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GOAT induced ghrelin acylation regulates hedonic feeding

J.F. Davis [a,b,*], M. Perello [c], D.L. Choi [a,b], I.J. Magrisso [a,b], H. Kirchner [a,b], P.T. Pfluger [a,b], M. Tschoep [a,b], J.M. Zigman [c], S.C. Benoit [a,b]

[a] Department of Psychiatry, Metabolic Diseases Institute, University of Cincinnati, Cincinnati OH, USA
[b] Dept. of Int. Medicine, Metabolic Diseases Institute, University of Cincinnati, Cincinnati OH, USA
[c] Division of Hypothalamic Research, University of Texas Southwestern Medical Center, Dallas TX, USA

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ABSTRACT

Ghrelin is an orexigenic hormone that regulates homeostatic and reward-related feeding behavior. Recent evidence indicates that acylation of ghrelin by the gut enzyme ghrelin *O*-acyl transferase (GOAT) is necessary to render ghrelin maximally active within its target tissues. Here we tested the hypothesis that GOAT activity modulates food motivation and food hedonics using behavioral pharmacology and mutant mice deficient for GOAT and the ghrelin receptor (GHSR). We evaluated operant responding following pharmacological administration of acyl-ghrelin and assessed the necessity of endogenous GOAT activity for operant responding in GOAT and GHSR-null mice. Hedonic-based feeding behavior also was examined in GOAT-KO and GHSR-null mice using a "Dessert Effect" protocol in which the intake of a palatable high fat diet "dessert" was assessed in calorically-sated mice. Pharmacological administration of acyl-ghrelin augmented operant responding; notably, this effect was dependent on intact GHSR signaling. GOAT-KO mice displayed attenuated operant responding and decreased hedonic feeding relative to controls. These behavioral results correlated with decreased expression of the orexin-1 receptor in reward-related brain regions in GOAT-KO mice. In summary, the ability of ghrelin to stimulate food motivation is dependent on intact GHSR signaling and modified by endogenous GOAT activity. Furthermore, GOAT activity is required for hedonic feeding behavior, an effect potentially mediated by forebrain orexin signaling. These data highlight the significance of the GOAT–ghrelin system for the mediation of food motivation and hedonic feeding.

Introduction

Ghrelin is a feeding-related hormone involved in the homeostatic and hedonic regulation of food intake (Abizaid et al., 2006; Perello and Zigman, 2012; Perello et al., 2010; Tschop et al., 2000). Recent work implicates a necessity for ghrelin signaling in a variety of rewarding behaviors including food, alcohol and psychostimulant reinforcement (Jerlhag et al., 2009, 2010; McCallum et al., 2011; Perello et al., 2010; Skibicka et al., 2011, 2012). The mechanism of ghrelin action in the context of reward behavior likely occurs through activation of the ghrelin receptor (GHSR) (Jerlhag et al., 2009) within tegmental and cholinergic signaling systems which activate brain reward circuitry (Jerlhag et al., 2006, 2007; Skibicka et al., 2012). Recent advances in the physiology of ghrelin indicate that the post-translational addition of an acyl group to the third serine of ghrelin is required to render ghrelin maximally active at the GHSR receptor (Kojima et al., 2001; Matsumoto et al., 2001). This regulation is achieved through the action of ghrelin-*O*-acyltransferase, an enzyme produced in the enterocytes of the stomach (Gutierrez et al., 2008; Sakata et al., 2009; Yang et al., 2008). Any contribution of the ghrelin–GOAT system to the regulation of rewarding behavior is currently unknown. Here, we hypothesized that GOAT-mediated acylation of ghrelin is a critical modulator of food reward. To test this possibility, we examined operant performance and hedonic feeding behavior in GOAT-KO and GHSR-null mice following both metabolic and pharmacological manipulations that stimulate food reward behavior.

Experimental procedures

Animals

GHSR-null and GOAT-KO mice with their respective wild-type littermates were generated as reported previously (Kirchner et al., 2009; Zigman et al., 2005). Study animals were derived from crosses between heterozygous animals back-crossed >10 generations onto a C57BL6/J genetic background. Current studies were performed with male mice, which were housed in a 12-h light/dark cycle with regular chow (4 g% fat, diet #7001, Harlan-Teklad, Madison, WI) and water available *ad lib*, except when indicated. All animal procedures were carried out in accordance with NIH guidelines and adhered to the guidelines set forth by the Institutional Animal Care and Use Committee at the University of Cincinnati and/or UTSW.

