Psychiatry Research 272 (2019) 431–437

Contents lists available at ScienceDirect

Psychiatry Research

journal homepage: www.elsevier.com/locate/psychres

Total and acylated ghrelin plasma levels as potential long-term response markers in alcohol-dependent patients receiving high-dose of the GABA-B receptor agonist baclofen

Olga Geisel[a,*], Rainer Hellweg[a], Klaus-Dieter Wernecke[b], Klaus Wiedemann[c], Christian A. Müller[a]

[a] Charité – Universitätsmedizin Berlin, Department of Psychiatry, Campus Charité Mitte, Charitéplatz 1, 10117 Berlin, Germany
[b] Charité – Universitätsmedizin Berlin and Sostana GmbH Berlin, Berlin, Germany
[c] Department of Psychiatry, Universitätsklinikum Hamburg Eppendorf, Martinistr. 52, 20246 Hamburg, Germany

ARTICLE INFO

Keywords:
Alcohol dependence
Baclofen
BACLAD
Acylated ghrelin
Total ghrelin

ABSTRACT

The orexigenic hormone ghrelin is involved in the regulation of food intake and energy balance. Previous findings suggest its involvement in the modulation of mesolimbic reward pathways, thus potentially being relevant in the pathophysiology of substance use disorders such as alcohol dependence. In the present study, we assessed plasma levels of total and acylated ghrelin within the BACLAD trial, where alcohol-dependent patients received individually titrated high-dose baclofen (30–270 mg/d) within a randomized, placebo-controlled design. Plasma levels of total ghrelin and acylated ghrelin were measured at baseline, during treatment with individually titrated high-dose baclofen and after termination of the study medication within a timeframe of up to 20 weeks. Multivariate longitudinal non-parametric analysis revealed that plasma levels of total ghrelin significantly decreased in the group of abstinent patients receiving high-dose baclofen. In addition, plasma levels of total ghrelin correlated negatively with days of abstinence during treatment with high-dose baclofen. Plasma levels of acylated ghrelin increased during the study in the group of relapsed patients under baclofen and placebo treatment. These findings suggest that the long-term response to baclofen treatment in alcohol use disorder (AUD) might be monitored by assessing total and acylated ghrelin plasma levels.

1. Introduction

Ghrelin is an orexigenic proteo-hormone, which is secreted by entero-endocrine cells of the stomach (Cummings et al., 2001; Tschop et al., 2001). A proteolytical cleavage and acetylation converts preproghrelin to biologically active ghrelin (Banks et al., 2002; Cummings et al., 2001). Only acylated ghrelin is able to cross the blood-brain barrier (Arora and Anubhuti, 2006; Kojima et al., 1999; Tschop et al., 2000), and to modulate food intake and energy balance via growth-hormone secretagogue receptors in the hypothalamus and stimulation of the production of neuropeptid Y and agoutirelated protein (AGP) (Banks et al., 2002). The acylated form of ghrelin has been found to increase food intake, body weight (Arora and Anubhuti, 2006; Cummings et al., 2001; Wren et al., 2000) and plasma glucose levels (Hagemann et al., 2003).

Various studies indicate that ghrelin is involved in the regulation of mesolimbic pathways (Figlewicz et al., 2003; Hommel et al., 2006). Among other molecules, it modulates dopaminergic transmission (Abizaid et al., 2006) not only in food intake related pathways (Narayanan et al., 2010; van Zessen et al., 2012) but also in alcohol use disorder (AUD) (al'Absi et al., 2014; Kiefer et al., 2001; Koopmann et al., 2015; Koopmann et al., 2018; von der Goltz et al., 2010). With regard to AUD, there is growing evidence that ghrelin is also involved in the regulation of alcohol intake. However, findings are inconsistent, possibly related to varying states of alcohol use of the subjects included (Gomez et al., 2015; Gomez and Ryabinin, 2014; Jerlhag, 2008; Jerlhag et al., 2014; Lyons et al., 2008; Schneider et al., 2007). Preclinical studies suggest that exogenous ghrelin application might stimulate alcohol consumption (Gomez et al., 2015; Gomez and Ryabinin, 2014; Jerlhag et al., 2011; Kaur and Ryabinin, 2010; Stevenson et al., 2016). Furthermore, ghrelin and particularly acylated ghrelin plasma levels in patients with AUD seem to be positively associated with craving for alcohol (Addolorato et al., 2006a; Hillemacher et al., 2007; Koopmann et al., 2012; Leggio et al., 2012; Leggio et al., 2014; Suchankova et al., 2016a,b; Wurst et al., 2007). Leggio et al. found that ghrelin administration in severely affected alcohol-dependent patients led to increased

* Corresponding author.
E-mail address: olga.geisel@charite.de (O. Geisel).

https://doi.org/10.1016/j.psychres.2018.12.095
Received 20 June 2018; Received in revised form 9 November 2018; Accepted 18 December 2018
Available online 18 December 2018
0165-1781/

craving for alcohol, whereas no such effect was found for the desire of consuming food or drinking juice (Leggio et al., 2012). Koopmann et al. reported elevated plasma levels of acylated ghrelin in patients with AUD between day 1 and 14 of detoxification treatment (Koopmann et al., 2012). The same group also found significantly decreased levels of acylated ghrelin and craving in alcohol-dependent patients who drank 1 liter of water after alcohol cue exposure, whereas no such effect was observed in a control group. Recent studies reported higher alcohol consumption after ghrelin infusion in patients with severe AUD (Farokhnia et al., 2017) as well as an association of fasting serum ghrelin levels and alcohol intake in non-dependent subjects (Wittekind et al., 2018). Lee et al. performed a phase 1b human study with a novel ghrelin receptor inverse agonist and found that this compound reduced craving for alcohol when co-administered in patients with AUD (Lee et al., 2018). Thus, ghrelin secretion might represent a potential treatment target for AUD (Koopmann et al., 2017).

Ghrelin secretion is known to be modulated by the inhibitory neurotransmitter gamma-aminobutyric acid (GABA) (Ataie et al., 2013; Cruz et al., 2013; Jonaidi et al., 2012). GABA was also found to be involved in the regulation of appetite and food intake (Bonaventura et al., 2012; Delgado, 2013; Ebenezer and Baldwin, 1990; Stratford and Kelley, 1997) as well as in modulation of AUD (Koob, 2006) via ionotropic GABA-A or GABA-A-rho and particularly metabotropic GABA-B receptors (Roberto et al., 2003; Watanabe et al., 2002; Weiner and Valenzuela, 2006). GABA-B receptor agonists have been shown to modulate alcohol-related behaviors (Agabio and Colombo, 2014; Brown et al., 2015; Filip et al., 2015; Mirijello et al., 2015). Clinical studies on the topic, as the "Baclofen for the Treatment of Alcohol Dependence" (BACLAD) study, found the selective GABA-B receptor agonist baclofen to be effective in the maintenance of abstinence in AUD (Addolorato et al., 2006b, 2011; Müller et al., 2015), although also null results were reported (Rose and Jones, 2018). Taken together, ghrelin secretion might be an important modulator of AUD. The fact that secretion of ghrelin is also influenced by GABA-B receptors, which play an important part in the pathophysiology of AUD, led to our current hypothesis that the GABA-B receptor agonist baclofen might alter ghrelin secretion in patients with AUD and hereby influence the maintenance of abstinence. In the present study, we aimed to investigate ghrelin plasma levels during the BACLAD trial, where total and acylated ghrelin were measured at 3 timepoints in currently abstinent alcohol-dependent patients receiving individually titrated high-dose baclofen or placebo (Müller et al., 2015).

