Published in final edited form as:

Neuropharmacology. 2018 July 15; 137: 230–239. doi:10.1016/j.neuropharm.2018.04.011.

Neuroendocrine response to GABA-B receptor agonism in alcohol-dependent individuals: Results from a combined outpatient and human laboratory experiment

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Abstract

Gamma-aminobutyric acid (GABA), the main inhibitory neurotransmitter in the nervous system, plays an important role in biobehavioral processes that regulate alcohol seeking, food intake, and stress response. The metabotropic GABA-B receptor has been investigated as a potential therapeutic target for alcohol use disorder, by using orthosteric agonists (e.g., baclofen) and positive allosteric modulators. Whether and how pharmacological manipulation of the GABA-B receptor, in combination with alcohol intake, may affect feeding- and stress-related neuroendocrine pathways remains unknown. In the present randomized, double-blind, placebo-controlled study, thirty-four alcohol-dependent individuals received baclofen (30 mg/day) or placebo in a naturalistic outpatient setting for one week, and then performed a controlled laboratory experiment which included alcohol cue-reactivity, fixed-dose priming, and self-administration procedures. Blood samples were collected, and the following neuroendocrine markers were measured: ghrelin, leptin, amylin, glucagon-like peptide-1 (GLP-1), insulin, prolactin, thyroid-stimulating hormone, growth hormone, cortisol, and adrenocorticotrophic

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Declaration of interest: none

Conflict of Interest: The authors declare that they have no conflict of interest.

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hormone (ACTH). During the outpatient phase, baclofen significantly increased blood concentrations of acyl-ghrelin ($p=0.01$), leptin ($p=0.01$), amylin ($p=0.004$), and GLP-1 ($p=0.02$). Significant drug $\times$ time-point interaction effects for amylin ($p=0.001$) and insulin ($p=0.03$), and trend-level interaction effects for GLP-1 ($p=0.06$) and ACTH ($p=0.10$) were found during the laboratory experiment. Baclofen, compared to placebo, had no effect on alcohol drinking in this study (p 's > 0.05). Together with previous studies, these findings shed light on the role of the GABAergic system and GABA-B receptors in the shared neurobiology of alcohol-, feeding-, and stress-related behaviors.

Keywords

GABA; GABA-B receptor; Baclofen; Alcohol; Neuroendocrine Response; Appetitive Hormones; Stress Hormones

1. Introduction

Gamma-aminobutyric acid (GABA) acts as the main inhibitory neurotransmitter in the mammalian nervous system by binding to its ionotropic (GABA-A and GABA-C) and metabotropic (GABA-B) receptors (Watanabe et al., 2002). The GABAergic system plays a fundamental role in a variety of neurophysiological processes, including those related to alcohol seeking and consumption, and alterations in different components of the GABAergic system may result from chronic/excessive alcohol use (Koob, 2006). There is a complex pharmacological interplay between alcohol and GABA receptors; while alcohol potentiates GABA-A receptors to enhance GABAergic neurotransmission, a number of mechanisms chiefly mediated by GABA-B receptors dampen alcohol-induced activation of the GABAergic synapses (Roberto et al., 2003; Weiner and Valenzuela, 2006; Wu et al., 2005). GABA-B receptors are located at presynaptic, postsynaptic, and extrasynaptic sites, and show wide distribution in both peripheral and central nervous systems, including brain areas that mediate alcohol-related behaviors (e.g., ventral tegmental area, amygdala, and globus pallidus) (Castelli and Gessa, 2016; Watanabe et al., 2002). Potentiation of GABA-B receptors can antagonize alcohol reinforcement and intake; therefore, numerous preclinical and clinical experiments have investigated GABA-B receptor, via orthosteric agonists and positive allosteric modulators, as a promising pharmacological target to suppress alcohol craving and drinking (Agabio and Colombo, 2014; Brown et al., 2015; Filip et al., 2015; Mirijello et al., 2015; Phillips and Reed, 2014).

The integral role of GABAergic neurotransmission in modulating consummatory and addictive behaviors extends beyond alcohol use. In fact, the GABAergic system, particularly GABA-B receptors, are among the main neural substrates involved in metabolic homeostasis, appetite regulation, and food intake (Bonaventura et al., 2008; Delgado, 2013; Stratford and Kelley, 1997). Previous data indicate that pharmacological manipulation of the GABA-B receptors influences food intake/preference, and this effect is mediated primarily through central GABA-B receptors (Ebenezer and Baldwin, 1990; Ebenezer and Patel, 2004; Patel and Ebenezer, 2004). Some rodent and human studies have found increased feeding (De Oliveira et al., 2011; Ebenezer, 1996, 2012b; Ebenezer and Baldwin, 1990; Ebenezer

and Patel, 2004; Ebenezer and Prabhaker, 2007; Ebenezer and Pringle, 1992; Patel and Ebenezer, 2008a, b), while others show a decrease in food intake and weight (Arima and Oiso, 2010; Krolczyk et al., 2005; Sato et al., 2007; Wojnicki et al., 2006; Zarrindast et al., 1989) following GABA-B receptor agonism and/or positive allosteric modulation. Such inconsistent results may stem from the heterogeneity of the study designs and their samples (e.g., satiated versus food-deprived, obese versus normal weight). It has also been demonstrated that some appetite-regulating drugs modify the GABA signaling in the midbrain, through presynaptic GABA-B receptors, which ultimately alters the activity of dopaminergic neurons (Ledonne et al., 2009).

