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Copeptin in CCK-4-induced panic in healthy man: Sexual dimorphisms in secretion pattern and panic response, but no correlation of copeptin with panic symptoms

Michael Kellner[a,b,*], Peter Zwanzger[c,d], Rainer Rupprecht[e], Daniela Eser[d], Alexander Yassouridis[f], Klaus Wiedemann[a]

[a] University Medical Center Hamburg-Eppendorf, Dept. of Psychiatry and Psychotherapy, Hamburg, Germany
[b] Herford Hospital, Dept. of Psychiatry, Psychotherapy & Psychosomatics, Herford, Germany
[c] kbo-Inn-Salzach-Hospital, Wasserburg am Inn, Germany
[d] Ludwig Maximilian University, Dept. of Psychiatry and Psychotherapy, Munich, Germany
[e] University of Regensburg, Dept. of Psychiatry and Psychotherapy, Regensburg, Germany
[f] Max-Planck-Institute of Psychiatry, Munich, Germany

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ABSTRACT

Copeptin, the C-terminal part of the hypothalamic arginine vaspopressin (AVP) precursor, closely mirrors the production of AVP and was proposed as an easily measured novel marker of the individual stress level in man. First data in male volunteers proposed copeptin as a potential endocrine surrogate marker of cholecystokinin-tetrapeptide (CCK-4)-induced panic. We tried to replicate these pilot data and to extend them to the other sex.

46 healthy human subjects (29 men, 17 women) were given an intravenous bolus of 50 µg CCK-4. Basal and stimulated plasma copeptin was measured and panic symptoms were assessed using the Acute Panic Inventory (API).

Basal copeptin was significantly lower in women vs. men, while men showed a significantly higher CCK-4-induced increase of copeptin. In contrast, female subjects displayed a signifcantly higher increase of API ratings by CCK-4. No significant correlations of panic symptoms and copeptin release induced by CCK-4 could be found, neither in man, nor in women, nor in the total sample.

A sexual dimorphism in copeptin secretion and in panic response was demonstrated. Prior unexpected findings of copeptin release as an objective read-out of panic could not be replicated. The role of the vasopressinergic system in panic anxiety needs further study in panic patients and in healthy man, using also other panic provocation paradigms.

1. Introduction

Experimental induction of panic symptoms in healthy man and in panic patients by panicogenic agents offers the unique opportunity to investigate pathophysiological and psychopharmacologial aspects of panic under controlled conditions (Wiedemann et al., 2001; Kellner et al., 2009; Kellner, 2011). Cholecystokinin-tetrapeptide (CCK-4) is a safe and reliable panicogen for human use; while intravenous administration of 25 µg provokes a full blown panic attacks in 91% of panic patients and in only 17% of healthy controls, an increased dose of 50 µg elicits a panic attack in 47% of healthy subjects and in the rest of them substantial subthreshold panic symptoms are induced (Bradwejn et al., 1991a, b). Subjective panic responses to CCK-4 are paralleled by neuroendocrine changes, such as the secretion of the stress hormones cortisol and adrenocorticotropic hormone (ACTH). However, these parameters are not valuable as an objective „read out “of provoked panic symptoms in healthy young man, because there was no significant correlation between panic symptom intensity and neuroendocrine responses to CCK-4 (Flint et al., 2000; Eser et al., 2007).

Recently, Demiralay et al. (2017) published exciting preliminary data suggesting a role of the stress hormone copeptin as a potential surrogate marker of CCK-4-induced panic symptoms. Copeptin is the C-terminal part of the hypothalamic arginine vasopressin (AVP) precursor (CT-proAVP); it closely mirrors the production of AVP (which is a co-factor of corticotropin-releasing hormone in the activation of the pituitary-adrenocortical axis), it was proposed as a novel marker of the

* Corresponding author at: Herford Hospital, Dept. of Psychiatry, Psychotherapy & Psychosomatics, Schwarzenmoorstrasse 70, D-32049, Herford, Germany.
E-mail address: kellner@uke.uni-hamburg.de (M. Kellner).