2. Material and methods

This study was conducted within the "Baclofen for the Treatment of Alcohol Dependence (BACLAD)" trial (Müller et al., 2015) at the outpatient unit of the Department of Psychiatry and Psychotherapy at the Campus Charité Mitte of the Charité – Universitätsmedizin Berlin. Fifty-six patients were recruited from our in- and outpatient department as well as by spontaneous referral at the study site. Inclusion criteria for men and women were: (a) age of ≥18 and <65 years; (b) diagnosis of alcohol dependence according to ICD-10 (WHO, 1994) and DSM-IV-TR (APA, 2000), (c) an alcohol consumption of at least 2 heavy drinking days per week on average (men ≥5 drinks per day; women ≥4 drinks per day; 1 standard drink is equal to 12 g absolute alcohol) and an average overall alcohol intake of 21 drinks per week or more for men and 14 drinks per week or more for women during the 4 weeks before detoxification; (d) a completed in- or outpatient detoxification before randomization; (e) last alcohol consumption within 7–21 days before randomization; and (f) sufficient German language skills. Exclusion criteria were a) significant medical, other psychiatric (axis I diagnoses besides alcohol or nicotine dependence), or neurological conditions, epilepsy or epileptiform convulsions; b) current treatment with psychotropic drugs that could affect study outcome (i.e. sedatives, antidepressants, antipsychotics, anticonvulsants and alcohol relapse prevention with acamprosate, disulfiram, or naltrexone); c) intolerance to baclofen; d) pregnancy and/or currently breastfeeding; e) terminal renal failure; f) gastrointestinal ulcera; g) alanine aminotransferase (ALAT) or aspartate aminotransferase (ASAT) values 5 times the upper normal limit, bilirubin >1.9 mg/dl, International Normalized Ratio (INR) >1.6; and h) treatment mandated by a legal authority. The study was conducted in accordance with the principles of the Declaration of Helsinki and Good Clinical Practice and approved by the local ethics committee (substudy), the ethics committee of the state of Berlin (Landesamt für Gesundheit und Soziales Berlin) and the Federal Institute for Drugs and Medical Devices (Bundesinstitut für Arzneimittel und Medizinprodukte, BfArM). Written informed consent was obtained from all participants, please also see (Müller et al., 2015).

The 24-week, randomized, double-blind, placebo-controlled trial consisted of four intervals, i.e., the titration phase (up to 4 weeks, depending on the individually tolerated high-dose), high-dose phase (12 weeks), tapering phase (up to 4 weeks) and follow-up (4 weeks after termination of study medication). The full report of this study is shown elsewhere (Müller et al., 2015). Plasma concentrations of total ghrelin and acylated ghrelin were measured at baseline (t0), two weeks after reaching individually titrated high-dose treatment with baclofen/placebo (t1) and after termination of study medication (t2), up to 14 weeks after t1. The trial profile is shown in Fig. 1, which was also published in Geisel et al. (2016).

2.1. Assessments

Assessments were performed as described previously in Müller et al. (2015). Patients with AUD were administered Visual Analogue Scale of Craving (VASC) (Mottola, 1993), Obsessive Compulsive Drinking Scale (OCDS) (Anton et al., 1995), Hamilton Depression Scale (HAMD) and Alcohol Dependence Scale (ADS) as described in Müller et al. (2015). The VASC consists of a continuous horizontal line with 100 mm length and two endpoints [no desire (0) to very strong desire (100)] for the question "How strong is your desire for alcohol right now?".

The OCDS is a 14-items self-report instrument, which rates frequency of thoughts about drinking, efforts to resist / distress caused by those thoughts as well as frequency and intensity of drinking via a 0 "no symptoms" to 4 "marked symptoms" scale (Anton et al., 1995).

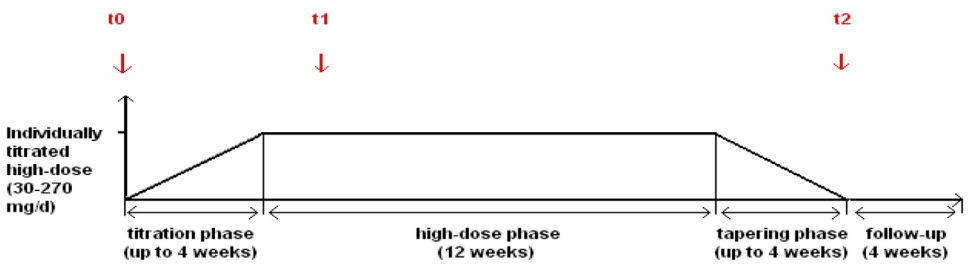

Fig. 1. Trial profile. Trial profile of the study with the timepoints of measurement t0–t2. Timepont t0: baseline; timepoint t1: 2 weeks after reaching individual high-dose; timepoint t2: end of medication phase.

2.2. Hormone assays

All blood samples were obtained by venipuncture at 9 a.m. after overnight fasting, were anti-coagulated with sodium EDTA (1 mg/ml whole blood), and then immediately cooled on ice (Geisel et al., 2016, 2015).

Plasma was separated in a centrifuge at 4000 g, and the aliquots were then immediately frozen and stored at −80°C until the time of analysis. Hormonal analyses were performed in the Neurobiology Lab of the Department of Psychiatry at the University Medical Center, Hamburg (KW).

The plasma concentration of total and acylated ghrelin were measured using radioimmunoassay techniques (DRG Instruments, Marburg, Germany) with the detection limit set to 105 pg/ml for total and 15 pg/ml for acylated ghrelin. The inter-assay coefficient of variation was less than 14%; the intra-assay coefficient was less than 10%, respectively.