* Corresponding author at: University of Cincinnati, Cincinnati, OH 45237-506, Room 234, Metabolic Diseases Institute, USA.
E-mail address: jon.davis@uc.edu (J.F. Davis).

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Diets

All mice were maintained on chow (Teklad, 3.41 kcal/g, 0.51 kcal/g from fat) throughout, unless otherwise noted. The hedonic feeding experiments utilized high-fat diet (HFD) (Research Diets, New Brunswick, NJ, 4.41 kcal/g, 1.71 kcal/g from fat) and the physiological assessment of GOAT deletion utilized a medium chain triglyceride diet (MCT) (Teklad, 3.9 kcal/g, 42.9 kcal/g from fat). Changes in acylated ghrelin were assayed in mice following a twenty-four hour fast.

Apparatus

Progressive ratio conditioning was conducted in four identical chambers (Model ENV307A, Med Associates Inc.) constructed of aluminum end walls and clear plexiglass sides and measuring 21.6×21.6×27.9 cm. A grid of 0.48-cm diameter stainless steel bars, spaced 1.9 cm apart, served as the floor of each chamber. A food cup was located on one end wall of each chamber inside a 5×5-cm recessed opening. Two portals were located approximately 5 cm to the left and right of the food cup and only the left portal was active and corresponded with food pellet delivery. All experimental events were controlled by computers located in an adjoining room running ABET software (Lafayette Instruments; Lafayette, IN).

Operant responding

Operant responding experiments were performed as previously indicated (Perello et al., 2010). Briefly, adult (10–12 weeks old) mice were trained to poke their nose into a lit portal to obtain a 20 mg HFD pellet. The effects of active ghrelin on operant responding were determined in three independent experiments: experiments 1 and 2 utilized 8 GOAT-KO, GHSR-null mice along with their respected wild-type littermates to determine the effects of endogenous ghrelin activity on operant responding. Experiment 3 was designed to test the effects of pharmacological addition of acylated ghrelin on operant responding in wild type and GHSR-null mice (n=7–8/group). Training and testing sessions were performed between 10:00 a.m. and 2:00 p.m.

During the training period, GHSR-null mice were restricted to 60% of their average daily food intake. Because caloric restriction results in impaired glucose homeostasis that is incompatible with life (Yang et al., 2008) GOAT-KO mice were trained under *ad lib* feeding conditions. For the training sessions, mice initially received pellets under a fixed ratio schedule and were then moved to a progressive ratio reinforcement schedule (Perello et al., 2010), whereby the response requirement for each successive pellet was progressively increased as follows: 5, 10, 20, 30, 50, 70, 100, 130, *etc.* This progressive ratio schedule was used through the rest of the test to evaluate the motivation to obtain HFD pellets. Breakpoint was defined as the last progressive ratio which an animal successfully completed to receive a reinforcement within a 10 min period. Mice were considered trained once they demonstrated a stable 10 min breakpoint for 3 successive days. Following training GHSR-null, GOAT-KO and wild-type mice were tested following a twenty four hour deprivation period to evaluate deprivation-induced responding in each group.

To examine the effects of active ghrelin on operant responding, *ad lib*-fed GHSR-null and wild-type littermates were tested after saline and ghrelin administration. Octanoylated ghrelin (Global Peptide, Fort Collins, CO, catalog # C-et-004) was suspended in saline and injected s.c. in a dose of 2 μg/g body weight in 150 μl. Mice were injected 10 min before placing them into the chambers, and their motivation to obtain HFD pellets was assessed according to the above progressive ratio schedule.

Plasma ghrelin measurement

Plasma acylated-ghrelin levels of mice were measured using an EIA kit according to the manufacturer's instructions (#10006307, Cayman Chemical Company, Ann Arbor, MI) as previously described (Sakata et al., 2009). To determine acyl-ghrelin levels in *ad lib* or fasted mice, blood from mice with *ad lib* access to regular chow and from mice that had been fasted for 24 h was collected from tails into tubes containing EDTA, on ice. The protease inhibitor p-hydroxymercuribenzoic acid (#12425, Sigma-Aldrich) was added to each sample at a final concentration of 1 mM. The samples were centrifuged and the resulting plasma was immediately treated with one-tenth volume of 1 N HCl, to prevent degradation of acylated ghrelin, and then stored at −80 °C until use. Acylated ghrelin levels were determined using ghrelin (rat acylated) EIA kits according to the manufacturer's instructions. The reported inter-assay variability for this EIA kit is 7.00 CV%, at a mean concentration of 24.9 pg/ml with 99.5 +/− 2.9 recovery.

