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Higher plasma levels of CT-proAVP are linked to less anxiety in men but not women with cardiovascular risk factors: Results from the observational Diast-CHF study

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ABSTRACT

Aim: Using data from the multicenter, observational Diast-CHF (Diagnostic Trial on Prevalence and Clinical Course of Diastolic Dysfunction and Heart Failure) study, this post-hoc analysis aimed at assessing the association between serum concentrations of C-terminal pro-arginine vasopressin (CT-proAVP) and anxiety in patients with cardiovascular risk factors.

Background: Animal studies have demonstrated that centrally released AVP is involved in the development of anxiety-like behaviors, however, it is unknown whether, also in humans, CT-proAVP used as a proxy for the co-secreted AVP is associated with self-reported anxiety.

Methods: In 1463 study participants with cardiovascular risk factors (mean age 66.7 ± 8.1 years, 51.3% males, mean left ventricular ejection fraction 59.8 ± 8.3%), serum concentrations of CT-proAVP were measured by means of an ELISA assay, and anxiety was assessed using the Hospital Anxiety and Depression Scale (HADS).

Results: Data showed that there was a significant and inverse correlation between HADS anxiety and CT-proAVP (rho = −0.074; p = 0.005). Serum CT-proAVP and the HADS anxiety differed between the two sexes: men displayed lower anxiety (4.7 ± 3.5 versus 5.5 ± 3.7) and had higher CT-proAVP levels (5.8 pmol/L, interquartile range 3.5–9.9 pmol/L versus 3.0 pmol/L, interquartile range 2.0–4.7) than women (both, p < 0.001). Using univariate ANOVA adjusted for age, body-mass index, estimated glomerular filtration rate, left ventricular ejection fraction, 6-minute walking distance, SF-36 physical functioning, and the natriuretic peptides NT-proBNP and MR-proANP, the interaction term sex*CT-proAVP was significantly associated with anxiety (p = 0.006). Further analysis showed that CT-proAVP was inversely related to anxiety only in men (B = −0.991; 95%CI = −1.650 to −0.331; p = 0.003), but not in women (p = 0.335).

Conclusion: In male study participants with cardiovascular risk factors, serum concentrations of CT-proAVP showed an inverse association with anxiety, which was independent from the severity of physical impairment.

1. Introduction

Anxiety affects the regulation of the cardiovascular system and may have an impact on the development and progression of heart diseases. Previous studies have suggested that the neuropeptide arginine vasopressin (AVP), also termed antidiuretic hormone (ADH), plays a role in the regulation of anxiety and stress responses by synergistically stimulating the hypothalamo-pituitary-adrenal (HPA) axis (Katan et al., 2008). The vasopressinergic regulation of the HPA stress responsiveness may have emotional implications affecting social recognition and anxiety responses. One study reported that serum CT-proAVP levels were higher in patients with suicide attempts than in healthy controls

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(Atescelik et al., 2017). Additionally, it was shown that intranasal administration of AVP displayed sex-specific differences in responses to pictures of same-sex models posing with various facial expressions, suggesting that, in humans, AVP may modulate anxiety (Thompson et al., 2006). In addition, CT-proAVP was identified as a clinically relevant biomarker for the prediction of prognosis in patients with heart failure (Alehagen et al., 2011; Maisel et al., 2011) and sepsis (Morgenthaler et al., 2007; Jochberger et al., 2009).

Laboratory studies using transgenic mouse lines suggested that AVP increased generalized anxiety levels through the vasopressinergic AVP-V1b receptor, which led to huddling (Mak et al., 2012; Bowen and McGregor, 2014). Knockout mice with a deficient AVP neurotransmission displayed lower blood pressure in both sexes and, in addition, anxiety-like behavior in females, whereas males were unaffected (Egashira et al., 2007; Herrera et al., 2011). However, other studies demonstrated that male mice lacking functional AVP-V1a receptor exhibit markedly reduced anxiety-like behavior and a profound impairment in social recognition, whereas female knockout mice displayed normal anxiety-like behavior (Bielsky et al., 2004, 2005). The biomarker CT-proAVP is cleaved from the larger pre-pro-vasopressin precursor in parvocellular neurons of the nuclei paraventricularis and supraopticus, where it is released in equimolar amounts with AVP as the 39-amino acid long, carboxy-terminal proAVP (CT-proAVP), also known as copeptin (Land et al., 1982). Due to its longer half-life, CT-proAVP is more stable in the circulation than the endogenous receptor ligand AVP and can be easily detected in immunological assays as a surrogate marker for AVP.