2.3. Statistics

Nonparametric tests were used for statistical analyses since sample sizes were small. Differences of hormone blood levels were determined by Kruskal-Wallis tests, Mann Whitney *U* tests for paired samples. Nonparametric multivariate analysis of longitudinal data in a two-factorial design (1st factor: treatment, 2nd factor: time) was applied for ghrelin plasma levels comparison over time across groups [nonparametric MANOVA], after adjusting for baseline measurements. A two-tailed p-value < 0.05 was considered statistically significant. Hereby, relative effects of ghrelin plasma levels alterations were calculated for abstinent and relapsed patients in the baclofen vs. placebo group. Relative effects (measured in a scale between 0 and 1, according to a probability) show ghrelin alterations in the different study groups relative to the other groups. Correlations were analyzed by Spearman's Rho. Statistical analyses were performed using IBM SPSS Statistics version 25 (SPSS Inc., Chicago, USA).

3. Results

The trial profile, as published by Geisel et al. (2016) and Müller et al. (2015) is presented in Fig. 1. Median and percentiles of plasma levels of total ghrelin and acylated ghrelin are presented in Table 2 for the baclofen and placebo group and in Table 3 for abstinent and relapsed patients of both treatment groups. Differing sample sizes were due to missing blood samples in some cases. For sociodemographic and clinical parameters please see Table 1, as already published in Müller et al. (2015) and Geisel et al. (2016). There were no significant differences in sociodemography or clinical features in the baclofen treatment group compared to placebo, especially concerning sex and smoking status ($p > 0.05$, see Table 1). Total ghrelin plasma levels correlated negatively with BMI at timepoint t0 (baseline) ($n = 53$, Spearman's-Rho $= -0.476$, $p = 0.0001$), acylated ghrelin plasma levels and total ghrelin in t1 and t2 did not correlate with BMI or other sociodemographic and clinical variables ($p > 0.05$). Abstinent and/or relapsed patients receiving baclofen or placebo did not differ in BMI (Mann Whitney *U* test, $p > 0.05$). Furthermore, neither significant changes of absolute plasma levels of total and acylated ghrelin nor associations to clinical and sociodemographic parameters have been found (Mann-Whitney *U* test, $p > 0.05$, Spearman's Rho, $p > 0.05$) beside those described in the following paragraph.

Multivariate non-parametric analysis for longitudinal data in a two-factor based design revealed that there was a significant relative decrease of plasma levels of total ghrelin over time in the baclofen and placebo group ($p = 0.001$); this relative decrease of total ghrelin plasma levels was significantly higher in the patient group receiving baclofen ($p = 0.00001$). In a comparison of abstinent and relapsed subjects in the baclofen/placebo group, the relative decrease of plasma levels of total ghrelin over time was highest in the group of abstinent patients receiving baclofen ($p = 0.004$, see Fig. 2). Furthermore, total ghrelin plasma levels at timepoint t1 correlated negatively with days of abstinence ($n = 19$, Spearman's Rho $= -0.512$, $p = 0.025$) in the baclofen group, indicating longer abstinence with lower total ghrelin plasma levels during treatment with high-dose baclofen. There were no other significant correlations of total and acylated ghrelin plasma levels in the baclofen or placebo group with VASC, OCDS or ADS and HAM-D scores, baclofen blood levels or other clinical variables (Spearman's Rho, $p > 0.05$). Multivariate non-parametric analysis did not reveal associations between acylated or total ghrelin plasma levels and brain-derived neurotrophic factor serum levels ($p > 0.05$).

Multivariate non-parametric analysis for longitudinal data in a two-factor based design showed that plasma levels of acylated ghrelin increased significantly during the study ($p = 0.0001$) in relapsed subjects under baclofen treatment, whereas no such effect was found in abstinent patients both under baclofen or placebo treatment (see Fig. 3). Plasma levels of acylated ghrelin were significantly higher at timepoint t2 in relapsed subjects of the baclofen group (see Table 3, median = 44 ng/ml, Kruskwal-Wallis test, $p = 0.046$) compared to all other study groups (abstinent patients receiving baclofen median = 17, abstinent and relapsed patients in the placebo group, both median = 15 ng/ml). At timepoint t1, acylated ghrelin plasma levels were in trend higher in the placebo group (median = 18 ng/ml) compared to the baclofen group (see Table 2, median = 15 ng/ml, Mann Whitney *U* test for paired samples, $p = 0.082$). Furthermore, we performed a multivariate analysis of total and activated ghrelin plasma levels during the study controlled for BMI, sex, smoking status and age, which did not reveal any different finding.

Table 1
Sociodemographic characteristics of the study participants, as published by Geisel et al. (2016) and Müller et al. (2015).

Characteristics	Placebo	Baclofen	*p* value
Sex [*n* (%)]			
Male	19 (67.9)	20 (71.4)	>0.05
Female	9 (32.1)	8 (28.6)	
Age (years) [mean ± SD (range)]	45.6 ± 7 (29–64)	47.4 ± 7 (32–59)	>0.05
Smoker [*n* (%)]	18 (64.3)	17 (60.7)	>0.05
Duration of hazardous alcohol consumption (years) [mean ± SD (range)]	11.5 (7.3)	13.9 (10.1)	>0.05
Alcohol consumption before inclusion (gram per day) [mean ± SD (range)]	191.6 (94.8)	206.2 (94.1)	>0.05
Height (m) [mean ± SD]	1.77 ± 0.08	1.73 ± 0.08	>0.05
Weight (kg) [mean ± SD]	77.6 ± 13.8	71.8 ± 12.3	>0.05
Body Mass Index (BMI, kg/m^2) [mean ± SD]	24.9 ± 4.0	23.8 ± 4.0	>0.05
Glucose blood levels (mg/dl) [mean ± SD]	81 ± 13.5	82.3 ± 12.3	>0.05

Differences in sociodemographic characteristics of the study participants were calculated by using the Exact Mann Whitney *U* Test and Fisher's exact test. No significant difference was found. SD: standard deviation.

4. Discussion

Our present investigation was conducted within the BACLAD study by Müller et al. (2015), where high-dose baclofen treatment was found to be effective for the maintenance of abstinence in patients with AUD. Our study revealed significant alterations in both, total and acylated ghrelin plasma levels during the trial. Total ghrelin plasma levels decreased significantly over time in abstinent patients during treatment with high-dose baclofen. Even though decreasing levels of total ghrelin were observed in all study groups, the effect was stronger under high-dose baclofen treatment and most pronounced in the sub-group of abstinent patients. Furthermore, total ghrelin plasma levels correlated