Appetitive/metabolic hormones such as ghrelin, leptin, amylin, and glucagon-like peptide-1 (GLP-1) not only control homeostatic feeding, but also regulate hedonic and addictive properties of food intake, mainly through interactions with the mesolimbic dopaminergic pathway (Narayanan et al., 2010; van Zessen et al., 2012). Notably, GABA-B-mediated processes are involved in both peripheral secretion of these hormones, as well as the mechanisms through which they act in the central nervous system (Ataie et al., 2013; Braun et al., 2004; Cruz et al., 2013; Evans et al., 2014; Fulton et al., 2006; Gameiro et al., 2005; Guimond et al., 2014; Jonaidi et al., 2012; Kahn and Minokoshi, 2013; Lee et al., 2015; Sato et al., 2005; Secher et al., 2014; Wang et al., 2007). Consistent with the biological and behavioral overlap between alcohol and food consumption (Volkow et al., 2012), growing evidences suggest that appetitive/metabolic hormones contribute to the neurobiology of alcohol craving and intake, and to the pathophysiology of alcohol use disorder (Addolorato et al., 2009; Engel and Jerlhag, 2014; Farokhnia et al., 2017a; Zallar et al., 2017).

Stress-related pathways have been shown to modulate both food and drug seeking behaviors, primarily through negative reinforcement mechanisms (al'Absi, 2018; Koob et al., 2014; Torres and Nowson, 2007). Dysregulation of the brain stress system is a key element involved in the development and maintenance of alcohol use disorder, and neuroendocrine mediators of stress response play a substantial role in this regard (Herman, 2012; Spanagel et al., 2014). For instance, at the pharmacological level, alcohol directly activates the hypothalamic-pituitary-adrenal (HPA) axis (Allen et al., 2011; Zhou and Kreek, 2014; Zimmermann et al., 2004). Together with other substrates, the HPA axis represents an important neuroendocrine pathway involved in both acute and chronic stress (Smith and Vale, 2006). Moreover, alterations in different components of the HPA axis facilitate the transition from mild-to-moderate alcohol drinking to excessive alcohol consumption and dependence, and may also contribute to the risk of relapse (Adinoff et al., 2005; Blaine and Sinha, 2017; Galesi et al., 2016; Kiefer et al., 2002a; Koob, 2010; Stephens and Wand, 2012). Accumulating evidence indicates that the GABAergic system, including GABA-B receptors, plays an important role in stress response/adaptation by regulating the underlying neural circuits and neuroendocrine pathways involved in this process (Cryan and Kaupmann, 2005; Hausler et al., 1993; Lux-Lantos et al., 2008; Pizzo et al., 2017).

While GABA-B receptor has been investigated as a potential therapeutic target for alcohol use disorder, human research is scarce on whether and how pharmacological manipulation of the GABA-B receptor may affect feeding- and stress-related hormones in alcohol-dependent individuals, and in combination with alcohol intake. This is an important set of questions,

especially in light of the overlap between alcohol-, food-, and stress-related biobehavioral correlates, and the established role of the GABA-B receptor in mediating this link. In the present study, we explored the neuroendocrine response to GABA-B receptor agonism in alcohol-dependent individuals, by using the prototypic selective agonist baclofen in a double-blind placebo-controlled fashion. Assessments were performed in two complementary settings: A) an outpatient phase during which participants received baclofen and maintained their regular lifestyle, including diet and alcohol drinking habits; B) a laboratory experiment which was conducted under well-controlled conditions (e.g. standardized food and alcohol intake) after baclofen dosing was at steady-state.

2. Materials and Methods

2.1. Study design and participants

The parent study (Farokhnia et al., 2017b) was a between-subjects, randomized, double-blind, placebo-controlled trial conducted at the National Institutes of Health (NIH) Clinical Center in Bethesda, MD (ClinicalTrials.gov - NCT01751386). The study protocol was approved and continuously reviewed by the NIH Addictions Institutional Review Board. Potential candidates were evaluated through a screening protocol at the National Institute on Alcohol Abuse and Alcoholism (NIAAA), and eligible individuals were enrolled in this study after providing written informed consent. Participants were non-treatment seeking individuals with a current diagnosis of alcohol dependence and high trait anxiety, as respectively determined by the Structured Clinical Interview for Diagnostic and Statistical Manual of Mental Disorders - Fourth Edition - Text Revision (DSM-IV-TR) Axis I Disorders (SCID) and the Spielberger State Trait Anxiety Inventory (STAI) - trait version. The study eligibility criteria are listed in Appendix S1.

