Alcohol and Alcoholism Advance Access published October 27, 2013

Alcohol and Alcoholism pp. 1–5, 2013 doi: 10.1093/alcalc/agt160

ORIGINAL ARTICLE

The Impact of Atrial Natriuretic Peptide on Anxiety, Stress and Craving in Patients with Alcohol Dependence

Anne Koopmann[1,†]*, Tagrid Leménager[1,†], Nadine Donata Wolf[1], Iris Reinhard[2], Derik Hermann[1], Jan Koch[1], Klaus Wiedemann[3] and Falk Kiefer[1]

[1]Department of Addictive Behavior and Addiction Medicine, Central Institute of Mental Health, Medical Faculty Mannheim/Heidelberg University, Mannheim, Germany, [2]Division of Biostatistics, Central Institute of Mental Health, Medical Faculty Mannheim/Heidelberg University, Mannheim, Germany and [3]Department of Psychiatry, University Hospital of Hamburg, Hamburg, Germany

*Corresponding author: Department of Addictive Behavior and Addiction Medicine, Central Institute of Mental Health, J5, D-68159 Mannheim, Germany. Tel: +49-621-1703-3731; Fax. +49-621-1703-3505; E-mail: anne.koopmann@zi-mannheim.de

[†]A.K. and T.L. contributed equally to this manuscript.

(*Received* 14 *July* 2013; *first review notified* 22 *August* 2013; *in revised form* 5 *September* 2013; *accepted* 13 *September* 2013)

Abstract — **Aims:** Atrial natriuretic peptide (ANP) is well known to modulate fluid and electrolyte homeostasis but also to counter-regulate hypothalamic-pituitary-adrenal (HPA) axis activity. Correspondingly, recent studies suggest an important role of ANP in the neurobiology of anxiety. Preclinical and clinical data now provide evidence for an involvement of ANP in the pathophysiology of addictive behavior. The present study aims to elucidate the effects of ANP on alcohol-dependent patients' anxiety, perceived stress and craving during alcohol withdrawal. **Methods:** A sample of 59 alcohol-dependent inpatients was included in the analysis. A blood sample was taken at day 14 of detoxification in order to assess the concentrations of ANP and cortisol in plasma. In parallel, we assessed patients' alcohol craving, using the Obsessive Compulsive Drinking Scale, as well as anxiety (State-Trait Anxiety Inventory). Patients' stress levels were assessed using the Perceived Stress Scale. **Results:** We found a significant negative association between patients' ANP plasma concentrations and anxiety, craving for alcohol and perceived stress. Regression analyses suggest that ANP is a significant predictor both for patients' perceived stress and for the severity of anxiety during early abstinence. The association of patients' ANP plasma levels and craving is suggested to be mediated by perceived stress. **Conclusion:** Our results suggest that the association of patients' ANP plasma levels and craving is mediated by their perceived stress. For this reason, intranasal application of ANP may prove to be a new avenue for the treatment of alcohol dependence in patients exhibiting high levels of perceived stress.

INTRODUCTION

Atrial natriuretic peptide (ANP) is a 28 amino acid peptide that is synthesized and secreted mainly by atrial myocytes and acts as a regulator of fluid and electrolyte homeostasis (Cantim and Genest, 1987). Via peripheral receptors, it induces natriuresis, diuresis and helps to lower blood pressure (de Bold *et al.*, 1981; de Bold, 1982). Additionally, ANP decreases intake of salt and water (Antunes-Rodriges *et al.*, 1985, 1986).

Several preclinical and clinical studies have demonstrated that ANP is also produced in the central nervous system (CNS) and that it is involved in the regulation of various endocrine, autonomic, cardiovascular and behavioral functions (Imura *et al.*, 1992). In men, ANP is directly involved in stress-hormone responses. It inhibits the release of corticotrophin-releasing hormone (CRH) (Antoni *et al.*, 1992) and corticotrophin (ACTH) (Fink *et al.*, 1991). Since CRH also stimulates the release of ANP, some have suggested that ANP may be part of a complex endocrine-feedback system (Tojo *et al.*, 1996; Colao *et al.*, 1998). Moreover, ANP impacts affective symptoms as well as symptoms of anxiety and plays a special role in the modulation and termination of stress responses and panic attacks (Bhattacharya *et al.*, 1996; Strohle *et al.*, 2001; Wiedemann *et al.*, 2001). ANP is also active in the regulation of anxiety and stress associated behavior, where it acts not only as a peripheral physiological antagonist of CRH effects (Liebsch *et al.*, 1995), but also within the CNS itself, where it counteracts CRH's centrally mediated anxiogenic effects (Wiedemann *et al.*, 2000).