To address the question of whether, also in humans, AVP is associated with anxiety, we measured serum concentrations of CT-proAVP in a sample of patients with cardiovascular risk factors. Using data from the observational, multicenter Diagnostic Trial on Prevalence and Clinical Course of Diastolic Dysfunction and Heart Failure (Diast-CHF) study, we tested the hypothesis that, in probands with cardiovascular risk factors, serum CT-proAVP is positively linked to self-assessed anxiety.

2. Methods

2.1. Study design

The baseline assessment of the multicenter, observational Diast-CHF trial was conducted in 2004 to 2006 as part of the nationwide German Competence Network Heart Failure, which aimed at assessing the prevalence of diastolic left ventricular dysfunction in medical outpatients treated by primary care physicians (Stahrenberg et al., 2010a,b). Study participants were medical outpatients aged 50–85 years. They were recruited if they had at least one cardiovascular risk factor for the development of diastolic heart failure, such as hypertension, diabetes mellitus, coronary heart disease, sleep apnea syndrome or a history of myocardial infarction. Exclusion criteria were unwillingness to give informed consent, insufficient understanding of the German language, and/or unavailability for logistic reasons. The primary care Diast-CHF study recruited in total 1935 participants and, as a control group, 208 healthy volunteers who were excluded from the present analysis, as were 49 participants with known atrial fibrillation and 136 subjects lacking echocardiographic parameters for diastolic dysfunction. Due to the lack of CT-proAVP measurements and incomplete data on psychometric assessment, another 79 participants were excluded. In the end, the study population analyzed in this work comprised 1463 participants with complete data. All participants received routine physical examination and a detailed echocardiogram for the assessment of systolic and diastolic function, including left ventricular end-diastolic diameter (LVEDD) and ejection fraction (LVEF). The study protocol was approved by all local institutional ethics committees and complied with the Declaration of Helsinki. All patients gave their written informed consent before being included in the study.

2.2. Assessment of anxiety and quality of life

The German version of the Hospital Anxiety and Depression Scale (HADS) was used for the assessment of anxiety (Herrmann-Lingen et al., 2011). This short self-rating questionnaire, initially developed for the assessment of both anxiety and depression in physically ill patients (Zigmond and Snaith, 1983), comprises 14 items on two subscales, with 7 items each for anxiety and depression. The severity of symptoms is rated on scales ranging from 0 to 3 for each item, and the values achieved for each subscale are then added, so that total values from 0 to 21 can be reached per subscale. Several of the items on the anxiety subscale cover the criteria of generalized anxiety disorder, while one item also addresses panic attacks. The HADS has been validated as a measure of clinically relevant anxiety and shows a good reliability with Cronbach's alpha of 0.80 (Herrmann-Lingen et al., 2011). To measure individual dimensions of health-related quality of life, we used the 36-item Short-Form Health Survey (SF-36), which was first evaluated in the Medical Outcomes Study (Ware and Sherbourne, 1992) and has since then emerged as one of the most widely validated generic instruments.

