin in mice but not rats along with corresponding species differences in the regulation of proglucagon mRNA expression. These data, combined with previous results, suggest a different mechanism of interaction between leptin and NTS proglucagon neurons in mice and rats. (*Endocrinology* 149: 492–497, 2008)

LEPTIN IS A HORMONE produced by adipose tissue and acts on the brain to regulate several physiological processes, including neuroendocrine function and energy balance (1–3). The most widely studied target of leptin has been the hypothalamus, specifically the arcuate nucleus (ARC). Within the ARC, leptin has been shown to affect energy balance through stimulation of catabolic neuropeptides such as αMSH [a product of the proopiomelanocortin (*pomc*) gene] and by inhibition of anabolic factors including neuropeptide Y and agouti-related peptide (4–7).

Leptin acts on the leptin receptor (ObRb), a member of the cytokine receptor superfamily, resulting in signaling through several pathways including a Janus kinase-signal transducer and activator of transcription (JAK-STAT) pathway (8). Leptin-dependent activation of STAT3 phosphorylation (P-STAT3) has been used as a reliable marker for the identification of neurons that express functional ObRb receptors (9, 10). Indeed, administration of recombinant leptin to rodents leads to robust and specific induction of P-STAT3 in regions of the hypothalamus that correspond with the anatomical localization of ObRb mRNA (11) and in known leptin-responsive neurons, such as POMC neurons (10).

Whereas action of leptin in the arcuate hypothalamus has been the most vigorously studied, leptin has important extraarcuate hypothalamic targets as well (10, 12–16). One site is the nucleus of the solitary tract (NTS) (10, 14, 16). The NTS relays interoceptive signals via vagal afferent neurons, including chemical and mechanical signals of gastrointestinal origin, to more rostral structures including the hypothalamus (17, 18). Peripheral leptin administration results in rapid induction of STAT3 phosphorylation in the NTS of mice and rats (10, 14, 16, 19), and microinjection of leptin directly into the NTS at doses that are ineffective when delivered to the brain ventricles leads to rapid inhibition of feeding (14). Moreover, balloon distension of the stomach in rats results in induction of c-Fos expression in neurons of the NTS that are also leptin responsive (20), suggesting that leptin acts in the NTS to modulate visceral sensory information to reduce meal size. The neurochemical identity of the leptin-responsive neurons in the NTS is therefore of interest to better understand their role in regulating energy balance. POMC neurons, which are key mediators of leptin action in the ARC of the hypothalamus (3, 21), are also present in the NTS (22–26) and have been considered potential leptin target cells. However, our previous work in mice demonstrated that the leptin-responsive neurons in the NTS do not include POMC neurons (27). The identity of these leptin-sensitive neurons of the NTS thus remains unknown.

Preproglucagon is expressed in pancreatic α-cells and L cells of the distal gut (28). Preproglucagon is cleaved to

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Abbreviations: AP, Area postrema; ARC, arcuate nucleus; DAB, diaminobenzidine; GLP, glucagon-like peptide; GLP-1R, GLP-1 receptor; i.c.v., intracerebroventricular; IHC, immunohistochemistry; IR, immunoreactive; JAK-STAT, Janus kinase-signal transducer and activator of transcription; m, mouse; NTS, nucleus of the solitary tract; POMC, proopiomelanocortin; P-STAT3, STAT3 phosphorylation; r, rat.

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produce glucagon, which is the major product in the pancreas, and is critical for stimulation of glucose production in the liver during fasting and/or starvation. In the gut, preproglucagon is processed into glucagon-like peptide (GLP)-1 and GLP-2 and released into circulation after nutrient ingestion and serves a variety of functions such as regulation of glucose homeostasis, gastric emptying, insulin release from pancreatic β-cells, and food intake (29, 30). Preproglucagon is also expressed the brain, specifically in neurons of the NTS in the caudal brain stem. Here the precursor is processed into GLP-1 and GLP-2. NTS proglucagon-producing neurons project to GLP-1 receptor (GLP-1R)-bearing neurons in hypothalamus [including the paraventricular hypothalamic nucleus, ARC, dorsomedial hypothalamus, and lateral hypothalamus] as well as caudal brain stem (including the NTS and area postrema) that are known to be important in energy balance regulation (29, 31). Whereas anorexic responses follow peripheral and central application of GLP-1R ligands, the functional relationship between the peripheral and the central proglucagon systems has yet to be fully defined.