Hedonic feeding

To determine the effects of GHSR or GOAT deletion on hedonic feeding behavior we utilized a feeding paradigm in which sated rodents voluntarily overconsume a palatable test diet (Choi et al., 2010). To do this, GHSR-null and GOAT-KO mice along with their wild type littermates (n=6–8/group) were exposed to a small amount of high fat diet (HFD; 4.41 kcal/g, 1.71 kcal/g from fat; Research Diets; New Brunswick, NJ) to prevent neophobia. All mice were food deprived for 21 h prior to testing. On the day of testing, chow food hoppers were weighed, placed in each cage and subsequently reweighed each hour for 2 h. Following the second hour of chow access, a separate set of food hoppers containing the HFD was weighed and placed in each cage next to the previously placed chow hoppers. Both sets of food hoppers were reweighed after 1 h.

qPCR

Mice were sacrificed *via* CO_2 asphyxiation, and brains were rapidly removed, frozen and stored at −80 °C until processing. The lateral hypothalamus (LH) and nucleus accumbens (NAcc) from each animal were microdissected using an AHP-1200CPV freezing plane (Thermoelectric Cooling America, Chicago, Il) which maintained a constant temperature of 12 °C throughout the dissection process. Total RNA from microdissected tissue was isolated by Trizol reagent (Invitrogen, Carlsbad, CA) and purified using the RNeasy Mini Kit (Qiagen, Valencia, CA) according to the manufacturer's instructions. The total RNA was treated to remove any potential genomic DNA contamination using RNase free DNase (Promega, Madison, WI), and was quantified using a NanoVue spectrophotometer (GE Healthcare, Cambridge, UK). RNA quality was confirmed by standard agarose gel electrophoresis. Complementary DNA (cDNA) was then reverse transcribed (RT) from 2 μg of total RNA by a mixture of random hexamers and oligo DT priming using the SuperScript III First Strand Synthesis Kit (Invitrogen, Carlsbad, CA). Non-retrotranscribed (no RT) reactions were also prepared from each sample to control for potential genomic DNA contamination. The cDNA and no RT controls were diluted, and 10 ng of template cDNA from each sample was used to measure mRNA expression of selected genes by real time quantitative PCR utilizing the MyIQ Real-Time PCR Detection System (Bio-Rad, Hercules, CA). Triplicate measurements for each sample were run on standard iCycler 96 well plates, along with no template controls (NTC) to detect potential cross contamination, in 20 μl reaction volumes consisting of 10 μl 2× iQ Sybr Green Supermix (Bio-Rad, Hercules, CA), 1 μl of 0.2–0.5 μM each primer, 3 μl DEPC water, and 5 μl of template. All qPCR reactions included a melt curve analysis to ensure specificity of signal. Relative expression for each gene of interest was calculated by extrapolation to a

standard curve individually run on each plate and derived from serial dilutions of a pooled sample of reference cDNA, and normalized to relative expression of the reference gene acidic ribosomal phosphoprotein 36B4 for gene expression in both hypothalamus and nucleus accumbens.

Primers

The following forward and reverse primer sequences (IDT, San Diego, CA) were used to amplify rat prepro-orexin (Acc.# NM_013179); orexin-1 receptor (Acc.# NM_013064) and orexin-2 receptor (Acc.# NM_013074), respectively: prepro-orexin: 5′-TTC CTT CTA CAA AGG TTC CCT-3′, 5′-GCAACAGTTCGTAGAGACGGCAG-3′; orexin receptor 1: 5′-CCCCACACTCATGGAAAGAC-3′, 5′-GGGACAGTAACCACGTCCAC-3′; and orexin receptor 2: 5′-GTGCTTGTGACCATCACCTG-3′, 5′-TGGCT GTGCTCTTGAACATC-3′.

Blood glucose

Following 21 h of fasting GOAT-KO mice were placed into restrainer tubes and blood was collected from the tail vein into EDTA-coated tubes containing p-hydroxymercuribenzoic acid (final concentration, 1 mM) to determine fasting levels of glucose within both cohorts. Next, food was returned for 4 h and 200 μl of blood was sampled at 120 min. Blood glucose concentrations were measured on a Medisense Optium glucometer (Abbot Laboratories, Chicago, IL).