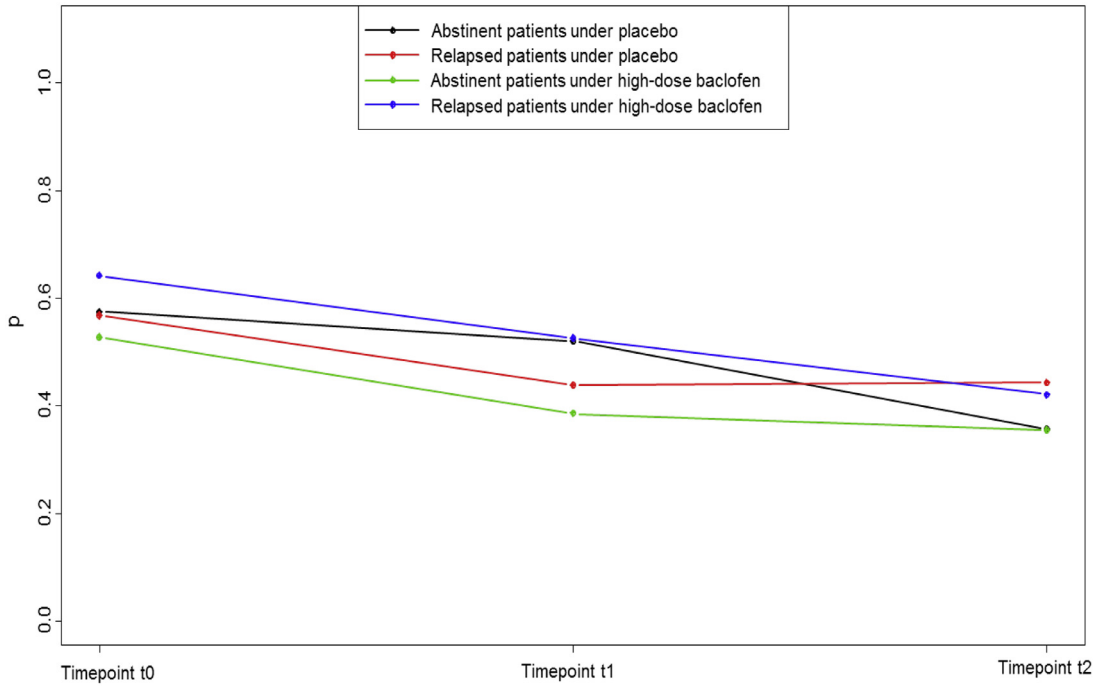

Fig. 2. This figure shows relative effects of alterations of total ghrelin plasma levels in abstinent and relapsed patients in the baclofen/placebo treatment group, calculated by multivariate non-parametric statistics in a longitudinal design. Y-axis represents probability (p). All study groups showed a significant decrease of total ghrelin plasma levels ($p = 0.003$). The decrease was especially prominent in the group of abstinent patients under high-dose baclofen treatment ($p = 0.004$).

negatively with days of abstinence in patients under high-dose baclofen treatment indicating that subjects with lower total ghrelin plasma levels under high-dose baclofen treatment were longer abstinent during the trial. Plasma levels of acylated ghrelin rose highest in relapsed patients under high-dose baclofen but were in trend higher during placebo than during baclofen treatment.

Abstinent patients under high-dose baclofen treatment were considered as responders to the medication. We hypothesize that a decrease of total ghrelin plasma levels during treatment with high-dose baclofen might indicate a longer abstinence duration. Vice versa, an increase of acylated ghrelin might be associated with a higher relapse risk. Thus, repeated measurements of both forms of ghrelin might potentially be useful for treatment-response monitoring in patients with AUD receiving baclofen.

However, findings of other studies are equivocal regarding the course of ghrelin plasma concentrations and outcome. In contrast to our findings, a study by Kim et al. revealed that patients with AUD, who were abstinent for at least 4 weeks or longer had higher ghrelin plasma

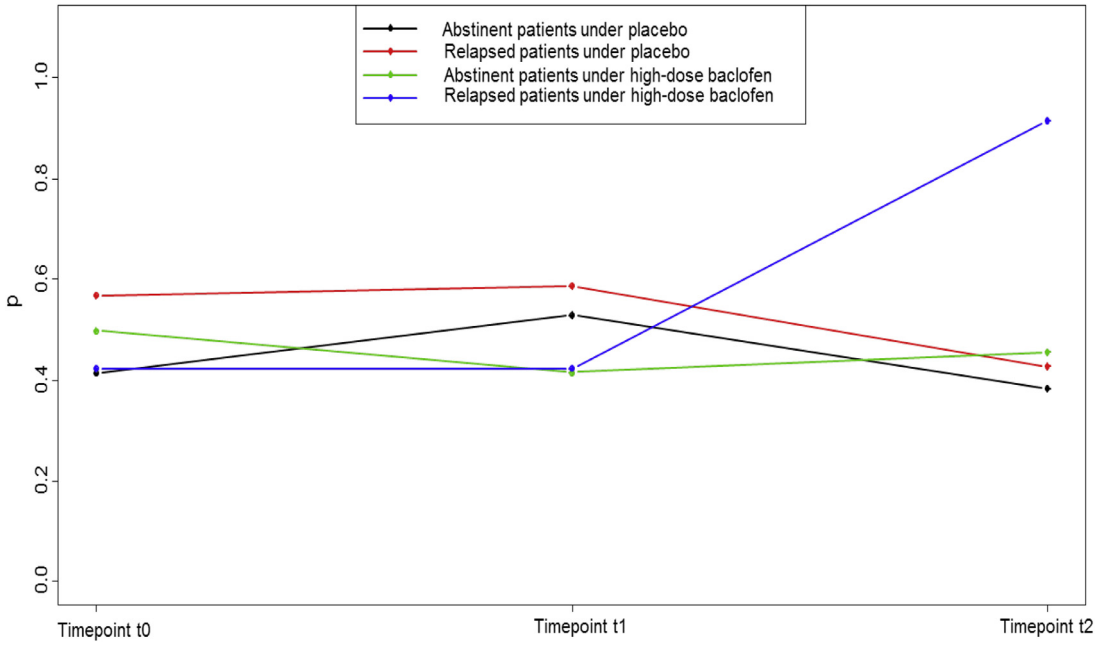

Fig. 3. This figure shows relative effects of alterations of acylated ghrelin plasma levels in abstinent and relapsed patients in the baclofen/placebo treatment group, calculated by multivariate non-parametric statistics in a longitudinal design. Y-axis represents probability (p). Plasma levels of acylated ghrelin increased significantly over time in the group of relapsed patients under high-dose baclofen treatment ($p = 0.000$).

Table 2
This table shows medians and percentiles of plasma levels of total and acylated ghrelin in pg/ml in the groups of patients with AUD receiving baclofen or placebo. Timepoint description: t0 = baseline, t1 = two weeks after reaching individual high-dose, t2 = end of medication phase.

Study group		Total ghrelin in pg/ml			Activated ghrelin in pg/ml		
		t0	t1	t2	t0	t1	t2
Placebo	*n*	26	18	9	25	17	11
	Median	**1377**	**1007**	**1184**	**18**	**18**	**15**
	Percentiles	854	616	647	15	15	15
	25	1377	1007	1184	18	18	15
	50	1628	1680	1352	23	27	22
	75						
Baclofen	*n*	27	19	14	22	17	13
	Median	**1393**	**1064**	**1005**	**15**	**15**	**18**
	Percentiles	1003	735	804	15	15	15
	25	1393	1064	1005	15	15	18
	50	1680	1378	1260	22	18	24
	75						

Table 3
This table shows medians and percentiles of plasma levels of total and acylated ghrelin in pg/ml in the groups of abstinent and relapsed patients with AUD receiving baclofen or placebo. Timepoint description: t0 = baseline, t1 = two weeks after reaching individual high-dose, t2 = end of medication phase.