Proglucagon neurons have previously been shown to express ObRb mRNA by *in situ* hybridization in mice (32). Furthermore, GLP-1 immunoreactive (IR) neurons have been reported to coexpress c-Fos after central leptin administration in rats (19). Functionally, forebrain and caudal brain stem ventricular [intracerebroventricular (i.c.v.)] injection of GLP-1 (33, 34) and GLP-2 (35) reduces food intake acutely in rodents, and long-term ventricular administration of GLP-1 lowers body weight in rats (36). In addition, leptin has been shown to augment hypothalamic GLP-1 peptide content in rats and mice (37). Interestingly, i.c.v. administration of a GLP-1/2 receptor antagonist, exendin 9–39, acutely attenuates leptin's inhibition of food intake and body weight in rats (32), altogether suggesting that proglucagon neurons mediate some of leptin's actions on energy balance.

Based on the anatomical location of both proglucagon neurons and leptin-responsive cells in the NTS and the attenuation of leptin anorexia with GLP-1R antagonism, we hypothesized that GLP-1 neurons in the NTS express functional leptin receptors and are thus direct targets of leptin. To evaluate this hypothesis, we performed double-labeling studies to assess colocalization of GLP-1 and P-STAT3 in both leptin-treated mice and rats. Furthermore, we examined the regulation of NTS proglucagon mRNA by leptin in both species.

Materials and Methods

Materials

Recombinant mouse leptin was purchased from Dr. A. F. Parlow (National Institute of Diabetes and Digestive and Kidney Diseases, Torrance, CA). Supplies for immunohistochemistry (IHC) were purchased from Sigma-Aldrich (St. Louis, MO) and the ABC Vectastain Elite kit was purchased from Vector Laboratories (Burlingame, CA). The phospho-specific (Y705)-STAT3 rabbit antibody was obtained from New England Biolabs (Beverly, MA), the GLP-1 (7–36) rabbit antibody was from Peninsula Laboratories Inc. (San Carlos, CA), and the donkey-antirabbit antibody was from Jackson ImmunoResearch Laboratories (West Grove, PA). Fluorescent donkey antirabbit immunoglobulin conjugates were from Molecular Probes (Eugene, OR), and donkey serum was from Invitrogen Life Technologies, Inc. (Carlsbad, CA).

Animals

Male Sprague Dawley rats (80–100 g, 4–5 wk of age) and male C57BL/6 mice (5–6 wk of age) were purchased from Charles River Laboratories, Inc. (Wilmington, MA). Animals were housed in a 14-h light, 10-h dark cycle with *ad libitum* access to tap water and chow, unless otherwise described. The animal procedures were used in accordance with the guidelines and approval of the Harvard Medical School and Beth Israel Deaconess Medical Center and the University of Pennsylvania's Institutional Animal Care and Use Committees.

Leptin stimulation and IHC

After 24 h of fasting (food withheld but water available), rats (n = 4) and mice (n = 4) were injected ip with leptin (4.0 mg/kg body weight) or vehicle (PBS) and anesthetized 45 min later with ketamine (100 mg/kg body weight) and xylazine (10 mg/kg body weight) (20, 27). Transcardiac perfusion/fixation with formalin, removal of the brain, postfixation, and cryoprotection were performed as described earlier (9). Brains were cut in 25-μm coronal sections, collected in five series, and stored at −20 C until further use. Single or double P-STAT3 and GLP-1 IHC was performed as described below. Brain sections were pretreated with citrate buffer for 30 min at 80 C. P-STAT3 IHC was performed as described earlier (9). In brief, free-floating tissue sections were incubated with the P-STAT3 antibody (1:4000). Sections were incubated with biotinylated antirabbit antibody (1:1000), followed by avidin-biotin-complex labeling, and developed with nickel-diaminobenzidine (DAB), generating a brown-black nuclear precipitate. In double-IHC studies, cytoplasmic fluorescent IHC for GLP-1 was performed consecutively by incubating sections with the anti-GLP-1 antibody (1:2500). On the next day, sections were incubated with a fluorescent-labeled secondary antibody generating green fluorescence. Results were visualized using either fluorescent light (GLP-1) or bright-field light (P-STAT3) and captured with a digital camera (AxioCam; Carl Zeiss, Thornwood, NY) mounted on a Zeiss microscope (Axioscope2; Carl Zeiss). Adobe Photoshop software (Adobe, San Jose, CA) was used to merge fluorescence and bright-field photographs via red-green-blue channels to visualize double-labeled cells.