Statistics

Data were analyzed using STATISTICA 6.0 for Windows (StatSoft; Tulsa, OK). The breakpoints for each mouse in each condition were calculated as the average of the collected data in independent days. Then, these values were used to calculate the average of each genotype in each condition. We used paired *t*-test to compare the different breakpoints for the different treatments in each genotype and analysis of variance (ANOVA) to assess the effects of genotype. The effects of genotype on hedonic feeding were analyzed using an analysis of variance (ANOVA) in combination with least significant differences (LSD) *post hoc* comparisons to assess the source of significant main effects. $P<0.05$ was considered statistically significant.

Results

Endogenous ghrelin

We first confirmed that the caloric restriction protocol used in this study resulted in the expected physiological increases in acyl ghrelin. The overnight fasting protocol produced a significant increase in acylated ghrelin levels in both wild type and GHSR-null mice (12.1 ± 1.3 and 13.6 ± 1.7 pg/ml) compared to *ad lib*-fed levels (7.1 ± 0.8 and 6.8 ± 0.7 pg/ml, for wild type and GHSR-null mice, respectively, $p<0.01$) (Fig. 1A).

Operant responding

The effects of physiologically increasing ghrelin on operant responding was achieved by withholding access to food for 24 h, and was evidenced by a two-fold increase in acylated ghrelin (Fig. 1A). Following this twenty four hour deprivation period, wild type mice showed a significant increase of the breakpoint responding compared to *ad lib*-fed mice ($p<0.05$) (Fig. 1B). Next, we assessed the effects of this same dietary manipulation on operant responding in mice with attenuated ghrelin signaling. The 24 h deprivation protocol had no effect on raising acylated ghrelin in GOAT-KO mice (data not shown) and also was unable to induce a statistically significant increase in breakpoint responding in GOAT-KO mice (Fig. 2B). In contrast, GHSR-null mice did display significant increases ($p<0.05$) in breakpoint responding following the fasting period compared to the *ad lib*-fed condition (Figs. 1C–D). Although acyl-ghrelin levels did rise in GHSR-null mice in the setting of the 24-h fast, it has been shown here (see below) and previously that they are unable to mount an orexigenic or food reward response to acyl-ghrelin (Perello et al., 2010).

To determine if active ghrelin is sufficient to increase breakpoint responding we administered a 2 μg/g s.c. dose of acyl-ghrelin to mice trained to lever press for high fat pellets. Consistent with previous reports (Perello et al., 2010), wild type mice receiving acyl-ghrelin 10 min prior to placement into the operant chamber, showed a significant increase of the breakpoint ($p<0.001$) (Fig. 2A). GHSR-null mice receiving acyl-ghrelin did not show changes in the breakpoint as compared to saline-treated GHSR-null mice suggesting that the effects of active ghrelin on operant responding are mediated by the GHSR receptor (Fig. 2B).

Hedonic feeding

Whereas the above operant responding task assessed the effects of fasting-induced rises in ghrelin or administered ghrelin on food reward behavior, we also wanted to better model reward-based eating that occurs in humans that are fully sated. Thus, in our "Dessert Effect" test, we calorically restricted mice, allowed them to become sated on regular chow, and then assessed their intake of a palatable HFD "dessert" (Choi et al., 2010, 2012). GOAT-KO and GHSR-null mice were used to determine the effect of disturbed ghrelin signaling on the overconsumption of HFD. Wild type and GOAT-KO mice displayed increased consumption of the HFD relative to the last hour of re-feeding. Interestingly, wild type mice consumed significantly more HFD following re-feeding than did their GOAT-KO littermates, suggesting that active ghrelin levels regulate overconsumption of the HFD "dessert" in this model ($p<0.05$, Fig. 2C). When compared to their wild-type littermates, GHSR-null mice consumed a similar amount of the HFD (Fig. 2D).

Blood glucose/body weight

Since GOAT activity mediates homeostatic glucose responses, more specifically, life-threatening falls in blood glucose levels following severe caloric deprivation (16) we next wanted to determine if the behavioral effects observed here in GOAT-KO mice might be directly related to altered blood glucose rather than absent acyl-ghrelin. Thus, we examined plasma glucose levels in GOAT-KO and wild type mice following fasting and re-feeding. GOAT-KO and wild type littermates displayed similar plasma glucose levels following an overnight fast and upon re-feeding (Figs. 2D–E). In addition, one week of access to a diet that induces endogenous levels of acyl-ghrelin and promotes body weight gain led to increased body weight gain in wild-type but not in GOAT-KO mice (Fig. 2F).