In recent years, preclinical and clinical data have shown ANP to be involved in alcohol-related pathologies as well. In mice, the intracerebroventricular (ICV) injection of an antiserum against ANP was found to intensify hyperexcitability during alcohol withdrawal, whereas ICV injections of ANP were found to have the opposite effect (Kovacs, 1993). Additionally, Mutschler *et al.* (2010) found mice lacking a functioning natriuretic peptide receptor A (NPR-A) to show increased stress-induced alcohol intake as well as aggravated neurobehavioral alcohol-withdrawal symptoms (Mutschler *et al.*, 2010). Corresponding human studies reported dysregulations of the ANP plasma level after acute and chronic alcohol injection as well as during alcohol withdrawal. Gianoulakis *et al.* (1997) showed healthy men's ANP plasma levels to increase following acute alcohol ingestion. In contrast, alcohol-dependent patients' ANP plasma levels were lower during detoxification compared with healthy, abstinent controls (Kiefer *et al.*, 2002a). Hillemacher *et al.* (2009) found the expression of ANP mRNA to be significantly elevated in alcohol-dependent patients, while the methylation of promoter-related ANP DNA was significantly decreased. Furthermore, the authors found promoter-related DNA methylation to be significantly negatively correlated with patients' level of alcohol craving.

Since ANP-related psychopathology is possibly mediated via its counter-regulating effect on HPA-axis activity, results of studies dealing with the association between cortisol-stress response and alcohol intake related behavior are potentially interesting. Former clinical studies showed that duration of abstinence in alcohol-dependent patients was positively related to cortisol stress response (Kiefer *et al.*, 2002b; Adinoff *et al.*, 2005; Starcke *et al.*, 2013). These results indicate that ANP might modulate craving and relapse indirectly via its effects on the stress-hormone system in abstinent alcohol-dependent patients.

In order to further elucidate the influence of the natriuretic peptide system on relapse-inducing conditions in early-abstinent

alcohol-dependent patients, we tested the hypothesis whether the effects of ANP on alcohol craving are mediated by or associated with stress and anxiety in this population or not. To this end, we assessed hormonal and psychometric criteria in 59 alcohol-dependent patients of both genders on day 14 of detoxification treatment.

MATERIALS AND METHODS

Participants

We studied 59 individuals meeting *DSM*-IV criteria for alcohol dependence. All individuals were inpatients at the Department of Addictive Behavior and Addiction Medicine at the Central Institute of Mental Health in Mannheim, Germany, where they had been admitted for detoxification treatment. Patients did not meet diagnostic criteria for any other current psychiatric disorders, including abuse and dependence on illegal drugs. We chose to include only patients who had not taken any psychotropic medications for at least 3 months prior to the study, with exceptions for low-dose administrations of either Mirtazapine (max. 15 mg) or Doxepine (max. 25 mg) as sleep aids. If necessary, patients received benzodiazepines (diazepam or lorazepam) for detoxification in progressively smaller dosages between Days 1 and 4 of inpatient treatment.

The study protocol was approved by the local ethics committee (authorization number: 2008-214N-MA). Written informed consent was obtained from all participants, in accordance with the Declaration of Helsinki, after they had been presented with detailed information on the study's purpose and procedures.