Cell counting

One of the five brain series from each animal was subjected to single or double IHC as described above. Sections were organized in a rostral-to-caudal manner according to the rat brain atlas. Nomenclature and assignment of subnuclei within the NTS were done according to Herbert *et al.* (38). All sections in the series were then examined by dark-field and/or fluorescent microscopy to identify single- and/or double-labeled cells. All brain sections from the hindbrain that contained positive cells were analyzed. Cell counts were obtained from both hemispheres in each section. To estimate the total cell numbers in the entire brain region, bilateral cell counts from one series of each animal were multiplied by five.

Microdissection and real-time RT-PCR

Male Sprague Dawley rats (n = 14) and C57BL/6 mice (n = 10), were fed *ad libitum* and treated with vehicle (PBS, 2 times daily, ip), deprived of food but not water and treated with PBS (2 times daily, 50 μl, ip), or deprived of food but not water and treated with leptin (2 mg/kg, 2 times daily, ip). After 48 h, rats and mice were deeply anesthetized (100 mg/kg ketamine and 10 mg/kg xylazine), brains were removed, and 1-mm sagittal sections were cut using a cooled rat or mouse brain matrix (ASI Instruments, Houston, TX). Tissue from the caudal brain stem that included the entire NTS was obtained using a scalpel under a magnifying glass as described earlier (27). The tissues were snap frozen in liquid nitrogen and stored at −80 C until further use. Total RNA was isolated from the tissue blocks using RNA STAT-60 (Tel-Test Inc., Friendswood, TX). Five hundred nanograms of total RNA were used for reverse transcription (RT-PCR kit; CLONTECH, Palo Alto, CA). Quantification of proglucagon was carried out by real-time PCR using the Mx3000P system (Stratagene, La Jolla, CA). Real-time PCR was performed in a 96-well plate according to the manufacturer's instructions with minor alterations. The primers (Invitrogen) and probes (Biosearch

Technologies, Novato, CA) were designed with the assistance of PrimerExpress software as follows: mouse (m) GLP-1, forward (5′-TGGCAGCACGCCCTTC-3′), mGLP-1R (5′-GCGCTTCTGTCTGGGA-3′), and mGLP-1P [5′-6-carboxy-fluorescein (Fam)-AGACACAGAGGAGAACCCCAGATCATTCC-BHQ-1–3′]; rat (r) GLP-1, forward, (5′-TGGCAGCAtGCCCCTC-3′), rGLP-1R (5′-GTGGTTCTGTCTGGGA-3′), and rGLP-1P [5′-6-carboxy-fluorescein (Fam)-AGACACgGAGGAGAACgCCAGATCATTCC-BHQ-1–3′]. PCRs were run in a volume of 25.0 μl using 1.0 μl cDNA. A standard curve was generated from duplicate measurements of serial dilutions of brainstem cDNA.

Statistical analyses

All results are given as the mean ± SEM unless otherwise specified. Probabilities of chance differences between groups were calculated by one-way ANOVA (StatView version 5.0.1; SAS Institute Inc., Cary, NC). Means were then compared by *post hoc* analyses using Fisher's protected least significant difference test.

Results

Anatomical localization of leptin-responsive neurons in the NTS of mice and rats

We first determined the detailed anatomical location of leptin-responsive neurons in the NTS. Fasted mice and rats were given leptin (4 mg/kg, ip) or vehicle (PBS) and perfused after 45 min. IHC was then performed for P-STAT3, a well-defined marker of leptin receptor signaling in first-order neurons. In the hindbrain, leptin-responsive (P-STAT3 positive) neurons were found primarily in the medial subnucleus of the NTS at the level of the area postrema (AP) in both rats and mice (Fig. 1), however with some differences between the species. First, a significant number of leptin-responsive neurons were also found in the AP of rats but not mice (Fig. 1). Second, the distribution of leptin-responsive neurons extended more caudally relative to the AP in the NTS of the mouse than the rat. Third, a few P-STAT3-positive cells were present in the dorsal motor nucleus of the vagus and in the lateral NTS of the mouse, areas devoid of P-STAT3 in leptin-treated rats.