2.3. Laboratory measurements

Venous blood samples were drawn and collected in tubes from the antecubital vein of the study participants after 15 min of rest in a prone position. Heparinized and EDTA samples were immediately centrifuged, and supernatants were subsequently stored at −80 °C, until further use in a specialized laboratory. The biomarker CT-proAVP was measured with a commercially available chemiluminescence sandwich assay (B.R.A.H.M.S. AG, Hennigsdorf, Germany) using antibodies against the two peptides PATV17 and PLAY17 (Morgenthaler et al., 2006, 2008). The CT-proAVP sandwich immunoassay has a functional assay sensitivity of 2.25 pmol/L and a measuring range of 2.25–1215 pmol/L. In a healthy population, the median value of CT-proAVP was reported to be 4.2 pmol/L (range < 2.25–13.8 pmol/L), the 99th percentile was 13.5 pmol/L, and most healthy individuals (97.5%) had concentrations of copeptin detectable by this assay (Morgenthaler et al., 2006). Serum concentrations of N-terminal pro-B-type natriuretic peptide (NT-proBNP) were determined using a non-competitive electrochemiluminescence immunoassay (Elecsys, Roche Diagnostics, Mannheim, Germany) (Karl et al., 1999). The assay incorporated a biotinylated polyclonal NT-proBNP antibody directed against amino acids 1–21 and a secondary polyclonal antibody conjugated to a ruthenium complex, which specifically binds to amino acids 39–50 (Mueller et al., 2003). Mid-regional pro-atrial natriuretic peptide (MR-proANP) was measured using an immunoluminometric assay (SERISTRA from B.R.A.H.M.S AG) based on two polyclonal affinity-purified sheep antibodies directed against different peptide fragments of proANP (Morgenthaler et al., 2004).

2.4. Statistical analysis

Demographic and clinical data of the study population are shown as means and standard deviations or frequencies and percentages. Plasma levels of CT-proAVP and other biomarkers were logarithmically transformed prior to analysis in order to approach normal distribution, which was tested using the Shapiro-Wilk test. In general, laboratory variables were not distributed normally even after log transformation. In view of this lack of normal distribution, we used the Spearman´s correlation for examining the bivariate relationship between CT-proAVP and HADS anxiety. Differences between the CT-proAVP serum concentrations and HADS anxiety in men versus women were tested using the Wilcoxon-Mann-Whitney U test. A fully adjusted univariate ANOVA model with parameter estimation was performed with HADS anxiety as dependent variable including the interaction term of sex*CT-proAVP. This model was adjusted for age, body mass index (BMI),

Table 1
Baseline characteristics of the study cohort.

Group	Study group		Men		Women	
	n	Mean/SD/%/Median	n	Mean/SD/%/Median	n	Mean/SD/%/Median
Age (years)	1463	66.7 ± 8.1	751	66.31 ± 7.9	712	67.3 ± 8.1
Married (%)	1400	67.9	718	77.2	682	58.1
Body mass index (kg/m^2)	1453	29.3 ± 5.0	747	29.1 ± 4.7	706	29.4 ± 5.0
Heart rate (beats/min)	1457	70.0 ± 12.0	747	69.6 ± 12.1	710	70.7 ± 11.9
Systolic blood pressure (mmHg)	1460	149.4 ± 21.1	749	149.8 ± 21.1	711	148.9 ± 21.2
Diastolic blood pressure (mmHg)	1460	84.1 ± 11.9	749	84.9 ± 11.6	711	83.2 ± 12.1
6-min walking distance (m)	1351	509 ± 110	690	530 ± 110	661	488 ± 106
Hypertension (%)	1463	89.1	751	88.5	712	89.6
Hyperlipidemia (%)	1463	44.5	751	44.6	712	44.4
Diabetes mellitus (%)	1463	26.9	751	30.6	712	23.0
Coronary heart disease (%)	1463	20.6	751	27.3	712	13.6
Previous myocardial infarction (%)	1463	10.0	751	14.2	712	5.5
Sleep apnea (%)	1463	7.1	751	10.3	712	3.8
Current smokers (%)	1461	10.9	750	12.0	711	9.7
LVEF (%)	1447	59.8 ± 8.3	742	58.3 ± 8.9	705	61.4 ± 7.2
NYHA class						
0	1448	89.0	742	90.4	706	87.5
I	1448	2.7	742	2.4	706	3.0
II	1448	5.3	742	4.6	706	6.1
III/IV	1448	3.9	742	2.5	706	3.4
ASE grade						
0	1354	16.5	675	16.1	679	16.9
I	1354	60.2	675	57.9	679	62.4
II	1354	22.7	675	25.3	679	20.2
III/IV	1354	0.5	675	0.6	679	0.4
Neurohumoral activation						
CT-proAVP (pmol/L)	1463	4.1[1] (2.6;7.7)[2]	751	5.8[1] (3.5;9.9)[2]	712	3.0[1] (2.0;4.7)[2]
NT-proBNP (pg/mL)	1331	105.7[1] (53.2;221.4)[2]	682	100.6[1] (44.9;249.3)[2]	649	110.9[1] (62.4;206.3)[2]
MR-proANP (pmol/L)	1462	92.2[1] (64.7;136.9)[2]	751	94.1[1] (60.8;142.2)[2]	711	61.1[1] (67.3;131.5)[2]
Psychometric variables						
HADS-A score	1430	5.0 ± 3.7	730	4.7 ± 3.5	700	5.5 ± 3.7
SF-36 Physical functioning score	1437	72.3 ± 24.7	736	76.3 ± 22.5	701	68.0 ± 26.1
Medication						
ACE inhibitor (%)	1439	45.8	746	46.6	693	44.9
AT1 receptor antagonist (%)	1439	17.9	746	18.2	693	17.6
Beta-blocker (%)	1439	50.0	746	49.3	693	50.8
Diuretics (%)	1439	53.9	746	57.2	693	50.4
Statins (%)	1439	30.1	746	30.6	693	30.3
ASS (%)	1439	34.7	746	35.9	693	33.5
Insulin/and or oral antidiabetics	1439	24.2	746	23.0	693	25.2