qPCR

Previous studies suggest that the central orexin system may regulate ghrelin action on reward-based feeding behavior (Perello et al., 2010). To examine this possibility we investigated transcriptional levels of orexin and its associated receptors in wild type and GOAT-KO mice following a twenty-four hour fast. In the hypothalamus no differences were observed in the expression of orexin or orexin-1 receptor, Figs. 3A–B; there was a trend of reduced orexin-2 receptor in GOAT mice following an overnight fast however this did not reach statistical significance (Fig. 3C). All other orexin-related transcripts were unaltered in the LH of GOAT mice. GOAT-KO displayed decreases in orexin related gene transcripts following a twenty-four hour fast. In particular, GOAT mice displayed significantly less orexin-1 receptor

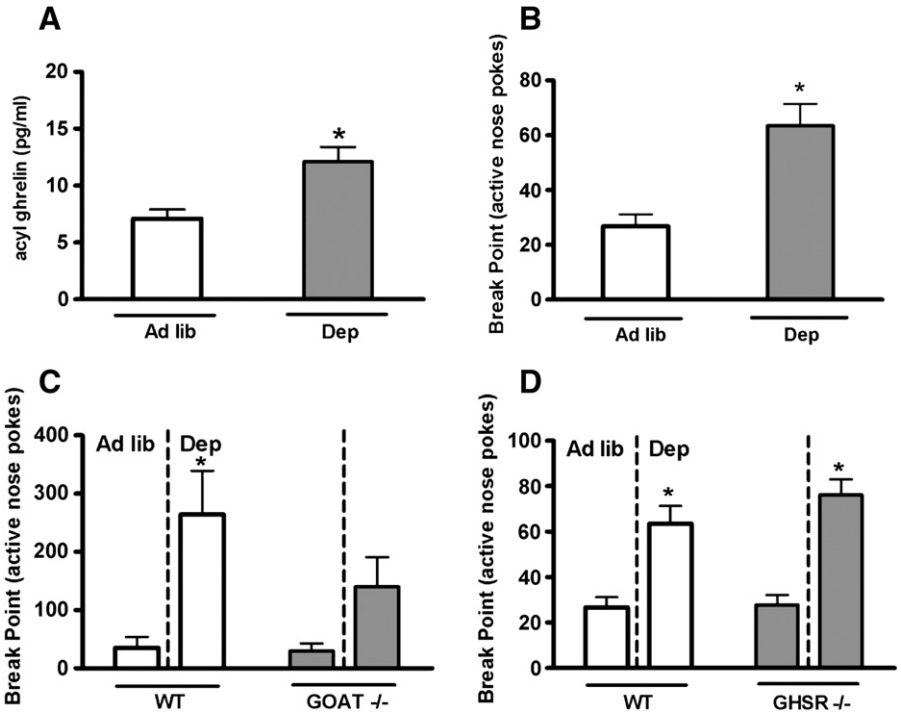

Fig. 1. Illustrated are acyl-ghrelin levels following A) a twenty-four hour fast. This figure also depicts breakpoint responding under a progressive ratio of reinforcement expressed as active nose pokes in B) wild-type, C) GOAT-KO or D) GHSR-null mice under *ad lib* and fasting conditions. *Ad libitum* (*Ad lib*), deprivation (Dep). * indicates $p<0.05$.

mRNA ($F_{(1,5)}=8.12$, $p<0.05$) compared to wild-type mice in the NAcc (Fig. 3D). There were no differences in the expression of orexin receptor-2 in the NAcc, Fig. 3E.

Discussion

The goal of the current study was to test the hypothesis that the process of ghrelin acylation regulates food reward. From this effort, several notable findings emerged. Negative metabolic status is a potent stimulator of acyl-ghrelin, a state correlated with enhanced motivation, and dependent on endogenous GOAT activity. Acyl-ghrelin augments operant behavior, and the nature of this regulation is dependent on signaling through GHSR, the only known ghrelin receptor. Furthermore, mice with disrupted acyl-ghrelin display decreased hedonic feeding and transcriptional changes in brain reward circuitry that parallels the observed deficiencies in motivated behavior. Collectively, these findings highlight