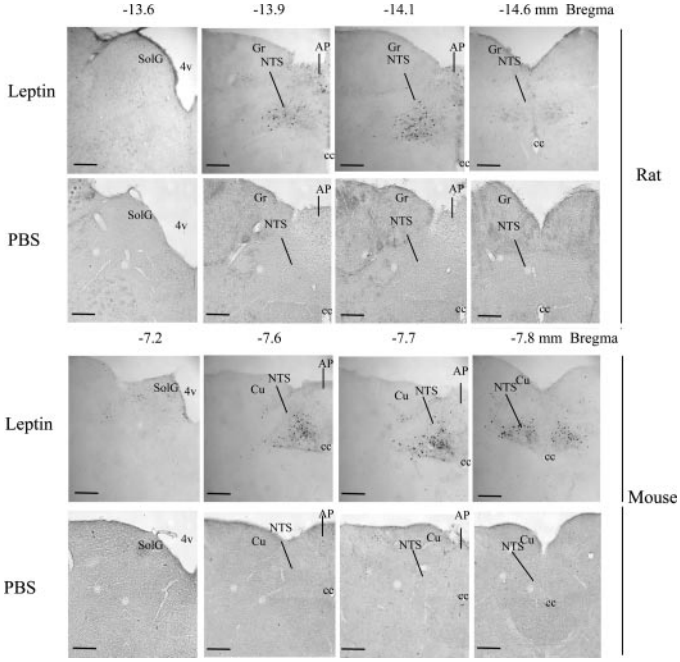

FIG. 1. Anatomical localization of leptin-responsive neurons in the NTS of rats and mice. Male Sprague Dawley rats and C57BL/6 mice were injected with leptin ip or PBS for 45 min. Series of coronal hindbrain sections were subjected to P-STAT3 DAB IHC. Shown are representative photomicrographs of P-STAT3 DAB IHC in the NTS of leptin-treated rats (*top row*) and leptin-treated mice (*third row*). A matched series of PBS-treated rats and mice were, respectively, shown in the *second row* and *bottom row*. All sections are ordered in a rostral to caudal manner (see Bregma levels). cc, Central canal; Cu, cuneate nucleus; Gr, gracile nucleus. *Scale bar*, 200 μm.

Localization of GLP-1 neurons in the NTS of mice and rats

We next investigated the localization of GLP-1 neurons in the NTS by fluorescence IHC. Overall, GLP-1 IR cells were more widely dispersed in rats, compared with mice. In the mouse, the majority of GLP-1-positive neurons were densely localized to the medial NTS at the levels of the AP and just caudal to the AP. Consistent with previous reports (39, 40), GLP-1-positive cells in rats were more scattered and found in the lateral and dorsal parts of the NTS (Fig. 2, A and B, respectively). In rats, GLP-1 neurons were also found more caudal to the level of the AP and in the commissural region of the NTS. The results in rats are consistent with more detailed analyzes reported earlier (39–41). In summary, the