2.2. Study procedures

A detailed description of the study has been previously reported (Farokhnia et al., 2017b). Briefly, as outlined in Figure 1, this study consisted of two consecutive phases: an outpatient phase and a human laboratory experiment.

During the outpatient phase, participants received oral baclofen or matched placebo in a double-blind fashion for 7 days (+1–8 days to allow flexibility). On Day 1, baseline assessments were done, and the study drug was dispensed according to each participant's randomization code. The initial dose of baclofen was 15 mg/day (5 mg t.i.d.; titration phase) for 3 days, followed by 30 mg/day (10 mg t.i.d.; target dose) until the laboratory experiment (Table S1).

The laboratory experiment was conducted during a full-day visit. All procedures followed a consistent timetable across participants as shown in Figure 1. Participants came to the clinic after taking the first drug dose at home. Behavioral questionnaires were completed, two standardized meals were provided (400 kcal each, details in Appendix S2), and the second drug dose was administered. An optional smoking break was also given, as the last opportunity to smoke cigarettes, approximately one hour before the laboratory experiment started. Participants were escorted to a private bar-like room at noon for the laboratory

experiment, which consisted of three consecutive procedures: Alcohol Cue-Reactivity (ACR), Alcohol Priming (AP), and Alcohol Self-Administration (ASA) (Farokhnia et al., 2017b). The ACR included a water trial followed by two alcohol trials. Each trial took three minutes during which participants were exposed to visual, tactile, olfactory, and proprioceptive stimuli associated with the beverage, and at the end of each trial, their alcohol craving was assessed. The next procedure was AP, for which a fixed-dose priming drink designed to raise the blood alcohol concentration to 0.03 g/dL was served, and participants were asked to drink it within five minutes. Estimated total body water was used to calculate the appropriate alcohol content for this priming drink (Watson, 1989). Finally, for the ASA procedure, two trays with four mini-drinks each were presented over a 2-hour period, and participants had the choice to drink as many mini-drinks as they wanted; an alternative reinforcer (i.e., \$3.00 for each mini-drink not consumed) was provided. The alcohol content of each mini-drink was calculated to increase the blood alcohol concentration by approximately 0.015 g/dL. A safety limit of breath alcohol concentration (BrAC) 0.12 g/dL was set, which would result in halting the session; this scenario did not occur in this study. During the AP and ASA procedures, self-reported questionnaires were used to assess alcohol craving, subjective response to alcohol, and mood/anxiety symptoms. Upon completion of the experimental procedures, participants were monitored until their BrAC reached 0 g/dL and were kept overnight in an inpatient facility. The following morning, safety assessments were performed, and participants were discharged.

2.3. Blood collection, processing, and assays

Blood samples were obtained at five time-points during the course of this study (Figure 1): (1) Pre-Drug: beginning of the outpatient phase (baseline) before the first dose of the study drug (baclofen or placebo) was administered, (2) Post-Drug/Pre-Experiment: end of the outpatient phase after the study drug was administered for at least one week, but before any experimental procedure started, (3) Post-ACR: 10 minutes after the ACR procedure was completed, (4) Post-AP: 10 minutes after the priming drink was consumed, (5) Post-ASA: 10 minutes after the ASA session was finished. At the beginning of the laboratory experiment visit, a saline lock intravenous catheter was inserted into the antecubital fossa of the non-dominant arm for multiple blood samplings. Prior to each visit, participants were asked to abstain from alcohol for 24 hours and to start fasting at midnight. Therefore, the first two sampling time-points (Pre-Drug and Post-Drug/Pre-experiment) were performed after overnight fasting. In addition, as described above, the timing of procedures and calorie content of the meals served during the laboratory experiment visit was standardized across all participants. Blood concentrations of the following hormones were measured at each time-point: ghrelin, leptin, amylin, GLP-1, insulin, prolactin, thyroid-stimulating hormone (TSH), growth hormone (GH), cortisol, and adrenocorticotrophic hormone (ACTH).

Details regarding blood sampling, processing, and analysis are presented in Figure S1. Briefly, peripheral venous blood was collected into four tubes at each time-point. Plasma and serum samples were extracted from two tubes, pipetted into microtubes, and stored in a -80°C freezer until analysis. The other two tubes were transferred to the NIH Clinical Center Department of Laboratory Medicine (DLM), as whole blood samples, immediately after collection. Total ghrelin was measured in stored plasma aliquots via a sandwich

Enzyme-Linked Immunosorbent Assay (ELISA). A bead-based multiplex ELISA was also used to simultaneously quantify the following analytes in the stored plasma samples: active ghrelin (here referred to as acyl-ghrelin), leptin, active amylin (here referred to as amylin), active GLP-1 (here referred to as GLP-1), and insulin. All samples were run in duplicates and the assays were carried out in accordance with the manufacturer's instructions. Measurements of prolactin, TSH, GH, cortisol, and ACTH were performed at the NIH Clinical Center DLM using Food and Drug Administration (FDA) cleared assays. For more details, see Figure S1.