Study group			Total ghrelin in pg/ml			Activated ghrelin in pg/ml		
			t0	t1	t2	t0	t1	t2
Placebo	**Abstinent**	*n*	4	4	2	4	2	3
		Median	**1332**	**1331**	**996**	**15**	**20**	**15**
		Percentiles	963	522	723	15	15	15
		25	1332	1331	996	15	20	15
		50	1601	1680		62		
		75						
	Relapsed	*n*	22	14	7	21	15	8
		Median	**1427**	**984**	**1184**	**20**	**18**	**15**
		Percentiles	825	616	570	15	15	15
		25	1427	984	1184	20	18	15
		50	1628	1608	1373	23	27	22
		75						
Baclofen	**Abstinent**	*n*	11	11	11	11	9	10
		Median	**1221**	**1062**	**998**	**16**	**15**	**17**
		Percentiles	1003	672	849	15	15	15
		25	1221	1062	998	16	15	17
		50	1406	1268	1161	22	20	19
		75						
	Relapsed	*n*	16	8	3	11	8	3
		Median	**1610**	**1244**	**1218**	**15**	**16**	**44**
		Percentiles	970	803	532	15	15	25
		25	1610	1244	1218	15	16	44
		50	1680	1616		20	18	
		75						

levels than healthy controls and that ghrelin plasma levels correlated positively with the duration of abstinence (Kim et al., 2005). Kraus et al. showed that ghrelin plasma levels were higher in healthy controls than in either active drinking patients with AUD or in early states of abstinence in AUD; no correlation between ghrelin plasma levels and craving (measured by OCDS) was found in their study (Kraus et al., 2005). In contrast, Addolorato et al. found that ghrelin levels in actively drinking patients with AUD were decreased in comparison to healthy controls (Addolorato et al., 2006a). Furthermore, the study revealed a positive association of ghrelin blood levels and craving. Hillemacher et al. found a trend for a positive correlation of ghrelin blood levels and self-rated craving in Lesch's type 1 patients with AUD, but not in other Lesch's types (Hillemacher et al., 2007). Wurst et al. found a significant relationship of OCDS-subscores in female patients with AUD, but not in male (Wurst et al., 2007). Taken together, those studies are exemplary for the divergence in research on ghrelin levels in AUD. Direct comparisons to our present results are difficult, particularly due to varying study settings. Most studies investigated ghrelin alterations in AUD in comparison to healthy subjects and performed single-timepoint comparisons of absolute ghrelin levels. In the present study, we only compared patients with AUD during treatment with baclofen/placebo, without including healthy controls. Furthermore, we assessed baseline-adjusted alterations of total and acylated ghrelin plasma levels over time via relative effects.

We did not find any direct associations of ghrelin with craving as measured by VASC or OCDS, which is partially in line with the findings by Kraus et al. (2005), Hillemacher et al. (2007) and Wurst et al. (2007), but not with the majority of previous findings suggesting a positive association of ghrelin blood levels and craving in AUD (Koopmann et al., 2018).

Interestingly, baclofen serum levels and abstinence rates of the different study groups were not correlated with craving in the BACLAD trial too (Müller et al., 2015). Thus, lacking associations of ghrelin plasma levels with craving are not contradicting our assumption that response to baclofen might be associated with total and acylated ghrelin plasma levels alterations.

A study by Leggio et al. reported that ghrelin levels decreased in relapsed patients with AUD and increased over time in abstinent patients with AUD within a study setting similar to the present trial (Leggio et al., 2012). Forty-two of the included patients with AUD were taking part in a placebo-controlled baclofen treatment study (Addolorato and Leggio, 2010; Leggio et al., 2012). The authors did not find any effects of baclofen treatment on ghrelin blood levels and focused on differences of abstinent and relapsed subjects. Interestingly, they hypothesized that baseline ghrelin levels might be relapse predictors in their study: higher baseline ghrelin levels indicated a higher risk of relapse, whereas lower ghrelin levels at baseline indicated a longer duration of abstinence (Leggio et al., 2012). These results are consistent with our finding of negative associations of ghrelin plasma levels with duration of abstinence, thereby supporting our assumption that low ghrelin levels might be associated with maintenance of abstinence during treatment with high-dose baclofen. The negative results of Leggio et al. regarding ghrelin blood levels alterations during treatment with baclofen might potentially be related to the different study designs. In the BACLAD study, individual doses up to 270 mg baclofen per day were used, whereas Leggio et al. used doses ranging between 30–60 mg per day and did not assess levels of acylated ghrelin (Leggio et al., 2012).

Our results support the notion that alterations of ghrelin plasma levels during treatment with individually titrated baclofen might reflect a general improvement or a changed status of the AUD during pharmacological treatment. Based on the lacking association of ghrelin plasma levels with baclofen blood levels, a direct effect of baclofen on ghrelin plasma levels seems unlikely. Ralevski et al. recently suggested that social drinkers with higher ghrelin blood levels are more sensitive to reward and show higher levels of impulsivity (Ralevski et al., 2018). This finding is in line with the assumption that ghrelin modulates stress and reward pathways, thereby being involved in the development and maintenance of substance use disorders (Zallar et al., 2017). One might speculate that baclofen modulates reward pathways in patients with AUD, thereby supporting abstinence, which might in turn be reflected in decreasing ghrelin plasma levels over time. This hypothesis might also explain the quite unexpected finding of decreasing total ghrelin levels in the placebo group of our study, which still was abstinent over a significant period of time compared to patients with AUD without interventions at all.

Current research on ghrelin tends to be more consistent and indicates that ghrelin signaling affects alcohol seeking in humans since studies by Farokhnia et al. or Wittekind et al. showed that exogenous and endogenous ghrelin modulates alcohol consumption in patients with AUD (Farokhnia et al., 2017; Wittekind et al., 2018). The review by Zallar et al. points out that upregulation of the ghrelin system might

increase alcohol craving. However, acute and chronic exposure to drugs seems to influence ghrelin regulation differently (Zallar et al., 2017).

Taken together, our findings point out that repeated measurement of total and acylated ghrelin plasma levels during baclofen treatment in patients with AUD might potentially be used as a long-term treatment response marker. Here, decreasing total ghrelin plasma levels over time might indicate maintenance of abstinence during treatment with high-dose baclofen, whereas an increase of acylated ghrelin over time might be associated with relapse. Thus, the regulation of the ghrelin secretion is a complex process, its potential role as a response marker in baclofen treatment in AUD needs to be assessed in future studies.