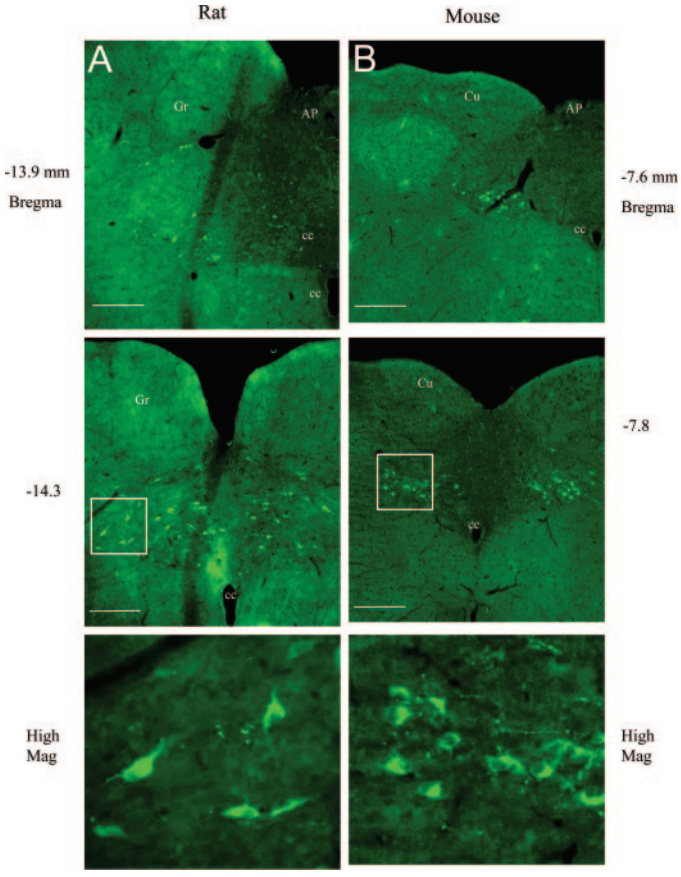

FIG. 2. Anatomical localization of GLP-1-producing neurons in the NTS of rats and mice. Shown are representative images of GLP-1 fluorescence IHC in hindbrain sections from rats (A) and mice (B). All sections are ordered in a rostral to caudal manner (see Bregma levels). The *last row of images* represent higher magnifications of the *boxed areas* above. cc, Central canal; Cu, cuneate nucleus; Gr, gracile nucleus. *Scale bar*, 200 μm.

population of GLP-1 neurons in the rat was more diffuse than the mouse (Fig. 2). In the entire NTS of both the mouse and rat, we estimated a total of 351 ± 70 and 497 ± 19 GLP-1 neurons, respectively (n = 4 rats and n = 4 mice). The number of GLP-1 neurons observed in the rat is consistent with previous reports showing similar or slightly larger numbers (40, 41). We analyzed only GLP-1 neurons in the NTS in which the majority of GLP-1 neurons are located. We did not analyze other regions reported to contain limited numbers of GLP-1-IR cells, such as the medullary reticular nucleus (41), because leptin does not signal in those regions. Furthermore, the distribution patterns and number of proglucagon neurons were confirmed by *in situ* histochemical hybridization analyzes of proglucagon mRNA expression in both species (data not shown).

GLP-1 is colocalized with P-STAT3 in leptin-treated mice but not leptin-treated rats

We next performed double labeling of P-STAT3 and GLP-1 in leptin-treated (4 mg/kg, ip) mice and rats. P-STAT3 and GLP-1 immunoreactive cells were found in the NTS of both rats and mice. Remarkably, we did not observe any colocalization between P-STAT3 and GLP-1 in any of the rat sections we examined (0% ± 0) (Fig. 3A), whereas P-STAT3 was induced in all of the GLP-1 neurons of all of the mouse sections we studied (100% ± 0) (Fig. 3, B and C). In mice, GLP-1-positive cells accounted for 40% (n = 4 mice and n = 4 rats) of all P-STAT3 IR cells.