Results are presented as means and standard deviations, percentages or frequencies, [1]median, [2]25 and 75 quartiles of the biomarkers. ANP = atrial natriuretic peptide, ASE = American Society of Echocardiography, BNP = B-type natriuretic peptide, DD = diastolic dysfunction, HF = heart failure, HADS = Anxiety subscale of the Hospital Anxiety and Depression Scale, NYHA = New York Heart Association, LVEF = left ventricular ejection fraction, SD = standard deviation.

estimated glomerular filtration rate (eGFR), left ventricular ejection fraction (LVEF), 6-min walking distance, SF-36 physical functioning, and the natriuretic peptides NT-proBNP and MR-proANP. All analyses were performed using the software program SPSS version 25 (SPSS Inc., Chicago, IL, USA). A p value of < 0.05 was considered statistically significant.

3. Results

Approximately half of the study population was male (51.3%), and the mean age of the participants was 66.7 ± 8.1 years (Table 1). Due to the inclusion criteria, the majority of all patients (83.4%) were suffering from echocardiographically documented diastolic dysfunction, as judged by an American Society of Echocardiography grade of ≥I. Less than 10% of all participants were suffering from heart failure symptoms with New York Heart Association (NYHA) class II or higher. The most frequent cardiovascular risk factor was arterial hypertension, which was observed in 1303 participants (89.1%). The mean HADS anxiety score in the total study population was 5.0 ± 3.7, and was statistically lower in men (4.7 ± 3.5) than in women (5.5 ± 3.7, $p < 0.001$). The median CT-proAVP concentration was 4.1 pmol/L in the whole study group (interquartile range: 2.6 and 7.7 pmol/L), 5.8 pmol/L in men (interquartile range: 3.5 and 9.9 pmol/L) and 3.0 pmol/L in women (interquartile range: 2.0 and 4.7 pmol/L). The median level of NT-proBNP was 105.7 pg/mL (interquartile range: 53.2 pg/mL and 221.4 pg/mL), and the median MR-proANP was 92.2 pmol/L (interquartile range: 64.7 and 136.9 pmol/L). A detailed characterization of the study population is presented in Table 1.