Hormonal measures

Blood samples were obtained from all patients 2 weeks after detoxification (day 14). We drew 30 ml of blood on Day 14, at 8.00 a.m. before breakfast, after the patient had fasted overnight, and under standardized conditions (e.g. sitting patient, puncture of the cubital vein). These blood samples were used for routine laboratory testing, including gamma glutamyl transferase (γGT), glutamic oxaloacetic transaminase (GOT) and glutamate pyruvate transaminase (GPT) as well as determining plasma concentrations of ANP, cortisol, sodium and calcium. For measuring ANP and cortisol plasma concentrations, all blood samples were anticoagulated using sodium EDTA (1 mg/ml whole blood), and then immediately ice-cooled. Plasma concentrations of ANP were measured with a radioimmunoassay kit (DRG-Instruments, Marburg, Germany). The detection limit was 0.5 pg/ml. The inter-assay coefficient of variation was <11.6%; the intra-assay coefficient was <8.6%. Plasma cortisol was determined by modified commercial radioimmunoassay with coated tube techniques (DRG-Instruments, Marburg, Germany). The inter-assay coefficient of variation was <7.0%, the intra-assay coefficient was <5%. Minimum detectable amounts were 1 ng/ml plasma.

Psychometric measures

Psychometric measurements were obtained at day 14 of detoxification when both acute alcohol physical withdrawal and withdrawal-medication effects were tapered off. These data were acquired at the same time as the blood samples. We investigated the severity of alcohol dependence symptoms by using the Alcohol Dependence Scale (ADS; Skinner and Horn, 1984). The ADS is a self-rating questionnaire consisting of 25 items including alcohol-withdrawal symptoms, impaired control over drinking, awareness of compulsions to drink, increased alcohol tolerance and salience of drink-seeking behavior. Craving was rated using the Obsessive Compulsive Drinking Scale (OCDS; Anton *et al.*, 1996, German-language version: Mann and Ackermann, 2000). The 14 items of the OCDS evaluate alcohol-related thoughts, psychosocial disturbances and impaired control of drinking. The five possible responses per item (0–4) reflect increasing symptom intensity. Two subscales—'obsessions' and 'compulsions'—are used to summarize items 1–6 and 7–14, respectively. The obsession subscale is believed to represent the cognitive preoccupation with alcohol of subjects suffering from an alcohol use disorder, while the compulsion subscale is thought to account for the behavioral and motivational aspects of alcohol consumption (Mann and Ackermann, 2000). We chose the State-Trait Anxiety Inventory (STAI) to investigate anxiety (Spielberger *et al.*, 1970, German-language version: Laux *et al.*, 1981) with its subscales for state anxiety (STATE-G) and trait anxiety (TRAIT-G). Subjective experience of stress was measured using the Perceived Stress Scale (PSS, Cohen *et al.*, 1983).

Statistical analysis

Data analyses were conducted using PASW 18 for Windows. Statistical results were reported as means and standard deviations (SD). Correlations were analyzed using Pearson's correlation coefficients. Results were considered statistically significant if their P-value was <0.05.

Linear regression analyses were applied to identify the influence of ANP as well as cortisol plasma concentrations, stress (measured using the PSS) and self-rated anxiety (measured using the STAI) on craving (measured using the OCDS). In order to assess whether perceived stress (PSS) or anxiety (STAI-State) mediates the effect of ANP or cortisol plasma levels on craving (OCDS sum score), we performed in a first step two univariate regression analyses, investigating the effect of ANP as well as cortisol plasma concentrations on state anxiety as well as on perceived stress. In a second step, multiple linear regression analyses were performed in order to assess the effects of the particular hormone level and anxiety as well as the effects of the particular hormone level and perceived stress on craving (OCDS sum score). Mediation effects were proved using the Goodman II formula (Goodman, 1960).

RESULTS

Participants

Patient characteristics—including age, duration of alcohol dependence, number of previous detoxifications, severity of alcohol addiction measured using the ADS, level of daily cigarette consumption, breath-alcohol content, as well as routine laboratory tests (including γGT, GOT and GPT) at Day 14 of the detoxification—are provided in Table 1. Additionally, Table 1 shows patients' plasma levels of sodium and calcium at Day 14.

Hormonal measures

At Day 14 of detoxification, the average ANP plasma level of the 59 participants was 54.0 ± 55.0 pg/ml and the average cortisol plasma level was 232.8 ± 89.4 ng/ml.