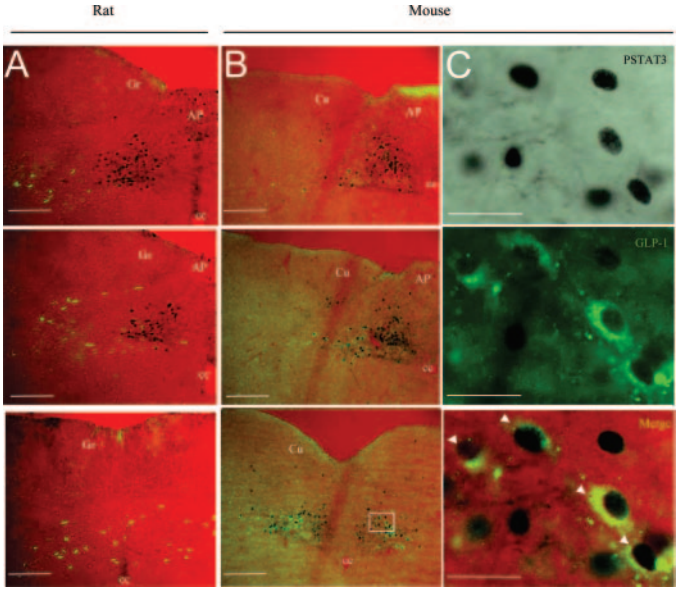

FIG. 3. Leptin induces P-STAT3 in 100% GLP-1-positive cells in the NTS of mice but not rats. Male Sprague Dawley rats or C57BL/6 mice were injected with leptin ip for 45 min. Series of coronal brain sections were subjected to combined P-STAT3 DAB and GLP-1 fluorescence double IHC. A and B, Shown are three representative merged photomicrographs from double IHC analyses of rats and mice, respectively. All sections are ordered in a rostral to caudal manner (*from top to bottom*). C, Shown are high magnifications (*top*, P-STAT3 DAB IHC; *middle*, GLP-1 green fluorescence IHC; *bottom*, merged photomicrograph from the double IHC) of the area marked in bottom of B. cc, Central canal; Cu, cuneate nucleus; Gr, gracile nucleus. Scale bars (A and B), 200 μm; (C), 50 μm.

Leptin stimulates NTS proglucagon mRNA expression in mice but not rats

With the disparate colocalization of GLP-1 and leptin-responsive neurons between mice and rats, we next investigated the potential regulation of proglucagon mRNA expression by leptin and fasting in both mice and rats. Caudal brain stem tissues were harvested from mice and rats from three groups: fed, fasted, and fasted+leptin (2 mg/kg, ip, twice daily). Proglucagon mRNA expression was then measured by quantitative RT-PCR. Fasting reduced the expression of proglucagon mRNA in mice but did not affect expression in rats (Fig. 4). Leptin administration to fasted mice resulted in proglucagon expression similar to fed mice. Leptin did not affect proglucagon expression in rats (Fig. 4).

Discussion

To elucidate the neurochemical identity of leptin-responsive neurons in the NTS, we here investigated the hypothesis that proglucagon neurons were targets of leptin and thus performed single- and double-labeling studies of P-STAT3 and GLP-1 immunoreactivity in the caudal brain stem of leptin-treated rats and mice. We found striking species differences in the regulation of proglucagon neurons by leptin between the species. Specifically, all proglucagon neurons in mice are directly responsive to leptin signaling and the fall in proglucagon mRNA by fasting is prevented by exogenous leptin administration. In stark contrast, none of the proglucagon neurons in rats respond to leptin as measured by P-STAT3 and consistent with this, proglucagon mRNA is not regulated by leptin. Because GLP-1 has been reported play a role in mediating leptin's inhibition of food intake in rats (32, 37, 42), our data suggest divergent regulation of the central proglucagon system in rats and mice.

We show that peripherally administered leptin induces phosphorylation of STAT3 in the NTS in anatomical patterns that are relatively similar in the mouse and rat, with the vast majority of P-STAT3 staining concentrated in the medial NTS at the level of the AP. This acute peripheral leptin administration is used to model tonic leptin action in the hindbrain. In the mouse but not the rat, a limited number of scattered

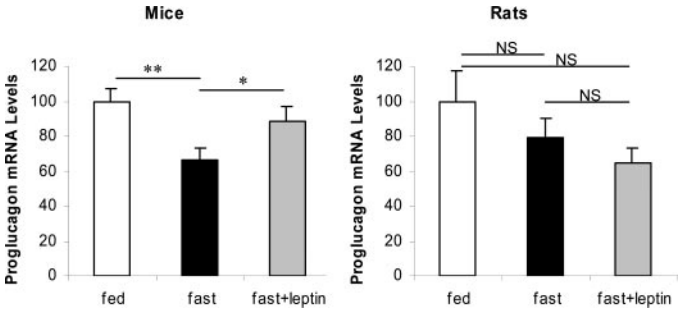

FIG. 4. Proglucagon mRNA is regulated by fasting and leptin in the NTS of mice but not rats. Male Sprague Dawley rats and C57BL/6 mice were fed *ad libitum* and treated with vehicle (PBS), fasted (48 h) and treated with vehicle (PBS), or fasted (48 h) and treated with leptin (2 mg/kg, two times daily, ip). Tissues from the caudal brainstem that included the entire NTS were dissected. Shown are real-time PCR results for proglucagon mRNA. Data are means ± SEM, n = 10 in each mice group, n = 14 in each group of rats. *, $P < 0.05$; **, $P < 0.01$. NS, Not significant.

P-STAT3-positive cells are present in the lateral regions of NTS, in caudal regions of the NTS relative to the AP and in the dorsal motor nucleus of the vagus at the level of the AP. In contrast, a significant number of leptin-responsive neurons were detected within the AP of rats but not in mice. Combined, these anatomical data suggest functional differences in leptin action in the hindbrain of mice and rats.