We found significant, positive correlations between CT-proAVP and NT-proBNP (rho = 0.174, $p < 0.001$) as well as CT-proAVP and MR-proANP (rho = 0.148, $p < 0.001$). Spearman's correlation analysis showed significant negative associations between CT-proAVP and HADS anxiety (rho = −0.074, p = 0.005) (Fig. 1). Similar results were obtained for the correlations between anxiety and either NT-proBNP (rho = −0.072, p = 0.009) or MR-proANP (rho = −0.080, p = 0.002).

In univariate ANOVA adjusted for age, body-mass index, estimated glomerular filtration rate, left ventricular ejection fraction, 6-minute walking distance, SF-36 physical functioning, and the natriuretic peptides NT-proBNP and MR-proANP, parameter estimation showed that the interaction term sex*CT-proAVP was significantly associated with anxiety (p = 0.006). A regression model revealed that CT-proAVP was inversely related to anxiety only in men (B = −0.991; 95%CI = −1.650 to −0.331; p = 0.003, Table 2) but not in women (p = 0.335), suggesting sex-specific differences in the association of this neuropeptide with anxiety.

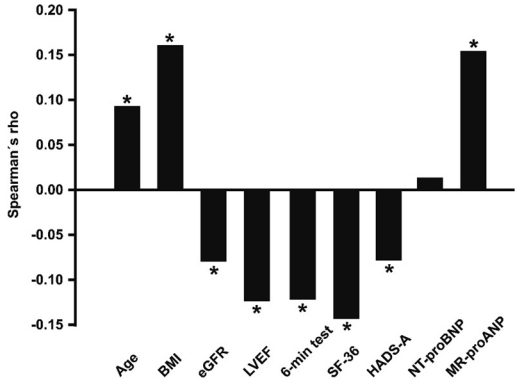

Fig. 1. Spearman's correlation coefficients of CT-proAVP with medical status, self-rated SF-36 (36-Item Short Form Health Survey) physical functioning, and self-rated HADS (Hospital Anxiety and Depression Scale) anxiety subscale. Asterisks indicate statistical significance correlation. Abbreviations: BMI = body mass index, eGFR = estimated glomerular filtration rate, LVEF = left ventricular ejection fraction, 6 min test = 6-min walking test, NT-proBNP = N-terminal pro-B-type natriuretic peptide, MR-proANP = mid-regional pro-atrial natriuretic peptide.

4. Discussion

The main finding of this post-hoc analysis from the Diast-CHF study is that, in a cohort of patients with cardiovascular risk factors, the serum concentrations of CT-proAVP are inversely correlated with self-assessed anxiety. This overall effect differs by sex and can be observed in men only but not in women. These data demonstrate that higher levels of circulating CT-proAVP are associated with less anxiety in men. This may suggest that this neuropeptide either modulates neural pathways involved in the regulation of anxiety or that higher anxiety states lead to suppression of AVP secretion. A third possibility would be that both, AVP secretion and anxiety depend on a third factor. As far as we were able to control for potential confounders such as age and cardiac disease severity, the observed relationship remained stable, suggesting that it was at least independent of those variables. In adjusted analyses, the two natriuretic peptides NT-proBNP and MR-proANP which have been reported previously to show inverse associations with anxiety (Meyer et al., 2015b; Fangauf et al., 2018) did not exhibit an additional predictive effect. Thus, this secondary analysis of the Diast-CHF study demonstrated that there was an inverse and significant association between CT-proAVP and anxiety modulated by gender. The generally lower serum CT-proAVP concentrations and higher anxiety levels in women as compared to men may account for these gender-specific effects. Due to their low circulating CT-proAVP levels and their smaller variance, putative anxiolytic properties of AVP as determined by its proxy CT-proAVP may not be detectable in our female study participants. However, the fact that anxiety was higher in women may at least in part be due to their lower CT-proAVP levels. Conversely, their lower CT-proAVP levels might be due to their anxiety. Both of these possible explanations would be in line with our primary finding of an inverse relationship and in clear contrast to the initial hypothesis. Accordingly, the higher serum CT-proAVP concentrations in male participants may have contributed to their lower overall anxiety (or vice versa). The larger variance of CT-proAVP found in men may have facilitated the detection of the inverse association within the male subgroup.