Table 1. Sample characteristics

	Total sample ($n = 59$) M ± SD
Age	46.5 ± 9.9
Duration of alcohol dependence (in months)	176.9 ± 143.8
Number of inpatient detoxifications	3.3 ± 4.3
ADS	16.9 ± 7.2
Cigarettes/day	17.8 ± 14.0
γGT (U/l)	161.5 ± 186.1
GOT (U/l)	33.3 ± 12.6
GPT (U/l)	34.5 ± 17.0
Sodium (mmol/l)	139.7 ± 2.4
Calcium (mmol/l)	2.4 ± 0.12

Data are given as arithmetic mean ± standard deviation (SD).
γGT, gamma glutamyl transferase; GOT, glutamic oxaloacetic transaminase; GPT, glutamate pyruvate transaminase.

Table 2. Relationships between ANP plasma levels and psychological symptoms

	ANP		Cortisol	
Day 14	*r*	*P*	*r*	*P*
OCDS sum score	−0.27*	0.04	−0.04	0.81
OCDS obsession subscale	−0.19	0.19	−0.05	0.74
OCDS compulsion subscale	−0.30*	0.03	−0.02	0.87
STATE-STAI	−0.29*	0.04	−0.11	0.45
TRAIT-STAI	−0.22	0.12	−0.06	0.67
PSS	−0.29*	0.04	−0.03	0.84

OCDS, Obsessive Compulsive Drinking Scale; PSS, Perceived Stress Scale; STATE-STAI, state anxiety subscale of the State-Trait Anxiety Inventory; TRAIT-STAI, trait-anxiety subscale of the State-Trait Anxiety Inventory; *r*, Pearson correlation coefficient; *p*, level of significance, $*P < 0.05$.

Psychometric testing

At Day 14 of detoxification, the average craving as measured by the OCDS sum score was 17.6 ± 9.6. On the OCDS subscales for obsession and compulsion, the participants' mean scores were 4.71 ± 4.2 and 7.1 ± 5.0, respectively. On the same measuring day, participants showed an average state anxiety of 38.0 ± 12.6 as well as a trait anxiety of 40.0 ± 11.0, as gathered using the STAI. Patients' mean score on the PSS was 16.8 ± 6.8.

Relationships between ANP plasma levels and psychological symptoms

We found significant inverse correlations between patients' ANP plasma concentrations and their OCDS sum score as well as between their ANP plasma levels and their scores on the OCDS Compulsion subscale. However, we were not able to show a significant correlation between patients' scores of the OCDS Obsession subscale and their ANP plasma concentrations (see Table 2). Furthermore, we found a significant inverse correlation between patients' ANP plasma levels and their scores on the state-anxiety subscale of the STAI. In contrast, we found no significant correlations between patients' ANP plasma levels and their scores on the trait-anxiety subscale of the STAI (see Table 2). Additionally, we were able to show a significant inverse correlation between patients' ANP plasma levels and their scores on the PSS (see Table 2).

At Day 14, we could not find any correlation between patients' cortisol plasma concentrations and their OCDS scores, as well as between patients' cortisol plasma levels and their scores on the state and trait-anxiety subscales of the STAI. Finally, we were not able to show a significant correlation between patients' cortisol plasma concentrations and their scores on the PSS (see Table 2).

Regression analyses

We employed distinct linear regression models in order to analyze any potential effects of ANP plasma concentrations on clinically relevant alcohol craving (as measured using the OCDS sum scores), state anxiety (as measured with the STATE-STAI) and perceived stress (as measured using the PSS). ANP accounts for 8.3% of the variance of patients' scores on the state-anxiety subscale of the STAI (adjusted $R^2 = 0.065$, $P < 0.001$) and for 7.9% of the variance in their PSS sum scores (adjusted $R^2 = 0.061$, $P < 0.001$). Additionally, univariate regression analyses revealed significant effects of state anxiety and perceived stress on craving (state anxiety on craving: adjusted: $R^2 = 0.312$, $P < 0.001$; perceived stress on craving: adjusted $R^2 = 0.428$, $P < 0.001$). Furthermore, we observed a statistical trend toward a direct influence of ANP plasma concentrations on craving (adjusted $R^2 = 0.073$, $P = 0.056$). But since this result failed only by a narrow margin to achieve the necessary significance level, ANP remained involved in the following mediation analyses.

To further investigate if the effects of ANP plasma levels on patients' alcohol craving are mediated by their perceived stress (PSS) or by their state anxiety, we performed two multiple linear regression models including ANP plasma levels and patients' perceived stress respectively their state anxiety as predictors.