The following limitations need to be discussed. Since most studies did not assess total and acylated ghrelin or medication effects on levels of ghrelin in AUD, direct comparisons with the present findings are not fully applicable and further research is needed to discriminate alterations of both forms of ghrelin in the blood. Further limitations to our preliminary results have to be addressed. The sample sizes of our groups were small, resulting in low statistical power. At timepoint t2 some sample sizes were lower than 10, therefore only relative, baseline adjusted effects were calculated and absolute comparisons were not performed. In addition, three timepoints within 20 weeks are presumably not sufficient to draw final conclusions on longitudinal effects of a pharmacological intervention on ghrelin plasma levels. We did not assess blood glucose levels to prove that patients were fasting. Finally, we included male and female patients in our study, but the subgroups were too small to control for potential confounding effects.

Declaration of interest

Olga Geisel, Klaus Wiedemann, Rainer Hellweg and Christian A. Müller have no conflicts of interest.

Acknowledgments

We would like to thank our study nurse Olga Vitlif for her dedicated work and Iris Remmlinger-Marten for excellent technical assistance.

References

Abizaid, A., Liu, Z.W., Andrews, Z.B., Shanabrough, M., Borok, E., Elsworth, J.D., Roth, R.H., Sleeman, M.W., Picciotto, M.R., Tschop, M.H., Gao, X.B., Horvath, T.L., 2006. Ghrelin modulates the activity and synaptic input organization of midbrain dopamine neurons while promoting appetite. J. Clin. Invest. 116 (12), 3229–3239.

Addolorato, G., Capristo, E., Leggio, L., Ferrulli, A., Abenavoli, L., Malandrino, N., Farnetti, S., Domenicali, M., D'Angelo, C., Vonghia, L., Mirijello, A., Cardone, S., Gasbarrini, G., 2006a. Relationship between ghrelin levels, alcohol craving, and nutritional status in current alcoholic patients. Alcohol Clin. Exp. Res. 30 (11), 1933–1937.

Addolorato, G., Leggio, L., 2010. Safety and efficacy of baclofen in the treatment of alcohol-dependent patients. Curr. Pharm. Des. 16 (19), 2113–2117.

Addolorato, G., Leggio, L., Agabio, R., Colombo, G., Gasbarrini, G., 2006b. Baclofen: a new drug for the treatment of alcohol dependence. Int. J. Clin. Pract. 60 (8), 1003–1008.

Addolorato, G., Leggio, L., Ferrulli, A., Cardone, S., Bedogni, G., Caputo, F., Gasbarrini, G., Landolfi, R., Baclofen Study, G., 2011. Dose-response effect of baclofen in reducing daily alcohol intake in alcohol dependence: secondary analysis of a randomized, double-blind, placebo-controlled trial. Alcohol Alcohol 46 (3), 312–317.

Agabio, R., Colombo, G., 2014. GABAB receptor ligands for the treatment of alcohol use disorder: preclinical and clinical evidence. Front. Neurosci. 8, 140.

al'Absi, M., Lemieux, A., Nakajima, M., 2014. Peptide YY and ghrelin predict craving and risk for relapse in abstinent smokers. Psychoneuroendocrinology 49, 253–259.

Anton, R.F., Moak, D.H., Latham, P., 1995. The obsessive compulsive drinking scale: a self-rated instrument for the quantification of thoughts about alcohol and drinking behavior. Alcohol Clin. Exp. Res. 19 (1), 92–99.

APA, 2000. Fourth ed. American Psychiatric Association, Washington DC Text Revision (DSM-IV-TR).

Arora, S., Anubhuti, 2006. Role of neuropeptides in appetite regulation and obesity–a review. Neuropeptides 40 (6), 375–401.

Ataie, Z., Babri, S., Ghahramanian Golzar, M., Ebrahimi, H., Mirzaie, F., Mohaddes, G., 2013. GABAB receptor blockade prevents antiepileptic action of ghrelin in the rat hippocampus. Adv. Pharm. Bull. 3 (2), 353–358.

Banks, W.A., Tschop, M., Robinson, S.M., Heiman, M.L., 2002. Extent and direction of ghrelin transport across the blood-brain barrier is determined by its unique primary structure. J. Pharmacol. Exp. Ther. 302 (2), 822–827.

Bonaventura, M.M., Crivello, M., Ferreira, M.L., Repetto, M., Cymeryng, C., Libertun, C., Lux-Lantos, V.A., 2012. Effects of GABAB receptor agonists and antagonists on glycemia regulation in mice. Eur. J. Pharmacol. 677 (1–3), 188–196.

Brown, K.M., Roy, K.K., Hockerman, G.H., Doerksen, R.J., Colby, D.A., 2015. Activation of the gamma-aminobutyric acid type B (GABA(B)) receptor by agonists and positive allosteric modulators. J. Med. Chem. 58 (16), 6336–6347.

Cruz, M.T., Herman, M.A., Cote, D.M., Ryabinin, A.E., Roberto, M., 2013. Ghrelin increases GABAergic transmission and interacts with ethanol actions in the rat central nucleus of the amygdala. Neuropsychopharmacology 38 (2), 364–375.

Cummings, D.E., Purnell, J.Q., Frayo, R.S., Schmidova, K., Wisse, B.E., Weigle, D.S., 2001. A preprandial rise in plasma ghrelin levels suggests a role in meal initiation in humans. Diabetes 50 (8), 1714–1719.

Delgado, T.C., 2013. Glutamate and GABA in appetite regulation. Front. Endocrinol. (Lausanne) 4, 103.

Ebenezer, I.S., Baldwin, B.A., 1990. Effect of intracerebroventricular administration of the GABAB-receptor agonist baclofen on operant feeding in satiated pigs. Br. J. Pharmacol. 101 (3), 559–562.

Farokhnia, M., Grodin, E.N., Lee, M.R., Oot, E.N., Blackburn, A.N., Stangl, B.L., Schwandt, M.L., Farinelli, L.A., Momenan, R., Ramchandani, V.A., Leggio, L., 2017. Exogenous ghrelin administration increases alcohol self-administration and modulates brain functional activity in heavy-drinking alcohol-dependent individuals. Mol. Psychiatry.

Figlewicz, D.P., Evans, S.B., Murphy, J., Hoen, M., Baskin, D.G., 2003. Expression of receptors for insulin and leptin in the ventral tegmental area/substantia nigra (VTA/SN) of the rat. Brain Res. 964 (1), 107–115.

Filip, M., Frankowska, M., Sadakierska-Chudy, A., Suder, A., Szumiec, L., Mierzejewski, P., Bienkowski, P., Przegalinski, E., Cryan, J.F., 2015. GABAB receptors as a therapeutic strategy in substance use disorders: focus on positive allosteric modulators. Neuropharmacology 88, 36–47.