Preproglucagon, the precursor for GLP-1, is produced within the central nervous system almost exclusively in the NTS (31). Our analyses of the localization of GLP-1 IR neurons in the NTS of mice and rats revealed marked species differences in the anatomical distribution of these neurons. In contrast to P-STAT3 expression, GLP-1 was expressed in a more diffuse pattern in the rat than the mouse. In the mouse, GLP-1 IR was almost exclusively localized to the medial NTS. On the other hand, GLP-1 neurons in the rat were more scattered with few cells in the medial subnucleus of the NTS. Our data demonstrating localization of GLP-1 in leptin-responsive neurons in the mouse but not the rat is therefore at the anatomical level mostly explained by a difference in the localization of GLP-1 neurons between the species, rather than differences in localization of leptin-inducible P-STAT3-positive neurons.

Consistent with our findings in mice, a previous study by Goldstone *et al.* (32) showed that preproglucagon mRNA-expressing cells also possess ObRb mRNA by double *in situ* hybridization. Furthermore, we demonstrated here that in the mouse, fasting decreased proglucagon mRNA, and leptin blocked the fasting-induced decrease in expression. Consistent with these data, Goldstone *et al.* (37) found that GLP-1 peptide levels in the hypothalamus were increased after leptin peripheral administration to food-restricted mice.

In the rat, Elias *et al.* (19) reported colocalization of GLP-1 with c-Fos after peripheral leptin administration by double IHC. The activation of c-Fos, but not P-STAT3, by leptin in rat proglucagon neurons may be explained by signaling via indirect neuronal circuits that have yet to be elucidated. Additionally, we demonstrated species-dependent differences in the regulation of preproglucagon mRNA by fasting and leptin in the caudal brainstem. Contrasting with the mouse, neither fasting nor fasting plus leptin affected proglucagon mRNA expression. Similarly, Goldstone *et al.* (37) demonstrated that rat proglucagon mRNA expression in rats was not affected by i.c.v. leptin. Combined, these data are consistent with a putative mechanism by which regulation of proglucagon mRNA may involve activation of STAT3 but that activation of c-Fos is not sufficient to influence proglucagon mRNA, although this hypothesis requires direct testing. When considered in isolation, these data suggest a role of proglucagon neurons in leptin action in mice but not rats. However in contrast to this scenario, GLP-1/2 antagonists have been reported to attenuate leptin's effect on food intake in rats (32), suggesting that proglucagon neurons may be regulated by leptin through indirect pathways and mediate some of leptin's actions in this species.

Previous work demonstrated that gastric distension induces c-Fos in the NTS of rats (43) and that many of these c-Fos neurons also express P-STAT3 after leptin administration (20). Moreover, fourth ventricular administration of leptin synergistically inhibits the short-term food intake of rats whose stomachs are distended to a level that would have no behavioral effect in the absence of leptin (20). These findings establish a population of NTS neurons that is stimulated by both leptin and gastric distention in the rat and strongly suggests that leptin action in the NTS is sufficient to modulate meal size (20). Furthermore, because gastric distension has previously been demonstrated to induce c-Fos in GLP-1 IR NTS neurons of rats (44) and because we demonstrate here that leptin-responsive neurons do not possess GLP-1 in the rat, we conclude that gastric distension induces c-Fos in at least two distinct populations of NTS neurons in the rat: one that responds to leptin and does not express GLP-1 and another population that expresses GLP-1 but does not respond directly to leptin. Studies are needed to examine gastric distension in mice and the relationship between leptin action and GLP-1 neurons in this species. This will determine whether some GLP-1 neurons in mice might be regulated by both gastric distension and leptin, a finding that would further support the notion of divergent leptin action via the proglucagon system to influence feeding behavior in mice and rats.

Taken together, the data demonstrates different signaling by leptin in proglucagon neurons in mice and rats. In the mouse, GLP-1 neurons are leptin responsive, and leptin stimulates preproglucagon mRNA, consistent with direct action of leptin on these cells. In contrast, rat GLP-1 neurons are not leptin responsive, and leptin has no effect on the expression of preproglucagon mRNA. Our results suggest that the proglucagon system is regulated differently by leptin in mice and rats.

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