In the first model we found the sum score of the STATE-STAI and the plasma concentration of ANP to account for 34% (adjusted $R^2 = 0.32$) of the variance in OCDS sum score ($F(2) = 12.59$, $P < 0.0001$). In this model, only the sum score of the STATE-STAI showed a statistically significant influence on the sum score of the OCDS. In the second model we found the PSS sum score and the plasma concentration of ANP to account for 45% (adjusted $R^2 = 0.43$) of the variance in OCDS sum score ($F(2) = 19.62$, $P < 0.0001$). In this model, only the sum score of the PSS had a statistically significant influence on the sum score of the OCDS.

Proving the mediation effects of the PSS sum score respectively the STATE-STAI on the association between ANP plasma levels and alcohol craving by using the Goodman II formula, only the mediation effects of the PSS reached statistical significance. Figure 1 depicts the details of the two mediation models.

Linear regression models assessing the effects of cortisol plasma levels on craving, state anxiety and perceived stress did not show significant results. Therefore, we refrained from further analyses.

DISCUSSION

A main result of this study was to show ANP plasma concentrations to be inversely correlated with craving, perceived stress and state anxiety in early-abstinent, alcohol-dependent patients. While there are direct associations between ANP plasma concentrations and both anxiety and patients' perceived stress, ANP plasma levels affect craving only indirectly, through their influence on patients' perceived stress.

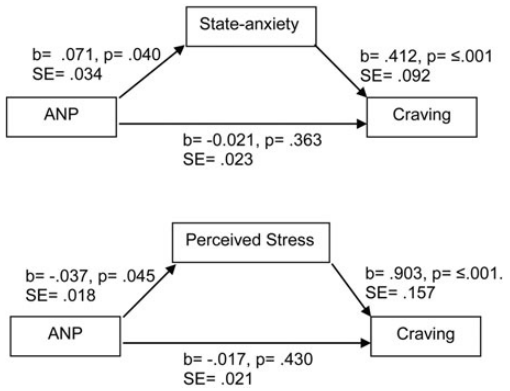

Fig. 1. Association between ANP plasma levels, anxiety, stress and craving. *b*, b coefficient; *p*, level of significance; SE, standard error; $*P < 0.05$. Craving as measured by the OCDS; perceived stress as measured by the PSS; state anxiety as measured by the STATE-STAI.

These results support the transfer of previous preclinical findings to humans. In one such study, Kovacs (1993) showed that injecting ethanol-fed mice with an ICV injection of ANP antiserum significantly intensified handling-induced convulsions, an indicator of withdrawal in mice. In contrast, the author found that an ICV injection of α-ANP reduced the severity of handling-induced convulsions. Another study by Bidzseranova *et al.* (1992) showed ICV injection of ANP in rats to have anxiolytic-like effects in paradigms such as the elevated plus maze. Additionally, Mutschler *et al.* (2010) demonstrated mice lacking a certain functional ANP receptor (NPR-A) to show both increased stress-induced alcohol intake and aggravated neurobehavioral alcohol-withdrawal symptoms compared with wild-type mice, even though basal alcohol intake did not differ between mice lacking a functional NPR-A receptor and their wild-type littermates.

Also in humans, stress is a major environmental factor affecting voluntary alcohol intake (Heilig and Egli, 2006; Uhart and Wand, 2009). In humans, an altered activity of the hypothalamic-pituitary-adrenocortical (HPA) axis—which represents the endocrine backbone of behavioral stress response—was shown repeatedly during alcohol withdrawal (Valimaki *et al.*, 1984; Heinz *et al.*, 1995; Kiefer and Wiedemann, 2004).

In line with the results of Sinha *et al.* (2011), reporting that recently sober alcohol-dependent patients showed higher basal ACTH- and cortisol plasma levels as well as smaller increases in these hormones during stress compared with healthy controls, we did not find a significant correlation between patients' perceived stress and their cortisol plasma levels. Furthermore, Junghanns *et al.* (2003) reported that in a sample of alcohol-dependent patients relapsers showed almost no cortisol responses in a stress test. Thus, these results indicate that cortisol might not be a valid marker to investigate perceived stress in alcohol-dependent patients due to a HPA axis dysregulation during alcohol withdrawal and early abstinence.