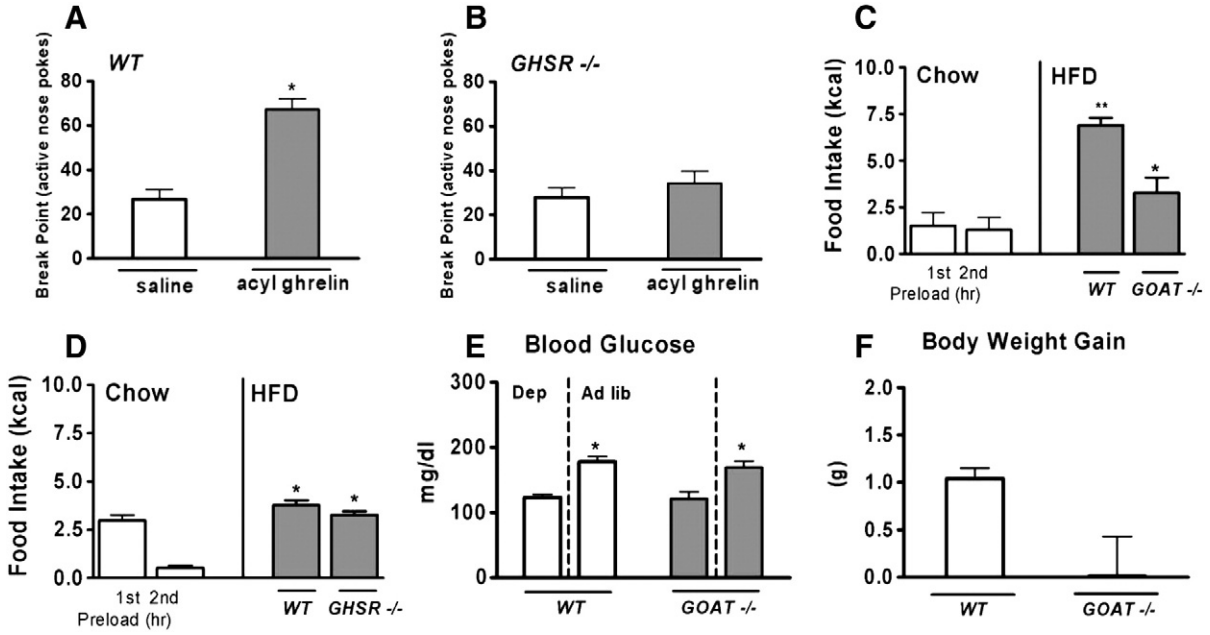

Fig. 2. Depicted is the breakpoint responding expressed as active nose pokes in A) wild-type or B) GHSR-null mice following acute administration of acyl-ghrelin and hedonic feeding behavior in C) GOAT or D) GHSR-null mice. This figure also illustrates E) blood glucose following an overnight fast and F) body weight gain from a medium chain triglyceride diet in wild-type and GOAT-KO mice. Preload refers to feeding from chow following fasting. * indicates $p<0.05$.