2.4. Statistical analysis

IBM SPSS Statistics for Windows (IBM Corp., Armonk, NY – Version 20.0) was used for data analysis. All data were examined for statistical outliers and normal distribution; outliers were removed, and if necessary, a logarithm transformation was applied. Values below the lower limit of quantitation (LLOQ) for each assay were set to 1/2 of the LLOQ (Keizer et al., 2015). Changes in neuroendocrine markers over the outpatient phase (= Post-Drug - Pre-Drug) were compared between the two study groups using independent samples *t*-tests. Repeated measurements of neuroendocrine markers during the laboratory experiment were analyzed using linear mixed-effects models with drug (baclofen or placebo), time-point (Pre-Experiment, Post-ACR, Post-AP, and Post-ASA), and drug $\times$ time-point interaction as the fixed effects, and individual subjects as the random effect. Age, gender, body mass index (BMI), and number of mini-drinks consumed were tested as potential covariates in the initial run of each model; significant covariates were retained in the final model analysis. Pairwise comparisons between estimated marginal means of fitted models were adjusted using the Bonferroni procedure. Severity of anxiety (trait STAI score) and alcohol dependence (number of SCID criteria for alcohol use disorder) were also tested as potential moderators of baclofen's effect on neuroendocrine outcomes. Measures of alcohol drinking during the outpatient phase (change in average drinks per drinking days, based on Timeline Followback four days prior to each visit) and the laboratory experiment (total number of mini-drinks self-administered during the ASA session) were compared between the two study groups, using one-way analysis of covariance (ANCOVA) after testing age, gender, and BMI as covariates. Independent samples *t*-test (for continuous variables) and χ^2 -test (for categorical variables) were used to compare the demographic characteristics, baseline blood tests, length of the outpatient phase, and type of alcohol between the two study groups. Significance level was set at $p < 0.05$ (two-tailed) for all analyses.

3. Results

3.1. Study sample

Out of 148 candidates screened, 39 individuals were enrolled and a total number of 34 participants completed the study procedures (baclofen: $n = 18$, placebo: $n = 16$ – Figure S2). The study sample was predominantly male and African American; the two study groups showed no significant differences in terms of demographic characteristics or baseline blood tests (Table S2).

3.2. Outpatient phase

Baclofen, compared to placebo, significantly increased blood concentrations of acyl-ghrelin ($t_{32} = -2.68$, $p = 0.01$), leptin ($t_{23.3} = -2.54$, $p = 0.01$), amylin ($t_{29} = -3.13$, $p = 0.004$), and GLP-1 ($t_{21.6} = -2.47$, $p = 0.02$) over the course of the outpatient phase (Figure 2). These effects were not significantly moderated by anxiety or alcohol dependence severity (p 's > 0.05 , data not shown). No significant differences were found between the two study groups for the other neuroendocrine markers (Table 1 and Figure S3).

Baclofen, compared to placebo, did not significantly affect participants' alcohol consumption during the outpatient phase [change in average drinks per drinking days, M (SEM): -0.91 (0.70) versus -0.19 (0.74) standard drinking units in the baclofen versus placebo group; $F_{1,28} = 3.86$, $p = 0.48$]. Similarly, no significant drug effect on body mass was detected [change in BMI, M (SEM): 0.10 (0.09) versus -0.08 (0.07) kg/m^2 in the baclofen versus placebo group; $F_{1,31} = 2.31$, $p = 0.13$]. The length of the outpatient phase was not significantly different between the two groups [M (SEM): 7.78 (0.49) days in the baclofen group, 7.69 (0.42) days in the placebo group; $t = 0.13$, $p = 0.89$]. There was no significant differences between the two groups in medication adherence according to pill count (Farokhnia et al., 2017b).

3.3. Laboratory experiment

Significant Drug $\times$ Time-point interaction effects were detected for amylin ($F_{3,78.3} = 5.8$, $p = 0.001$) and insulin ($F_{3,79.7} = 2.94$, $p = 0.03$) concentrations. Trend-level Drug $\times$ Time-point interaction effects were also found for GLP-1 ($F_{3,72.2} = 2.50$, $p = 0.06$) and ACTH ($F_{3,88.6} = 2.11$, $p = 0.10$) concentrations (Figure 3). Anxiety or alcohol dependence severity did not significantly moderate these effects (p 's > 0.05 , data not shown). No significant interaction effects were observed for the other neuroendocrine markers during the laboratory experiment (Table 2 and Figure S4).

There was no significant difference between the study groups in the total amount of alcohol self-administered during the ASA procedure [number of mini-drinks consumed, M (SEM): 4.11 (0.67) versus 4.50 (0.71) mini-drinks in the baclofen versus placebo group; $F_{1,32} = 1.28$, $p = 0.69$]. The type of alcohol chosen by participants and used during the laboratory experiment was not significantly different between the two groups ($X^2 = 2.47$, $p = 0.47$) (Table S3).