Geisel, O., Hellweg, R., Müller, C.A., 2016. Serum levels of brain-derived neurotrophic factor in alcohol-dependent patients receiving high-dose baclofen. Psychiatry Res. 240, 177–180.

Geisel, O., Panneck, P., Hellweg, R., Wiedemann, K., Müller, C.A., 2015. Hypothalamic-pituitary-adrenal axis activity in patients with pathological gambling and internet use disorder. Psychiatry Res. 226 (1), 97–102.

Gomez, J.L., Cunningham, C.L., Finn, D.A., Young, E.A., Helpenstell, L.K., Schuette, L.M., Fidler, T.L., Kosten, T.A., Ryabinin, A.E., 2015. Differential effects of ghrelin antagonists on alcohol drinking and reinforcement in mouse and rat models of alcohol dependence. Neuropharmacology 97, 182–193.

Gomez, J.L., Ryabinin, A.E., 2014. The effects of ghrelin antagonists [D-Lys(3)]-GHRP-6 or JMV2959 on ethanol, water, and food intake in C57BL/6 J mice. Alcohol Clin. Exp. Res. 38 (9), 2436–2444.

Hagemann, D., Meier, J.J., Gallwitz, B., Schmidt, W.E., 2003. Appetite regulation by ghrelin—a novel neuro-endocrine gastric peptide hormone in the gut-brain-axis. Z. Gastroenterol. 41 (9), 929–936.

Hillemacher, T., Kraus, T., Rauh, J., Weiss, J., Schanze, A., Frieling, H., Wilhelm, J., Heberlein, A., Groschl, M., Sperling, W., Kornhuber, J., Bleich, S., 2007. Role of appetite-regulating peptides in alcohol craving: an analysis in respect to subtypes and different consumption patterns in alcoholism. Alcohol Clin. Exp. Res. 31 (6), 950–954.

Hommel, J.D., Trinko, R., Sears, R.M., Georgescu, D., Liu, Z.W., Gao, X.B., Thurmon, J.J., Marinelli, M., DiLeone, R.J., 2006. Leptin receptor signaling in midbrain dopamine neurons regulates feeding. Neuron 51 (6), 801–810.

Jerlhag, E., 2008. Systemic administration of ghrelin induces conditioned place preference and stimulates accumbal dopamine. Addict. Biol. 13 (3-4), 358–363.

Jerlhag, E., Ivanoff, L., Vater, A., Engel, J.A., 2014. Peripherally circulating ghrelin does not mediate alcohol-induced reward and alcohol intake in rodents. Alcohol Clin. Exp. Res. 38 (4), 959–968.

Jerlhag, E., Landgren, S., Egecioglu, E., Dickson, S.L., Engel, J.A., 2011. The alcohol-induced locomotor stimulation and accumbal dopamine release is suppressed in ghrelin knockout mice. Alcohol 45 (4), 341–347.

Jonaidi, H., Abbassi, L., Yaghoobi, M.M., Kaiya, H., Denbow, D.M., Kamali, Y., Shojaei, B., 2012. The role of GABAergic system on the inhibitory effect of ghrelin on food intake in neonatal chicks. Neurosci. Lett. 520 (1), 82–86.

Kaur, S., Ryabinin, A.E., 2010. Ghrelin receptor antagonism decreases alcohol consumption and activation of perioculomotor urocortin-containing neurons. Alcohol Clin. Exp. Res. 34 (9), 1525–1534.

Kiefer, F., Jahn, H., Kellner, M., Naber, D., Wiedemann, K., 2001. Leptin as a possible modulator of craving for alcohol. Arch. Gen. Psychiatry 58 (5), 509–510.

Kim, D.J., Yoon, S.J., Choi, B., Kim, T.S., Woo, Y.S., Kim, W., Myrick, H., Peterson, B.S., Choi, Y.B., Kim, Y.K., Jeong, J., 2005. Increased fasting plasma ghrelin levels during alcohol abstinence. Alcohol Alcohol 40 (1), 76–79.

Kojima, M., Hosoda, H., Date, Y., Nakazato, M., Matsuo, H., Kangawa, K., 1999. Ghrelin is a growth-hormone-releasing acylated peptide from stomach. Nature 402 (6762), 656–660.

Koob, G.F., 2006. A role for GABA in alcohol dependence. Adv. Pharmacol. 54, 205–229.

Koopmann, A., Bez, J., Lemenager, T., Hermann, D., Dinter, C., Reinhard, I., Hoffmann, H., Wiedemann, K., Winterer, G., Kiefer, F., 2015. Effects of cigarette smoking on plasma concentration of the appetite-regulating peptide ghrelin. Ann. Nutr. Metab. 66 (2–3), 155–161.

Koopmann, A., Lippmann, K., Schuster, R., Reinhard, I., Bach, P., Weil, G., Rietschel, M., Witt, S.H., Wiedemann, K., Kiefer, F., 2017. Drinking water to reduce alcohol craving? A randomized controlled study on the impact of ghrelin in mediating the effects of forced water intake in alcohol addiction. Psychoneuroendocrinology 85, 56–62.

Koopmann, A., Schuster, R., Kiefer, F., 2018. The impact of the appetite-regulating, orexigenic peptide ghrelin on alcohol use disorders: a systematic review of preclinical