With our findings concerning the negative association between ANP plasma levels and both alcohol craving (as measured by the OCDS) and anxiety (as gathered using the STAI), we were able to confirm and generalize on the findings of a previous clinical study on alcohol-dependent patients by Kiefer *et al.* (2002a). The authors of this study had demonstrated detoxified patients whose ANP levels decreased during alcohol withdrawal to suffer from more intense craving than patients whose ANP plasma concentrations had increased. Additionally, the authors found a trend of elevated self-rated anxiety in alcohol-dependent patients whose ANP levels decreased during alcohol withdrawal.

By showing a significant negative association between patients' anxiety levels and their ANP plasma concentrations in our sample of alcohol-dependent patients in early abstinence, we demonstrated ANP to have anxiolytic-like effects in this group of patients. These findings are in line with previous clinical studies in non-alcohol-dependent patients. Wiedemann *et al.* (2001) showed the intravenous administration of 150 µg of ANP in nine patients suffering from a panic disorder to have an anxiolytic-like effect on panic attacks induced by cholecystokinin tetrapeptide (CCK-4). Additionally, Strohle *et al.* (2006) found increases of ANP plasma concentrations induced by aerobic exercise to reduce CCK-4-induced panic attacks in nine healthy subjects. Considered in the light of the aforementioned findings, the clinical observations, showing aerobic exercises to reduce craving in alcohol-dependent patients, might be at least partly explained by the aerobic exercise-induced increases of ANP plasma concentration.

By showing a significant negative association between patients' perceived stress and their ANP plasma concentrations in our sample of alcohol-dependent patients in early abstinence, we were able to show that ANP also modulates the level of perceived stress in this group of patients. Although the bidirectional interaction between the ANP plasma concentrations and the HPA-axis activity is well known (Fink *et al.*, 1991; Antoni *et al.*, 1992; Tojo *et al.*, 1996; Colao *et al.*, 1998), there are no former clinical studies investigating the association between ANP plasma concentrations and perceived stress in healthy controls and in patients suffering from a panic disorder or a depression. In addition to the aforementioned findings concerning the influence of ANP plasma concentrations on craving in alcohol-dependent patients during withdrawal, a previous study by Hillemacher *et al.* (2009) provides evidence that the promoter-related DNA methylation of the ANP gene is significantly negatively correlated with the extent of craving as measured by the OCDS. The authors also found the promoter-related DNA methylation of ANP to be lower in alcohol-dependent patients than in healthy controls, in addition to showing ANP mRNA expression to be significantly elevated in patients suffering from alcohol use disorder. Additionally, Kiefer *et al.* (2011) found the single nucleotide polymorphisms in an intron in the gene for GATA-binding protein 4 (GATA4)—which plays a role in controlling the ANP plasma concentration—to be associated with relapse within a 90-day medical treatment using acamprosate for alcohol-dependent patients. They were able to show that patients with a GG GATA4 genotype relapse significantly earlier than patients with an AA or AG genotype. In the present study—which found craving to be significantly negatively associated with ANP plasma levels, state anxiety and perceived stress—we did not analyze our sample's GATA4 genotypes or the promoter-related DNA methylation of their ANP genes, so we were unable to state whether either of these two factors influenced the interaction between their ANP plasma levels, perceived stress and anxiety. Further research is needed before such conclusions can be drawn.

In summary, our results present evidence that there is a complex interaction between early-abstinent alcohol-dependent patients' ANP plasma levels, anxiety, perceived stress and

alcohol craving, with the association between ANP plasma levels and craving being mediated by the influence of patients' ANP plasma levels on their perceived stress. One major problem in using these findings for therapeutic purposes is the confounding effects by systemic actions of ANP. The administration of ANP via the nose-brain pathway enables the peptide to access the brain compartment directly and avoid systemic side effects (Illum, 2000). For this reason, intranasal application of ANP, which has no significant systemic side effects, may prove a new and innovative avenue for treating alcohol dependence, especially in patients exhibiting high levels of perceived stress.

Funding — This study was supported by the Central Institute of Mental Health Mannheim, Germany. The Central Institute of Mental Health Mannheim had no further role in study design; in the collection, analysis and interpretation of data; in the writing of the report; or in the decision to submit the paper for publication.

Conflict of interest statement. None declared.

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