4. Discussion

The present study examined the effects of GABA-B receptor agonism on feeding- and stress-related neuroendocrine outcomes in the context of alcohol consumption and dependence. Over the course of the outpatient phase, blood concentrations of acyl-ghrelin, leptin, amylin, and GLP-1 were significantly increased in baclofen-treated participants compared to the placebo group. Since the outpatient phase was conducted in a naturalistic setting, participants had free access to alcohol and food. However, these neuroendocrine effects were observed despite no significant changes in self-reported drinking during this phase. While we did not collect data on food intake, participants were instructed to continue

their regular diet and habits during the outpatient phase (approximately one week); therefore, it is unlikely that other factors may have acted as significant confounders. In addition, both pre- and post-drug blood samples were collected around the same time in the morning, after overnight fasting, in order to limit the potential confounding effect of diurnal and/or feeding variation on drug-induced neuroendocrine changes.

Consistent with our results, a preclinical experiment found that systemic administration of baclofen increased blood concentrations of ghrelin in non-deprived rats; intraperitoneal injections of baclofen and ghrelin were also shown to have a synergistic effect on food intake (Ebenezer, 2012a). From a mechanistic standpoint, GABAergic system and GABA-B receptors mediate CNS-related functions of ghrelin, such as its antiepileptic capacity (Ataie et al., 2013) and interaction with alcohol effects (Cruz et al., 2013). GABA-mediated mechanisms play an integral role in leptin signaling as well. Leptin receptors are widely expressed on GABAergic neurons, particularly in brain regions responsible for homeostatic (e.g., hypothalamus) and hedonic (e.g., ventral tegmental area) food intake (Fujikawa et al., 2013; Fulton et al., 2006; Ovesjo et al., 2001; Xu et al., 2012). Collectively, previous data show that leptin potentiates GABAergic neurotransmission to regulate consummatory behaviors (Bogatyrev et al., 2017; Guimond et al., 2014; Krolczyk et al., 2005; Xu et al., 2012; Zupancic and Mahajan, 2011). Peripheral concentrations of leptin are generally correlated with adipose tissue volume, and a 12-week trial with baclofen significantly reduced leptin levels in obese individuals (Arima and Oiso, 2010). In comparison with the aforementioned study, participants received baclofen for a shorter period (one week) in the present study and no changes in BMI were detected over the course of the outpatient phase. While the two studies are not fully comparable, these inconsistent results suggest that the effects of GABA-B agonism on leptin levels may either proceed or be at least partially independent from potential effects on weight, a question requiring further investigation. Higher levels of leptin in the baclofen group were also found during the laboratory experiment of our study, as shown by a significant Drug (but no Drug $\times$ Time-point interaction) effect (Table 2 and Figure S4-C). Given the complex interaction between leptin and the nervous system, more studies are required to disentangle central versus peripheral elements of the leptin-GABA crosstalk.

Blood concentrations of amylin and insulin were influenced by baclofen in this study as implied by significant Drug $\times$ Time-point interaction effects during the laboratory experiment. Post-hoc analyses showed that after consuming the fixed-dose priming drink, amylin and insulin levels were increased in baclofen-treated individuals but decreased in the placebo group. Additionally, in the placebo group, blood concentrations of both hormones were higher after exposure to alcohol cues compared to baseline, but a similar increase was detected only for insulin in the baclofen group (Figures 3-A and 3-C). Blood samples were not collected between the pre-experiment time-point in the morning and the post-ACR time-point in the afternoon; therefore, we cannot fully parse the effect of time versus cue-reactivity on these neuroendocrine outcomes. However, the well-controlled laboratory setting enabled us to standardize the timing and content of all procedures/events (e.g., meals, smoking break, drug dosing, etc.) across all participants. Amylin and insulin are known as two closely linked metabolic hormones as they are co-secreted from pancreatic β -cells and play fundamental regulatory roles in glycemic control, energy homeostasis, and hunger/

satiety. Alcohol, on the other hand, considerably affects metabolic processes, being both a pharmacological agent and a source of calorie (Preedy et al., 1997; Steiner et al., 2015). Interestingly, in addition to their primary metabolic function, both amylin and insulin have been shown to modulate alcohol-related behaviors in animals and humans (Kalafateli et al., 2018; Leggio et al., 2008). Previous data illustrate that GABA is a key regulator of insulin release from pancreatic islets, and GABA-B receptor activation can stimulate or inhibit insulin secretion, depending on feeding status, timing of exposure, and other factors (Bonaventura et al., 2012; Braun et al., 2004; Faraji et al., 2011). Central effects of insulin in regulating energy balance, primarily through arcuate nucleus of hypothalamus, are also facilitated via GABAergic system and GABA-B receptors (Ito et al., 2013; Sato et al., 2005).

