Copeptin, a stable peptide derived from the vasopressin precursor, correlates with the individual stress level

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Abstract

BACKGROUND: During stress, vasopressin is a potent synergistic factor of CRH as a hypothalamic stimulator of the HPA axis. The measurements of CRH and vasopressin levels are cumbersome because of their instability and short half-life. Copeptin is a more stable peptide stoichiometrically released from the same precursor molecule. The aim of our study was to compare copeptin and cortisol levels in different stress situations.

METHODS: Three groups of patients with increasing stress levels were investigated: a) healthy controls without apparent stress (n=20), b) hospitalized medical patients with moderate stress (n=25) and c) surgical patients 30 minutes after extubation, with maximal stress (n=29). In all patients we assessed cortisol and copeptin levels. Copeptin levels were measured with a new sandwich immunoassay.

RESULTS: Cortisol levels in controls were (median, IQ range, 486 [397–588] nmol/L), not significantly different as compared to medical patients (438 [371–612] nmol/L, p=0.69). Cortisol levels in surgical patients after extubation were higher (744 [645–1062] nmol/L p<0.01 vs controls and medical patients). Copeptin levels in controls were 4.3 [3.2–5.5] pmol/L, which was lower as compared to medical patients (17.5 [6.4–24.1], pmol/L, p<0.001) and surgical patients after extubation (67.5 [37.8–110.0] pmol/L, p<0.001). The correlation between copeptin levels and cortisol was r=0.46, p<0.001.

CONCLUSION: Copeptin is a novel marker of the individual stress level. It more subtly mirrors different stress levels as compared to cortisol values.

ORIGINAL ARTICLE

INTRODUCTION

The two major peripheral limbs of the stress system are the hypothalamic-pituitary-adrenal (HPA) axis and the sympathetic nervous system [1,2]. Corticotropin- releasing hormone (CRH) and arginin vasopressin (AVP) also termed antidiuretic hormone (ADH), are the main secretagogues of the HPA-axis to produce ACTH and cortisol, respectively. Importantly, serum cortisol levels have been reported to be proportionate to the degree of stress, if the HPA axis is intact [3,4]. For a direct assessment of the individual stress level at a cerebral level, the measurement of CRH or AVP might offer an alternative. Unfortunately, the measurement of circulating AVP levels and CRH is challenging. Both, CRH and AVP are released in a pulsatile pattern, are unstable and rapidly cleared from plasma within minutes. AVP derives from a larger precursor peptide (pre-provasopressin) along with two other peptides, neurophysin II and copeptin. Copeptin is released in an equimolar ratio to AVP and is more stable in the circulation and easy to determine. Copeptin levels were found to closely mirror the production of AVP [5].

Large surgery can serve as a standardized physiological model for studying major stress [4]. Peri- and postoperative basal cortisol levels reflect the degree of surgical stress [6,7]. Peak cortisol levels are achieved in the immediate postoperative period, around the time of extubation [3,8]. The assessment and interpretation of cortisol levels is dependent on an intact anterior pituitary and adrenal gland, respectively.

This study compares copeptin levels with the cortisol response in order to identify subtle marker of the individual stress level at a hypothalamic level.

METHODS

Setting and Study population

The details of the study population with different levels of physical stress have been previously described [4,9,10]. Briefly we obtained ethical approval and the patients or their legal representatives gave written informed consent to participate in the study. The study population was divided in to three groups; Group A (no stress) contained 20 healthy control subjects, without apparent stress. Group B (moderate stress) consisted of 25 patients consecutively recruited from a wide and representative variety of patients having been hospitalized on the medical ward. They were on the medical ward for various reasons (i.e. acute diabetic complications [hyperglycemic crisis, hypoglycemia], infections [urosepsis, exacerbation of chronic obstructive lung disease, pneumonia], inflammations [acute pancreatitis or myocarditis], hypoventilation syndrome, myocardial infarction, congestive heart failure, unstable angina pectoris, cerebrovascular insult, decompensated liver cirrhosis, and ocular neuropathy). None of the patients showed clinical or laboratory features of acute or chronic adrenal insufficiency. In Group C, 29 stable surgical patients were consecutively recruited from the division of cardiothoracic surgery undergoing elective coronary bypass grafting under general anesthesia. These patients served as a standardized model for maximal stress, which is generally experienced after extubation [3,8]. For premedication midazolam 7.5mg was used in all patients. Anesthesia was induced with thiopentone (2–4mg/kg) and fentanyl (2–4ug/kg). Etomidate was not used for any patient. Intubation was facilitated with pancuronium (0.15 mg/kg). Before and during cardiopulmonary bypass anesthesia was maintained with isoflurane (0.5–1.5 MAC) and fentanyl. Antibiotic prophylaxis consisted of cefuroxime (1,5g, t.i.d.) for 48h. Surgery was done under normothermic conditions (i.e. resulting in cooling not lower than 35° Celsius). Cardiopulmonary bypass was started after heparin (350 IU/kg) and cylocapron 30 mg/kg. No blood transfusions were required. None of the patient's received corticosteroid treatment.