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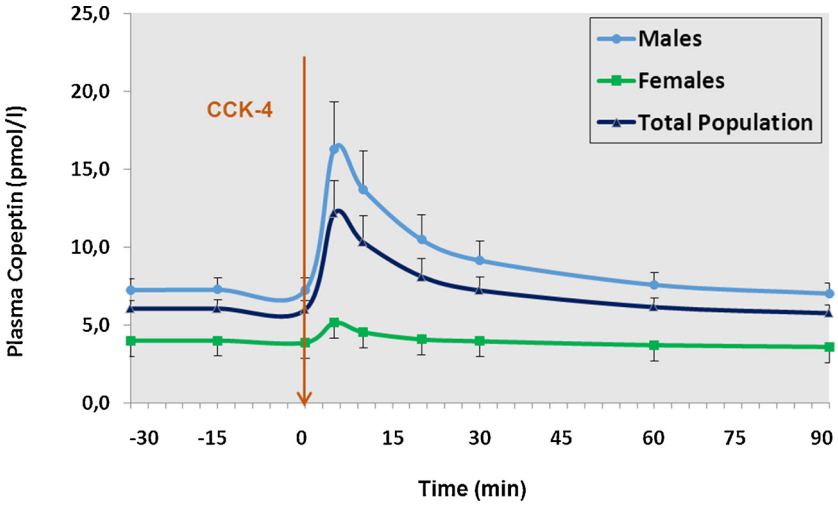

Fig. 1. Time concentration courses of plasma copeptin during a 50 µg CCK-4 challenge in 29 men, 17 women and in the total sample (mean ± SEM).

individual stress level in man and can be easily measured in plasma (Katan et al., 2008). In constrast, the reliable measurement of AVP is difficult because it is subject to preanalytical and analytical errors due to its short half-life time, its strong binding to platelets and its small size, which challenges detection by sandwich immunoassays (Dobsa and Edozien, 2013). In 29 male healthy subjects, Demiralay et al. (2017) demonstrated a rapid increase of plasma copeptin after 50 µg of CCK-4 and unexpectedly a significant correlation of CCK-4-induced panic symptoms (as measured by the Acute Panic Inventory (API)) and the increase of copeptin (area under the curve minus linear backgroud) emerged. We intended to replicate these findings in another sample of male healthy human volunteers undergoing a CCK-4 challenge. In addition, we studied a female sample in order to extend these findings to the other sex (representing the majority of panic patients) and to investigate gender effects of CCK-4-induced copeptin release and of provocation of panic symptoms.

2. Materials and methods

46 healthy human subjects of Caucasian origin (mean age 25.2 ± 0.7 yrs. (age range 19–42 yrs.)) were studied, among them 29 men (mean age 25.7 ± 0.8 yrs. (range 20–42 yrs.) and 17 women (mean age 24.2 ± 1.2 yrs. (range 19–35 yrs.)). This sample originated from three prior investigations on the effects of alprazolam (Zwanzger et al., 2003a), tiagabine (Zwanzger et al., 2003b) or repetitive transcranial magnetic stimulation (rTMS) (Zwanzger et al., 2007) on CCK-4-induced panic, from which remaining plasma specimens and respective psychometric ratings were generously provided, because currently worldwide CCK-4 is not available for human use. In detail, n = 27 evaluable subjects (with enough plasma volume left to perform reliable copeptin measurement at all time points) came from the initial CCK-4 challenge before alprazolam administration, n = 14 stemmed from the initial CCK-4 challenge before tiagabine and n = 5 arised from the CCK-4 challenge of the placebo condition of the rTMS study. Subjects with a history of mental or somatic disease had been excluded as decribed in detail in the papers cited above; none reported a history of mental illness in first-degree relatives or a history of spontaneous panic attacks. The protocols were approved by the local ethical review board. All subjects gave their written informed consent after the procedure had fully been explained.

50 µg CCK-4 (Clinalfa, Läufelfingen, Switzerland) dissolved in 2.5 ml NaCl 0.9% was given at 10:00 am as a bolus intravenous injection. Panic symptoms were assessed using the Acute Panic Inventory (API) (Dillon et al., 1987); ratings were performed one minute before CCK-4 („pre“) and five minutes after the challenge („post“, asking for the maximal symptom response after the injection). An intravenous catheter was inserted into a forearm vein 90 min prior to the challenge. Subjects were studied in a quiet, soundproof room in supine position. Blood samples were taken at −30, −15 and −1 min before the injection and 5, 10, 20, 30, 60 and 90 min after CCK-4 into prechilled tubes containing EDTA. They were placed on ice, plasma was immediatedly separated and stored permanently at −85° C until analysis.

Copeptin was measured using an immunofluorescent assay (KRYPTOR System, BRAHMS, Henningsdorf, Germany) with an analytical detection limit of 0.9 pmol/l and a measuring range from 0.9 to 500 pmol/l. Intra- and interassay coefficients of variation were below 7% in our hands. All specimens were analysed within one batch.