In addition to significantly increased levels of GLP-1 in the baclofen group during the outpatient phase, changes in GLP-1 concentrations during the laboratory experiment demonstrated different patterns between the two study groups, as indicated by a trend-level Drug $\times$ Time-point interaction effect. In both groups, GLP-1 levels were increased after the ACR procedure and decreased after the AP procedure. Notably, alcohol-induced changes were more prominent in the baclofen group, while greater cue-induced changes were observed in the placebo group (Table 2 and Figure 3-B). As an incretin produced chiefly by intestinal mucosa, GLP-1 regulates food intake and glucose homeostasis via both central (e.g., hypothalamus) and peripheral (e.g., pancreas) GLP-1 receptors. In addition, GLP-1 acts as a neuropeptide and is synthesized by preproglucagon neurons of the solitary nucleus (Larsen and Holst, 2005). GLP-1 receptors are widely expressed in the brain regions responsible for stress and reward processing, and growing evidence underscores the role of GLP-1 in alcohol seeking and other addictive behaviors (Egecioglu et al., 2013; Suchankova et al., 2015; van Bloemendaal et al., 2014). A close interaction also exists between GLP-1 signaling and GABAergic system as, on one hand, GLP-1 receptor activation enhances GABA formation and neurotransmission (Korol et al., 2015; Wang et al., 2007) and, on the other hand, GABA stimulates GLP-1 release from intestinal cells (Gameiro et al., 2005).

A trend-level Drug $\times$ Time-point interaction effect was also detected for ACTH during the laboratory experiment. The decrease in ACTH concentrations in the placebo group was flattened in baclofen-treated individuals, and post-hoc analysis showed a significant difference between the two study groups at the post-ASA time-point. A similar pattern was noticed for blood cortisol concentrations (Figure S4-J), and while the interaction term was not statistically significant, a trend-level Drug main effect on cortisol levels was detected (Table 2). Consistent with a previous experiment in rats (Hausler et al., 1993), these data suggest that central (e.g., pituitary) and not peripheral (e.g., adrenal gland) GABA-B receptors contribute to baclofen-induced activation of the HPA axis. HPA axis plays a key role in both acute and chronic neuroendocrine response to psychological and physiological stress, and alterations in different components of this pathway (e.g., corticotropin releasing factor, ACTH, and cortisol) are observed in chronic alcohol use (Smith and Vale, 2006; Stephens and Wand, 2012). Lower ACTH response has been reported in alcohol-dependent individuals, especially during early abstinence (Adinoff et al., 2005; Inder et al., 1995). Notably, similar to our results, previous studies have found that ACTH response to different pharmacological agents (e.g., naltrexone, acamprosate, naloxone, and ovine CRH), compared to placebo, is blunted in alcohol-dependent individuals (Adinoff et al., 2005; Inder

et al., 1995; Kiefer et al., 2002b; O'Malley et al., 2002). It is important to note that the present study did not employ a stress manipulation procedure per se, and all participants had high trait anxiety as an inclusion criterion. Therefore, the results may not be generalizable to other settings/participants without further investigation.

To our knowledge, this is the first study providing a comprehensive characterization of neuroendocrine response to GABA-B receptor agonism in an alcohol-dependent sample and in combination with alcohol intake. We evaluated a wide spectrum of feeding- and stress-related hormones under two different but complementary conditions: measurement of subacute changes in neuroendocrine markers during a naturalistic outpatient phase, followed by a well-controlled laboratory experiment during which acute neuroendocrine changes in response to alcohol cues, fixed-dose alcohol priming, and alcohol self-administration were assessed. It is important to note that baclofen did not affect alcohol drinking in this study, ruling out the possibility that the observed neuroendocrine effects might have been driven by changes in alcohol consumption. As another key player of the GABAergic system, GABA-A receptors are also involved in regulating various hormonal pathways (Inoue and Bains, 2014; Kakizawa et al., 2016; Kamatchi and Rathanaswami, 2012; Yoneda and Kuriyama, 1984) and mediate both pharmacological and behavioral effects of alcohol (Davies, 2003; Lobo and Harris, 2008). Future studies should also investigate the potential neuroendocrine effects of GABA-A receptor manipulation (e.g. by benzodiazepines) in alcohol-dependent individuals.

The findings of this study should be interpreted in light of its limitations. The enrolled sample had a relatively small size and was limited to alcohol-dependent individuals with high anxiety who were not seeking treatment for either condition. Participants' food and alcohol intake were not controlled during the outpatient phase, and factors other than the drug condition (baclofen versus placebo) may have contributed to the observed neuroendocrine effects. However, no significant differences were seen between the two groups at baseline (e.g., demographic characteristics, baseline blood tests) nor over the course of the outpatient phase (e.g., alcohol intake, BMI, length of treatment). Although the laboratory experiment was conducted under a standardized protocol, and no significant drug effect was detected on the study primary outcomes (Farokhnia et al., 2017b), we still took a conservative approach by analyzing relevant covariates (i.e., age, gender, BMI, and number of mini-drinks self-administered), in order to minimize the possible confounding effect of inter-individual variability on neuroendocrine outcomes. Baclofen dosage was selected based on some previous clinical studies with positive results (Addolorato et al., 2000; Addolorato et al., 2002; Addolorato et al., 2007; Leggio et al., 2013); consistent dosing across participants (as opposed to individually titrated dosage of baclofen) was needed to provide an accurate evaluation of neuroendocrine response to the drug. Therefore, this study does not provide information on whether and how higher doses of baclofen may affect blood concentrations of the reported neuroendocrine markers. Also, a fixed dose of alcohol was used as the priming and the number of mini-drinks during the ASA was limited to up to eight for safety reasons; therefore, the neuroendocrine effect of baclofen plus other doses of alcohol remains unknown. Finally, while having a placebo group enabled us to compare the drug effect between baclofen- and placebo-treated individuals, we did not have a non-alcoholic drink as a control condition. Future studies should have larger and more diverse