Our observation supports previous animal and psychological studies suggesting that the AVP-mediated signal transduction plays a role in the neurobiological mechanisms involved in the regulation of anxiety. While blood pressure-elevating catecholamines and angiotensin both induce enhanced stress responses and anxiety disorders (Arlt et al., 2003; Ströhle and Holsboer, 2003), much less is known about the actions of peptidergic neuro-modulators and circulating peptide hormones in protecting the heart from adverse effects of psychosocial stressors. In previous research using the same DIAST-CHF study cohort, we found inverse associations between anxiety and serum NT-proANP, NT-proBNP, MR-proANP, and MR-pro-adrenomedullin levels (Herrmann-Lingen et al., 2003; Meyer et al., 2015a,b; Meyer et al., 2015c). These data are in line with previous studies suggesting that ANP may function as a determinant in the neurobiology of anxiety disorders, possibly through counter-regulating HPA axis activity (Wiedemann et al., 2000; Ströhle et al., 2001; Wiedemann et al., 2001; Ströhle et al., 2006; Hodes and Lichtstein, 2014; Koopmann et al., 2014). Besides its modulating effects on fluid and electrolyte homeostasis, ANP is directly involved in stress-hormone responses by inhibiting the release of corticotropin-releasing hormone (CRH) and corticotropin (ACTH) (Fink et al., 1991; Antoni et al., 1992; Bhattacharya et al., 1996). The anxiolytic effect of ANP may shield the heart from the pro-arrhythmogenic potential of endogenously released hormones usually associated with elevated anxiety states and panic attacks.

A similar anxiolytic-like effect was previously proposed also for endothelin-1, a vasoconstrictive, 21-amino acid neuropeptide whose serum carboxy-terminal precursor fragment (CT-proET-1) concentration was associated with less anxiety, as shown from the Diast-CHF study (Meyer et al., 2015a). In line, decreasing the expression of endothelin-1 in the basolateral amygdala by lentiviral transduction enhanced anxiety-like behaviors in a mouse model, whereas the overexpression of endothelin-1 B-type receptors had an anxiolytic effect (Chen et al., 2017). Glutamatergic neurons in the basolateral amygdala

Table 2
Results from a regression model adjusted for the indicated confounders showing an association between anxiety and serum CT-proAVP only in male, but not female study participants.

	B (unstandardized)	95% confidence interval		T	p value
Dependent variable HADS anxiety (total model p < 0.001, adjusted R^2 = 0.103, F = 12.371)					
Age	−0.058	−0.093	−0.023	−3.222	0.001
BMI	−0.030	−0.086	0.026	−1.036	0.300
eGFR	−0.001	−0.013	0.011	−0.148	0.882
LVEF	0.014	−0.013	0.042	1.046	0.296
6-min walking distance	0.002	< 0.001	0.005	1.891	0.059
SF-36 PF	−0.051	−0.062	−0.040	−9.076	< 0.001
CT-proAVP (male)	−0.991	−1.650	−0.331	−2.948	0.003
CT-proAVP (female)	−0.443	−1.346	0.459	−0.964	0.335
NT-proBNP	−0.222	−0.966	0.539	−0.556	0.578
MR-proANP	−0.752	−2.273	0.755	−0.984	0.336

Abbreviations: BMI; body mass index, CT-proAVP; C-terminal pro-arginine vasopressin, eGRF; estimated glomerular filtration rate, HADS; Hospital Anxiety and Depression Scale, LVEF; left ventricular ejection fraction, MR-proANP; mid-regional pro-atrial natriuretic peptide, NT-proBNP; N-terminal pro-B-type natriuretic peptide, SF-36 PF; 36-Item Short Form Health Survey physical functioning.

express endogenous endothelin receptors and endothelin-1 modulates the excitability of pyramidal neurons in the amygdala, as demonstrated in whole-cell current-clamp recording in endothelin-1 shRNA-treated mice (Chen et al., 2017).