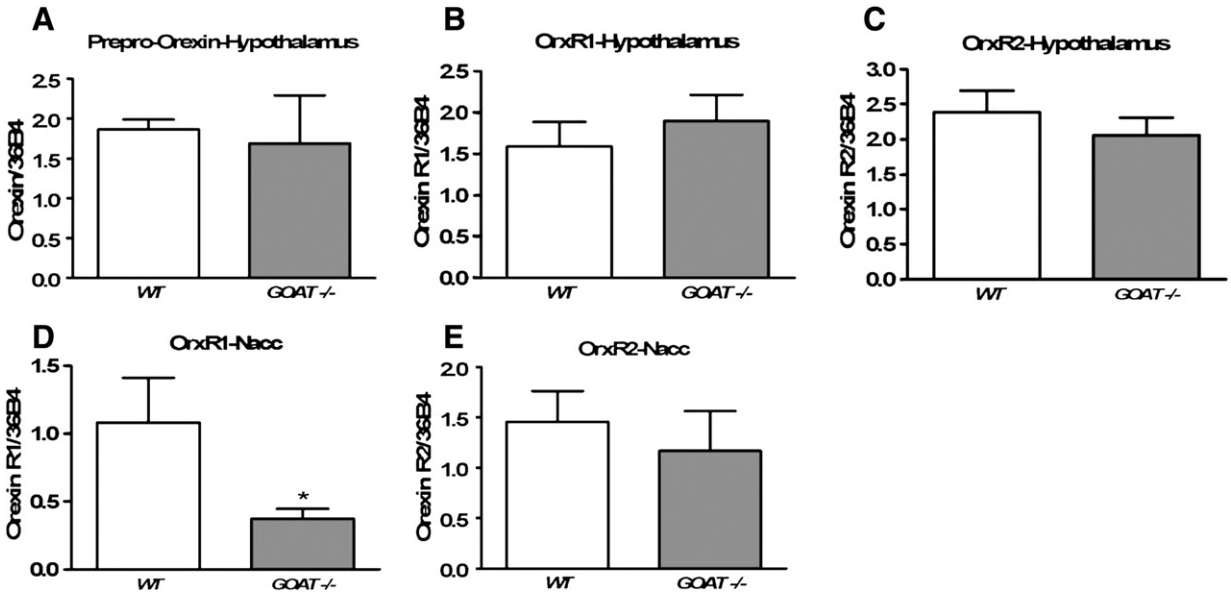

Fig. 3. Indicated is the expression of prepro-orexin, orexin-1 receptor (Orx1R) and orexin-2 receptor (OrxR2) in the hypothalamus (A–C) and nucleus accumbens (NAcc) (D–E) in wild-type and GOAT-KO mice following an overnight fast. * indicates $p<0.05$.

the significance of the ghrelin–GOAT system for the regulation of food motivation and hedonic feeding behavior.

Changes in metabolic status are perhaps the most physiologically relevant events regulating motivational state. Specifically, occurrences of caloric deprivation augment the motivation to obtain both food and drug-related reinforcers (Carroll and Meisch, 1980; Carroll et al., 1979). Of relevance, ghrelin levels increase in response to fasting (Kojima et al., 1999) indicating that metabolic status can be a potent regulator of ghrelin activity. A unique feature of the ghrelin molecule is its ability to be rendered active by an acyl modification to Ser3 of the endogenous peptide by the enzyme ghrelin-*O* acyltransferase (GOAT) (Gutierrez et al., 2008; Sakata et al., 2009; Yang et al., 2008). Similar to the ghrelin peptide, peripheral GOAT levels are increased following chronic under nutrition (Gonzalez et al., 2008). In the current study acyl-ghrelin peptide levels increased in plasma following both chronic caloric restriction and following a twenty-four hour fast indicating that states of negative energy balance promote activation of ghrelin peptide.

In terms of behavior, it is clear that ghrelin alters the rewarding properties associated with food and drug reinforcers (Jerlhag et al., 2009, 2010; McCallum et al., 2011; Perello et al., 2010; Skibicka et al., 2011, 2012). For example, ghrelin administration enhances the rewarding properties of high fat diet while ghrelin receptor antagonists abolish this effect (Perello et al., 2010). In addition ghrelin regulates the rewarding aspects of alcohol, cocaine and amphetamine (Jerlhag et al., 2009, 2010). However, the extent to which acyl-ghrelin participates in the regulation of reward is unknown. GOAT-KO mice displayed similar rates of responding in comparison to wild type littermates when tested under *ad libitum* feeding conditions. In contrast, GOAT-KO mice were unable to increase their levels of responding following a twenty four hour fast indicating that acyl-ghrelin may regulate metabolically induced increases in operant responding. To test this hypothesis directly, we examined progressive ratio responding after peripheral injection of acyl-ghrelin. Indeed, administration of acyl-ghrelin increased operant responding for high fat diet. This effect was absent in GHSR-null mice suggesting that the ability of acyl-ghrelin to elevate operant performance is dependent on signaling through GHSR. Collectively these data indicate that acyl-ghrelin mediates the augmenting effects of caloric deprivation on operant responding and is sufficient to induce increases in operant performance, an effect dependent on intact GHSR signaling.

Whereas operant performance represents a robust measure of food motivation, the propensity to consume food for its hedonic qualities is perhaps more relevant to humans. Importantly, ghrelin signaling has been implicated in the hedonic aspects of feeding behavior (Malik et al., 2008). The current study tested the ability of GOAT-KO and GHSR-null mice to selectively eat a high fat diet "dessert" following a period of caloric repletion and then re-feeding with regular chow. Consistent with previous reports (Choi et al., 2010, 2012), wild type mice displayed increased consumption of the HFD dessert following re-feeding indicating that the HFD was sufficient to induce consumption beyond homeostatic need. Notably, GOAT-KO mice consumed significantly less of the HFD dessert than did their wild-type counterparts, suggesting that acyl-ghrelin is involved in the regulation of hedonic based feeding behaviors. Unexpectedly, hedonic feeding in GHSR-null mice as assessed using the Dessert Effect test was similar to that observed in wild type littermates. This finding cannot be explained by the propensity to consume the HFD alone as GHSR-null mice consume significantly less HFD than littermate controls (Zigman et al., 2005) and HFD consumption in GOAT-KO mice is unaltered under *ad libitum* exposure to the diet (Zhao et al., 2010). However, a number of plausible explanations for the differences in responses within GOAT-KO and GHSR-null animals exist, including possible effects specific to deleting GHSR, which is known to have high levels of constitutive activity, and also potential effects of differing levels of desacyl-ghrelin (Toshinai et al., 2006). In fact, food reward responses in the setting of caloric restriction as assessed by a conditioned place preference are lacking in GHSR-null mice only upon a mild caloric restriction protocol and restored following a more severe caloric restriction (Perello et al., 2010) (*i.e.* 80% *vs.* 60% caloric restriction). Thus, another possible explanation is that compensatory changes following metabolic challenge may differ in GOAT-KO and GHSR-null mice. It is also likely that the differences in hedonic feeding observed between GOAT and GHSR-null mice may be mediated by an imbalance in hunger and satiety signaling mechanisms in brain stem regions. For example, it has been suggested that the constitutive nature of GHSR activity within the nucleus of the solitary tract (NTS) may represent a signaling set point for appetite regulation (Holst