samples and employ a fully factorial 2×2 design (baclofen versus placebo, alcohol versus placebo) to determine neuroendocrine changes following baclofen administration in the context of alcohol consumption and dependence.

In conclusion, GABA-B receptor agonism via baclofen administration in alcohol-dependent individuals altered blood concentrations of feeding- and stress-related hormones. Together with previous preclinical and clinical work, these findings point to an important role for GABA-B receptors in the shared neurobiology of alcohol-, feeding-, and stress-related behaviors.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank the clinical and research staff involved in data collection and support at the NIH Intramural Research Program, in particular, staff in the joint NIAAA/NIDA Section on Clinical Psychoneuroendocrinology and Neuropsychopharmacology, in the NIAAA clinical program of the Division of Intramural Clinical and Biological Research, and at the NIH Clinical Center (Nursing, Nutrition, and Pharmacy Departments). The authors would like to express their gratitude to the participants who took part in this study.

Funding: This work was supported by: 1) NIH intramural funding ZIA-AA000218 (Section on Clinical Psychoneuroendocrinology and Neuropsychopharmacology; PI: Leggio), jointly supported by the NIAAA Division of Intramural Clinical and Biological Research and the NIDA Intramural Research Program; and 2) Brain and Behavior Research Foundation (BBRF; formerly NARSAD) grant #17325 (PI: Leggio).

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Highlights

- Baclofen administration in an outpatient setting increased blood ghrelin, leptin, amylin, and GLP-1 concentrations
- Baclofen altered the pattern of change in blood amylin, insulin, GLP-1, and ACTH concentrations during a human lab experiment
- GABA-B receptors mediate the neuroendocrine correlates of alcohol-, feeding-, and stress-related behaviors

Fig. 1.

Schematic outline of the study procedures and blood sampling time-points.

Abbreviations: **ACR:** Alcohol Cue-Reactivity; **AP:** Alcohol Priming; **ASA:** Alcohol Self-Administration.

Fig. 2. Changes (= Post-Drug – Pre-Drug) in blood concentrations of (A) Acyl-Ghrelin, (B) Leptin, (C) Amylin, and (D) GLP-1 over the course of the outpatient phase.
Abbreviations: GLP-1: Glucagon-Like Peptide-1; **M:** Mean; **SEM:** Standard Error of the Mean.

Fig. 3.

Blood concentrations of (A) Amylin, (B) GLP-1, (C) Insulin, and (D) ACTH during the laboratory experiment. See Table 2 for pairwise comparisons.

Abbreviations: **ACR:** Alcohol Cue-Reactivity; **ACTH:** Adrenocorticotrophic Hormone; **AP:** Alcohol Priming; **ASA:** Alcohol Self-Administration; **GLP-1:** Glucagon-Like Peptide-1; **M:** Mean; **SEM:** Standard Error of the Mean.

Table 1

Changes in the blood concentration of neuroendocrine markers over the course of the outpatient phase

Outcome ^I	Placebo Group, M (SEM)	Baclofen Group, M (SEM)	Independent Samples <i>t</i> -test
Total Ghrelin, pg/ml	28.63 (64.35)	39.64 (40.60)	$t_{30} = -0.14, p = 0.88$
Acyl-Ghrelin, pg/ml	-25.67 (15.19)	20.14 (8.76)	$t_{32} = -2.68, p = 0.01$
Leptin, pg/ml	-234.87 (309.91)	1524.52 (625.83)	$t_{23,3} = -2.54, p = 0.01$
Amylin, pg/ml	-1.25 (0.88)	4.68 (1.55)	$t_{29} = -3.13, p = 0.004$
GLP-1, pg/ml	-4.30 (1.35)	5.21 (3.60)	$t_{21,6} = -2.47, p = 0.02$
Insulin, pg/ml	-403.89 (204.08)	-45.27 (146.98)	$t_{31} = -1.43, p = 0.16$
Prolactin, ng/ml	0.59 (0.54)	1.43 (0.48)	$t_{30} = -1.15, p = 0.25$
TSH, mIU/ml	0.23 (0.09)	0.07 (0.18)	$t_{23,7} = 0.70, p = 0.48$
GH (Log10), ng/ml	0.01 (0.13)	0.17 (0.07)	$t_{32} = -1.05, p = 0.30$
Cortisol, mcg/dl	0.55 (1.07)	0.61 (0.73)	$t_{32} = -0.04, p = 0.96$
ACTH, pg/ml	0.70 (2.79)	2.02 (1.23)	$t_{19,4} = -0.43, p = 0.66$

^I = Post-Drug – Pre-Drug

Abbreviations: **ACTH:** Adrenocorticotrophic Hormone; **GH:** Growth Hormone; **GLP-1:** Glucagon-Like Peptide-1; **M:** Mean; **SEM:** Standard Error of the Mean; **TSH:** Thyroid Stimulating Hormone.