For copeptin the following curve indicators were calculated: basal value (mean of the concentrations at −30, −15 and −1 min) and for the CCK-4-stimulated hormone secretion (in order to avoid carry-over effects of potential sex differences in basal levels) delta copeptin (maximal increase from basal value after CCK-4) and AUCnet copeptin (area under the curve from 5 to 90 min minus linear background, i. e. basal value). Panic symptoms were expressed as delta API (increase by the CCK-4 challenge, i. e. post- minus pre-ratings). For testing sex effects (men vs. women) analyses of covariance with sex as independent variable, the aformentioned indicators as dependent variables and age as covariate were performed. To approximate variance homogeneity the dependent variables had been ln-transformed before analysis. Alpha < 0.05 was set as the nominal level of significance. For post-hoc tests Bonferroni correction was applied. Correlations were determined and tested for significance by Spearman's correlation coefficients. All results are expressed by means ± SEM.

3. Results

3.1. Copeptin

The time concentration courses of copeptin for the male, the female and the total sample are depicted in Fig. 1. Basal copeptin was 7.2 ± 0.8 pmol/l in men and 4.0 ± 0.4 pmol/l in women. Analysis of variance with sex as influential factor and age as covariate demonstrated a significant sex difference in basal copeptin ($F(1,44) = 14.86$, $p < 0.0001$); age did not show a significant effect ($F(1,44) = 2.03$, $p = 0.161$).

Delta copeptin after CCK-4 was 9.1 ± 2.9 pmol/l in males and 1.3 ± 0.8 pmol/l in females, respective AUCnet copeptin was 36.0 ± 14.8 versus 3.0 ± 3.6. Multivariate analysis of variance with sex as influential factor, the copeptin indicators delta and AUCnet as dependent variables and age as covariate yielded a significant sex effect

Table 1
Acute Panic Inventory (API) before and after a 50 µg CCK-4 challenge (mean ± SEM).

	API pre-CCK-4	API post-CCK-4	delta API
Men (n = 29)	1.7 ± 0.4	16.0 ± 1.5	**14.2 ± 1.4***
Women (n = 17)	2.0 ± 0.5	20.5 ± 2.1	**18.5 ± 2.1***
Total sample (n = 46)	1.8 ± 0.3	17.7 ± 1.2	15.8 ± 1.2

* denotes a significant sex effect of API increase (please see text).

($F(2,43) = 6.05$, $p = 0.005$, effect size = 0.22, power = 86%). Post-hoc univariate F-tests showed a significant sex effect on delta copeptin ($F(1,44) = 6.52$, $p = 0.014$). For AUCnet copeptin no significant respective differences emerged ($F(1,44) = 3.70$, $p = 0.061$). The covariate age did not have a significant influence ($F(2,43) = 0.65$, $p = 0.936$).

In order to investigate whether CCK-4-induced copeptin secretion had been influenced by the three subsamples of our study population, we performed an additional analysis of covariance in which (beside age) also an index variable (sample index) characterizing the subsamples has been used as covariate. The obtained results are very similar to those stated above (global sex effect: $F(2,42) = 4.80$, $p = 0.013$, effect size = 0.18, power = 77%; simple sex effect on delta copeptin: $F(1,43) = 5.07$, $p = 0.029$; simple sex effect on AUCnet copeptin: $F(1,43) = 2.83$, $p = 0.099$). The covariates age and sample index did not reveal a significant influence ($F(4,84) = 0.13$, $p = 0.969$).

3.2. Acute panic inventory (API)

Table 1 shows mean values of pre-CCK-4, post-CCK-4 and delta API. Analysis of variance for delta API with age as covariate demonstrated a significant sex effect ($F(1,44) = 4.86$, $p = 0.033$). The covariate age did not show a significant effect ($F(1,44) = 0.88$, $p = 0.354$).

3.3. Correlations of copeptin and API

For the male sample correlation of AUCnet copeptin and delta API was $r = 0.24$ ($p = 0.20$). In the female sample the respective correlation was $r = -0.32$ ($p = 0.21$). Also in the total sample AUCnet copeptin and delta API did not show a significant correlation ($r = -0.03$, $p = 0.83$). Regarding delta copeptin and delta API, no significant correlations emerged (men: $r = 0.29$, $p = 0.13$, women: $r = 0.06$, $p = 0.83$, total sample: $r = -0.00$, $p = 0.98$).