and clinical data. Biol. Psychol. 131, 14–30.
Koopmann, A., von der Goltz, C., Grosshans, M., Dinter, C., Vitale, M., Wiedemann, K., Kiefer, F., 2012. The association of the appetitive peptide acetylated ghrelin with alcohol craving in early abstinent alcohol dependent individuals. Psychoneuroendocrinology 37 (7), 980–986.
Kraus, T., Schanze, A., Groschl, M., Bayerlein, K., Hillemacher, T., Reulbach, U., Kornhuber, J., Bleich, S., 2005. Ghrelin levels are increased in alcoholism. Alcohol Clin. Exp. Res. 29 (12), 2154–2157.
Lee, M.R., Tapocik, J.D., Ghareeb, M., Schwandt, M.L., Dias, A.A., Le, A.N., Cobbina, E., Farinelli, L.A., Bouhlal, S., Farokhnia, M., Heilig, M., Akhlaghi, F., Leggio, L., 2018. The novel ghrelin receptor inverse agonist PF-5190457 administered with alcohol: preclinical safety experiments and a phase 1b human laboratory study. Mol. Psychiatry.
Leggio, L., Ferrulli, A., Cardone, S., Nesci, A., Miceli, A., Malandrino, N., Capristo, E., Canestrelli, B., Monteleone, P., Kenna, G.A., Swift, R.M., Addolorato, G., 2012. Ghrelin system in alcohol-dependent subjects: role of plasma ghrelin levels in alcohol drinking and craving. Addict. Biol. 17 (2), 452–464.
Leggio, L., Zywiak, W.H., Fricchione, S.R., Edwards, S.M., de la Monte, S.M., Swift, R.M., Kenna, G.A., 2014. Intravenous ghrelin administration increases alcohol craving in alcohol-dependent heavy drinkers: a preliminary investigation. Biol. Psychiatry 76 (9), 734–741.
Lyons, A.M., Lowery, E.G., Sparta, D.R., Thiele, T.E., 2008. Effects of food availability and administration of orexigenic and anorectic agents on elevated ethanol drinking associated with drinking in the dark procedures. Alcohol Clin. Exp. Res. 32 (11), 1962–1968.
Mirijello, A., Caputo, F., Vassallo, G., Rolland, B., Tarli, C., Gasbarrini, A., Addolorato, G., 2015. GABAB agonists for the treatment of alcohol use disorder. Curr. Pharm. Des. 21 (23), 3367–3372.
Mottola, C.A., 1993. Measurement strategies: the visual analogue scale. Decubitus 6 (5), 56–58.
Müller, C.A., Geisel, O., Pelz, P., Higl, V., Kruger, J., Stickel, A., Beck, A., Wernecke, K.D., Hellweg, R., Heinz, A., 2015. High-dose baclofen for the treatment of alcohol dependence (BACLAD study): a randomized, placebo-controlled trial. Eur. Neuropsychopharmacol. 25 (8), 1167–1177.
Narayanan, N.S., Guarnieri, D.J., DiLeone, R.J., 2010. Metabolic hormones, dopamine circuits, and feeding. Front. Neuroendocrinol. 31 (1), 104–112.
Ralevski, E., Shanabrough, M., Newcomb, J., Gandelman, E., Hayden, R., Horvath, T.L., Petrakis, I., 2018. Ghrelin is related to personality differences in reward sensitivity and impulsivity. Alcohol Alcohol 53 (1), 52–56.
Roberto, M., Madamba, S.G., Moore, S.D., Tallent, M.K., Siggins, G.R., 2003. Ethanol increases GABAergic transmission at both pre- and postsynaptic sites in rat central amygdala neurons. Proc. Natl. Acad. Sci. USA 100 (4), 2053–2058.
Rose, A.K., Jones, A., 2018. Baclofen: its effectiveness in reducing harmful drinking, craving, and negative mood. A meta-analysis. Addiction 113 (8), 1396–1406.
Schneider, E.R., Rada, P., Darby, R.D., Leibowitz, S.F., Hoebel, B.G., 2007. Orexigenic peptides and alcohol intake: differential effects of orexin, galanin, and ghrelin. Alcohol Clin. Exp. Res. 31 (11), 1858–1865.
Stevenson, J.R., Francomacaro, L.M., Bohidar, A.E., Young, K.A., Pesarchick, B.F., Buirkle, J.M., McMahon, E.K., O'Bryan, C.M., 2016. Ghrelin receptor (GHS-R1A) antagonism alters preference for ethanol and sucrose in a concentration-dependent manner in prairie voles. Physiol. Behav. 155, 231–236.
Stratford, T.R., Kelley, A.E., 1997. GABA in the nucleus accumbens shell participates in the central regulation of feeding behavior. J. Neurosci. 17 (11), 4434–4440.
Suchankova, P., Engel, J.A., Jerlhag, E., 2016a. Sub-chronic ghrelin receptor blockade attenuates alcohol- and amphetamine-induced locomotor stimulation in mice. Alcohol Alcohol 51 (2), 121–127.
Suchankova, P., Nilsson, S., von der Pahlen, B., Santtila, P., Sandnabba, K., Johansson, A., Jern, P., Engel, J.A., Jerlhag, E., 2016b. Genetic variation of the growth hormone secretagogue receptor gene is associated with alcohol use disorders identification test scores and smoking. Addict. Biol. 21 (2), 481–488.
Tschop, M., Smiley, D.L., Heiman, M.L., 2000. Ghrelin induces adiposity in rodents. Nature 407 (6806), 908–913.
Tschop, M., Wawarta, R., Riepl, R.L., Friedrich, S., Bidlingmaier, M., Landgraf, R., Folwaczny, C., 2001. Post-prandial decrease of circulating human ghrelin levels. J. Endocrinol. Invest. 24 (6), RC19–RC21.
van Zessen, R., van der Plasse, G., Adan, R.A., 2012. Contribution of the mesolimbic dopamine system in mediating the effects of leptin and ghrelin on feeding. Proc. Nutr. Soc. 71 (4), 435–445.
von der Goltz, C., Koopmann, A., Dinter, C., Richter, A., Rockenbach, C., Grosshans, M., Nakovics, H., Wiedemann, K., Mann, K., Winterer, G., Kiefer, F., 2010. Orexin and leptin are associated with nicotine craving: a link between smoking, appetite and reward. Psychoneuroendocrinology 35 (4), 570–577.
Watanabe, M., Maemura, K., Kanbara, K., Tamayama, T., Hayasaki, H., 2002. GABA and GABA receptors in the central nervous system and other organs. Int. Rev. Cytol. 213, 1–47.
Weiner, J.L., Valenzuela, C.F., 2006. Ethanol modulation of GABAergic transmission: the view from the slice. Pharmacol. Ther. 111 (3), 533–554.
WHO, 1994. In: Dilling, H, Mombour, W, Schmidt, MH, Schulte-Warkwort, E. (Eds.), Internationale Klassifikation Psychischer Störungen ICD-10 Kapitel V (F) Forschungskriterien. Huber, Bern.
Wittekind, D.A., Kratzsch, J., Mergl, R., Enzenbach, C., Witte, A.V., Villringer, A., Kluge, M., 2018. Alcohol consumption is positively associated with fasting serum ghrelin in non-dependent adults: results from the population-based LIFE-adult-study. Psychoneuroendocrinology 97, 143–148.
Wren, A.M., Small, C.J., Ward, H.L., Murphy, K.G., Dakin, C.L., Taheri, S., Kennedy, A.R., Roberts, G.H., Morgan, D.G., Ghatei, M.A., Bloom, S.R., 2000. The novel hypothalamic peptide ghrelin stimulates food intake and growth hormone secretion. Endocrinology 141 (11), 4325–4328.
Wurst, F.M., Graf, I., Ehrenthal, H.D., Klein, S., Backhaus, J., Blank, S., Graf, M., Pridzun, L., Wiesbeck, G.A., Junghanns, K., 2007. Gender differences for ghrelin levels in alcohol-dependent patients and differences between alcoholics and healthy controls. Alcohol Clin. Exp. Res. 31 (12), 2006–2011.
Zallar, L.J., Farokhnia, M., Tunstall, B.J., Vendruscolo, L.F., Leggio, L., 2017. The role of the ghrelin system in drug addiction. Int. Rev. Neurobiol. 136, 89–119.