Table 2

Drug, Time-point, and Drug $\times$ Time-point effects on neuroendocrine outcomes during the human laboratory experiment

Outcome	Drug ¹ Main Effect	Time-point ² Main Effect	Drug $\times$ Time-point Interaction Effect
Total Ghrelin	$F_{1,30.7} = 0.002, p = 0.96$	$F_{3,87.7} = 4.92, p = 0.003$	$F_{3,87.7} = 1.50, p = 0.21$
Acyl-Ghrelin	$F_{1,32.5} = 1.27, p = 0.26$	$F_{3,89} = 19.79, p < 0.001$	$F_{3,89} = 1.30, p = 0.27$
Leptin	$F_{1,25.5} = 5.79, p = 0.02$	$F_{3,75.5} = 3.51, p = 0.01$	$F_{3,75.6} = 1.83, p = 0.14$
Amylin	$F_{1,28.7} = 1.16, p = 0.29$	$F_{3,78.3} = 1.29, p = 0.28$	$F_{3,78.3} = 5.8, p = 0.001^a$
GLP-1	$F_{1,23.2} = 1.00, p = 0.32$	$F_{3,72.2} = 10.06, p < 0.001$	$F_{3,72.2} = 2.50, p = 0.06^b$
Insulin	$F_{1,30.2} = 2.45, p = 0.12$	$F_{3,79.7} = 12.55, p < 0.001$	$F_{3,79.7} = 2.94, p = 0.03^c$
Prolactin	$F_{1,29.9} = 1.76, p = 0.19$	$F_{3,84.5} = 7.36, p < 0.001$	$F_{3,84.5} = 0.65, p = 0.58$
TSH	$F_{1,29.1} = 1.51, p = 0.22$	$F_{3,75.7} = 1.64, p = 0.18$	$F_{3,75.7} = 0.95, p = 0.41$
GH	$F_{1,29.7} = 0.50, p = 0.48$	$F_{3,77.7} = 9.51, p < 0.001$	$F_{3,77.7} = 1.38, p = 0.25$
Cortisol	$F_{1,30.2} = 3.10, p = 0.08$	$F_{3,87.9} = 26.71, p < 0.001$	$F_{3,87.9} = 1.42, p = 0.24$
ACTH	$F_{1,32.3} = 2.27, p = 0.14$	$F_{3,88.6} = 11.91, p < 0.001$	$F_{3,88.6} = 2.11, p = 0.10^d$

¹ Baclofen and Placebo;

² Pre-Experiment, Post-ACR, Post-AP, and Post-ASA

Pairwise Comparison:

^a Post-AP: Baclofen > Placebo ($p = 0.03$); Placebo: Post-CR > Pre-Experiment ($p = 0.007$); Placebo: Post-CR > Post-AP ($p = 0.005$); see also Figure 3-A.

^b Pre-Experiment: Baclofen > Placebo ($p = 0.01$); Baclofen: Post-CR > Post-AP ($p = 0.08$); Baclofen: Post-CR > Post-ASA ($p = 0.003$); Placebo: Post-CR > Pre-Experiment ($p = 0.001$); Placebo: Post-CR > Post-ASA ($p = 0.009$); Placebo: Post-AP > Pre-Experiment ($p = 0.009$); Placebo: Post-AP > Post-ASA ($p = 0.07$); see also Figure 3-B.

^c Post-AP: Baclofen > Placebo ($p = 0.01$); Post-ASA: Baclofen > Placebo ($p = 0.09$); Baclofen: Post-CR > Pre-Experiment ($p = 0.01$); Baclofen: Post-AP > Pre-Experiment ($p < 0.001$); Baclofen: Post-AP > Post ASA ($p = 0.02$); Placebo: Post-CR > Pre-Experiment ($p = 0.001$); Placebo: Post-CR > Post-ASA ($p = 0.01$); see also Figure 3-C.

^d Post-ASA: Baclofen > Placebo ($p = 0.02$); Placebo: Pre-Experiment > Post-AP ($p = 0.02$); Placebo: Pre-Experiment > Post-ASA ($p < 0.001$); Placebo: Post-CR > Post-ASA ($p < 0.001$); see also Figure 3-D.

Abbreviations: **ACR:** Alcohol Cue-Reactivity; **ACTH:** Adrenocorticotrophic Hormone; **AP:** Alcohol Priming; **ASA:** Alcohol Self-Administration; **GH:** Growth Hormone; **GLP-1:** Glucagon-Like Peptide-1; **M:** Mean; **SEM:** Standard Error of the Mean; **TSH:** Thyroid Stimulating Hormone.