4. Discussion

A sexual dimorphism of copeptin secretion was detected with significantly higher basal plasma levels and a significantly more pronounced increase after CCK-4 in men versus women. In contrast, a significantly higher increase of CCK-4-induced panic symptoms was shown in women versus men. A prior report in men demonstrating a significant correlation of provoked panic symptoms and increase of plasma copeptin could not be replicated although subjects 'age and many other methodological aspects (such as experimental surroundings, dose of CCK-4, panic questionnaire) were comparable. Furthermore, neither in women nor in the total sample such a correlation could be detected. However, CCK-4 had been injected five hours later in the day, i. e. at 15:00, in the study that we tried to replicate. Though, circadian studies did not show a consistent circadian rhythm of copeptin and suggested that copeptin levels can be determined irrespectively of the time of day (Darzy et al., 2010; Beglinger et al., 2017). Admittedly, this does not inform us about potential circadian differences concerning stimulated copeptin and may be a reason for non-replication of our previous findings.

Plasma copeptin concentrations before and after CCK-4 in our present male sample were in the same magnitude as in the previous initial study (Demiralay et al., 2017). Increases of vasopressin in plasma had been shown in control women and patients with premenstrual dysphoric disorder in response to CCK-4 (Le Mellédo et al., 2001) and in a mixed sex sample of healthy adults using another CCK-B receptor agonist, pentagastrin (Abelson et al., 2001). While Le Mellédo et al. (2001) did not report correlations of the behavioral and endocrine response to CCK-4, Abelson et al. (2001) observed that the vasopressin response to pentagastrin was significantly positively related to panic symptom intensity response (as per API ratings). However, they did not find differences between males and females in AVP levels.

Spanakis et al. (2016) using a psychosocial stressor (the Trier Social Stress Test) in healthy volunteers also showed significantly higher baseline copeptin levels in males compared to females, but the percent of increase in copeptin from baseline to peak levels was undistinguishable. Using an MDMA (ecstasy) challenge in healthy subjects, Simmler et al. (2011) also demonstrated significantly lower baseline copeptin levels in women than men, but only in women MDMA significantly elevated plasma copeptin. Kacheva et al. (2015) studying patients undergoing insulin tolerance testing for suspected pituitary dysfunction as well reported gender-specific differences of copeptin secretion with baseline levels significantly higher in men; stimulated copeptin levels were overall higher in males than in females. Already in healthy newborn human infants significantly increased basal and stressed plasma copeptin in boys compared with girls is present and further studies in healthy adult men show 1.2–1.8-fold higher baseline copeptin levels compared to women (Burckhardt et al., 2014). The influence of gonadal steroids in the mechanism of the sexual dimorphism in the secretion of vasopressin has been demonstrated (Share et al., 1988), but the physiological importance of these robust vasopressin and copeptin differences remains obscure. Sex-specific influences of vasopressin on human social communication have been reported (Thompson et al., 2006) and need further study in the context of panic anxiety.

Because of the influence of the menstrual cycle and of premenstrual syndrome (PMS) on the anxiety and panic response to CCK-4 (Le Mellédo et al., 1999), many studies in healthy volunteers had been performed in exclusively male samples. In the four published studies that included healthy men and women and statistically considered potential sex differences, no significant effects of gender in response to 50 µg CCK-4 were detected using the DSM-III-R/IV-derived Panic Symptom Scale (PSS) (Bradwejn et al., 1994; Shlik et al., 1997; Tõru et al., 2010, 2013). No information is available if the API is more sensitive than the PSS to detect sex differences of CCK-4-induced panic symptoms and further research is needed. In spontaneous panic attacks gender-based differences in occurring symptoms were reported (Sheikh et al., 2002). Future work should investigate how emotion regulation strategies, aversive life experiences, genetic predisposition, social learning contingencies, etc. influence sex differences in the experience and expression of experimentally induced panic (Kelly et al., 2006).

Additional limitations of our study include the missing information about the stages of menstrual cycle, of PMS symptoms and the intake of contraceptives in our female subjects. However, basal copeptin levels remain unchanged during the menstrual cycle (Blum et al., 2014). Our specimens had been safely stored deep-frozen for a long time, we did not measure osmolality, and our sample size was relatively small. Furthermore, the retrospective use of participants from three different studies with many methodological similarities but distinct aims is another limitation. Studies in clinical and non-clinical populations using also other panic provocation paradigms (e. g. sodium lactate, carbon dioxide, yohimbine, caffeine, etc.) are needed to further characterize the role of the vasopressinergic system in panic anxiety.

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Contributors

MK and KW conceptualized the study and wrote the manuscript, PZ, RR and DE supervised the data collection and reviewed the manuscript, AY perfomed the statistical analyses.

Declaration of Competing Interest

None.

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