and Schwartz, 2004). Moreover, recent reports indicate that NTS neurons which mediate satiety also project to the ventral tegmental area and nucleus accumbens to control food intake (Alhadeff et al., 2012). Therefore it is possible that differences in hedonic feeding observed between GOAT and GHSR mice may result from an imbalance of satiety and hunger signaling mechanisms within brain stem regions that project to brain reward regions involved in the control of hedonic feeding. However, each of these contentions requires further validation.

GOAT activity mediates glucose homeostatic responses to severe caloric restriction (Zhao et al., 2010), thus it is possible that the behavioral effects following deprivation were due to reduced availability of glucose in GOAT-KO mice. To rule out this potential confound we measured glucose following a twenty-four hour fast in addition to measuring metabolic responses to a diet known to stimulate endogenous GOAT activity. GOAT-KO mice displayed similar levels of plasma glucose after fasting and upon re-feeding indicating that the homeostatic response of glucose after a brief period of caloric deprivation is intact in GOAT deficient mice. In addition, one week of exposure to a diet rich in medium chain triglycerides, led to increased body weight gain in wild type but not in GOAT-KO mice which supports previous findings (Kirchner et al., 2009) and indicates that deletion of GOAT is physiologically relevant. Taken together these results suggest that diminished glucose homeostasis cannot account for the observed changes in behavior observed in GOAT-KO mice despite GOAT's obligatory role in the maintenance of long term energy homeostasis (Zhao et al., 2010).

To determine if deficiencies in central responses to ghrelin may account for the behavioral responses observed in GOAT-KO mice we examined transcriptional profiles of orexin and its receptors within the hypothalamus and nucleus accumbens. Recent evidence indicates that ghrelin exerts its effects on food reward through activation of the hypothalamic orexin system (Perello et al., 2010). Regarding food reward, the extra-hypothalamic expression of the orexin receptor-1 (ORX1R) has received particular attention for its ability to modulate hedonic-based feeding behaviors (Borgland et al., 2009; Choi et al., 2010; Zheng et al., 2007) and more recent evidence indicates that orexins may target the nucleus accumbens (NAcc) directly to modulate dopamine neurochemistry (Mukai et al., 2009). Thus we examined expression of prepro-orexin and its two receptors, orexin-1R and orexin-2R in the hypothalamus and nucleus accumbens of wild type and GOAT-KO mice. There were no differences in orexin or its associated receptors in the hypothalamus; interestingly, GOAT-KO mice displayed decreased ORX1R expression in the NAcc raising the possibility that diminished orexin signaling may account for the observed deficiencies in behavior however this idea requires further investigation.

Conclusion

In summary, changes in metabolic state and in particular those resulting in negative energy balance are physiological activators of the ghrelin–GOAT system leading to increases in plasma levels of acyl-ghrelin. Concomitant with the increase in acyl-ghrelin is an increase in the motivation to obtain food rewards. Our results indicate that this increase in motivation is regulated by endogenous GOAT activity and dependent on intact signaling through the GHSR receptor. Furthermore, ghrelin acylation is involved in the regulation of hedonic responses to calorically dense food rewards an effect that coincides with reduced orexin signaling in brain reward circuitry. Collectively these results describe a role for acyl-ghrelin signaling in the regulation of motivation and hedonics following changes in metabolic status. In humans, metabolic status is a potent modulator of executive function, a fact that is most apparent in patients with eating disorders who often display aberrant and disrupted decision making skills. Thus, ghrelin–GOAT signaling may represent a potential mechanism of interest toward the treatment of disordered eating and its associated consequences.

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