In summary, these observations suggest that a cocktail of neuropeptides typically elevated in heart disease, including ANP, endothelin-1 and adrenomedullin, attenuate anxiety-related responses, and may thus shield the heart from the adverse cardiac effects of psychosocial stressors. The occurrence of pro-arrhythmogenic episodes typically linked to anxiety-induced elevated catecholamine levels or vagal withdrawal may be blunted by elevated serum levels of neuropeptides with overall anxiolytic properties. The observations from the present study suggest that CT-proAVP may also fall into the category of peptidic neurohormones which modulate anxiety. However, the cross-sectional studies so far do not prove causality and it might also be possible that in anxiety states the secretion of the neuropeptides mentioned above is suppressed or both depend on a third factor.

Recently, Motoki et al. (2016) demonstrated increased plasma AVP levels in men as compared to women and suggested an effect of this neuropeptide on emotional processing and amygdala activation. In our analysis, the median of CT-proAVP was also significantly higher in men than in women. However, the sex-specific effects of AVP on amygdala functioning are unclear and require further investigation (Neumann and Landgraf, 2012; Motoki et al., 2016). Taylor et al. (2010) showed that AVP was elevated in men experiencing distress in the pair-bond relationship, but not in women. Furthermore, elevated plasma oxytocin was associated with distress in the pair-bond relationship for women, but not for men. AVP might appear to be the male counterpart of oxytocin, and is similar in molecular structure to oxytocin but possibly regulated by testosterone. There is a hypothesis that AVP regulates similar relationship functions in men as oxytocin serves in women (Taylor et al., 2000, 2010). Walum et al. (2008) suggested that genetic variation in the AVP receptor 1a gene (AVPR1A) is related to pair-bonding behavior in men. Thompson et al. (2004) indicated the possibility of sexually dimorphic effects of vasopressin and suggested that AVP may influence aggression in human males by biasing individuals to respond to emotionally ambiguous social stimuli.

Bamshad et al. (1993) reported on sex-specific differences in the vasopressin innervation of prairie voles (*Microtus ochrogaster*) and meadow voles (*Microtus pennsylvanicus*) with higher numbers of AVP-immunoreactive fibers in the lateral habenular nucleus and the lateral septum in males as compared to females regardless of the parental state. Sexually dimorphic patterns of AVP expression were also demonstrated in the rat brain (DiBenedictis et al., 2017). Moreover, testosterone removal by castration reduced the expression of AVP in the bed nucleus of the stria terminalis, which could be prevented by pharmacological treatment with testosterone (Auger et al., 2011). The altered AVP mRNA expression in the adult rat brain was associated with changes in the DNA methylation status of CpG sites on the AVP promoter.

The present work has some methodological limitations, which are mainly derived from the descriptive nature and the exploratory post-hoc design of the analysis. Due to the cross-sectional design of the study, no causal inferences can be made. In addition, the observed effects are small and not immediately clinically relevant. They do, however, add to the growing evidence demonstrating that multiple neural and humoral regulators typically elevated in heart diseases are involved in the pathophysiology of anxiety and may be differentially related to objective and subjective health outcomes. Since only a minority of patients from our sample had manifest heart failure, it remains to be tested whether the results of this study can be generalized to healthy subjects or patients with more severe heart failure.

In conclusion, in this secondary analysis of the observational Diast-CHF study, plasma levels of CT-proAVP were inversely related to anxiety in men and this association remained significant after adjustment for clinical confounders of the severity of physical impairment. Further research is needed to replicate this association and to decipher the physiological processes behind our observation.

Conflict of interest

CHL reports that he is receiving royalties from Hogrefe Huber Publishers for the German version of the HADS. During the last three years he has received lecture honoraria from Servier, Heel, and Novartis and an honorarium from Pfizer for serving on an advisory board. All other authors report no conflicts of interest.

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