Exclusion criteria were as follows: patients who received drugs that influence the hypothalamo-pituitary adrenal axis in the last 3 months [11]; patients with diseases affecting the adrenal or the pituitary gland; patients with known or suspected primary or secondary adrenal insufficiency; and patients receiving etomidate.

In Group A and Group B, patients were tested once between 6–9 a.m. Surgical patients in Group C were evaluated at three different time points - the morning (6-9 a.m.) before operation (no apparent stress) and 30 minutes after extubation (with maximal stress) as well as on the day after the operation.

Adrenal function in each participant was assessed by either low or standard ACTH tests performed between 0600–0900 h. Thereby, in all subjects, blood samples were taken at 0 min for the basal measurement of cortisol and ACTH and again at 30 and 60 min for the measurement of serum cortisol concentration after iv administration of 1 or 250 μg Synacthen (synthetic ACTH1–24), respectively.

Assay

We measured Copeptin serum levels with a new sandwich immunoassay as recently described in detail [12]. Briefly, this sandwich immunoluminometric assay works with two polyclonal antibodies which bind to the C-terminal region of pre-pro-AVP. One antibody is attached to polystyrene tubes and the other is labeled with acridinium ester for chemiluminescence detection. For the assay 50 μl of either serum or plasma are required, no extraction steps or other pre-analytical procedures like addition of protease inhibitors are necessary. The analytical detection limit is 1.7 pmol/L and the total precision of the assay (inter laboratory CV) was < 20% for copeptin concentrations across the calibration curve (up to 405 pmol/L). In 359 healthy individuals Copeptin plasma concentration had a median of 4.2 pmol/L (range, 1–13.8 pmol/L). The 97.5th percen-

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Table 1: Characteristics of the study group (Group A-C)

	Healthy controls (Group A, n=20)	Medical patients (Group B, n=25)	Surgical patients undergoing CABG (Group C, n=29)	
			preoperative	postoperative
Age (yr)	51(35–57)	64(56–79)	67(59–75)	
Sex (m/f)	10/10	15/10	28/1	
BMI (kg/m2)	23(21–24)	29.7(25–31)	27(24–28)	
Mean blood pressure (mmHg)		89(86–97)	90(83–93)	85(77–90)
Sodium (mmol/l)		141(138–144)	141(139–143)	138(136–138)
Potassium (mmol/l)		4.0(3.6–4.3)	4.0(3.7–4.3)	4.3(4.2–4.7)
Glucose (mmol/l)		5.8(5.3–7.8)	5.9(5.3–7.7)	7.8(7–9.1)

Demographic and laboratory characteristics of healthy controls (group A), medical patients (group B), and surgical patients undergoing coronary artery bypass grafting (CABG) (group C) before the operation and after surgery. To convert glucose from mmol/liter into mg/dl, multiply by 18. m, male; f, female. BMI denotes body mass index.

tile was 11.25 pmol/L, and the 2.5th percentile was 1.7 pmol/L. Of all 359 tested volunteers, only nine (2.5%) had a plasma copeptin concentration below the detection limit of the assay of 1.7 pmol/L. It is important to note that in contrast to mature AVP, copeptin is more stable in plasma or serum ex vivo. Ex vivo stability of copeptin (< 20% loss of recovery) was shown for serum and plasma for at least seven days at room temperature and 14 days at 4° C[12].

Statistical Analysis

We expressed discrete variables as counts (percentage) and continuous variables as means ± standard deviation (SD) or median [interquartile range] unless stated otherwise. Two-group comparison of normally distributed data was performed by Students t-test. For multigroup comparisons, one-way analysis of variance with least square difference for posthoc comparison was applied. For data not normally distributed, we performed the Mann-Whitney-U test. The Kruskal-Wallis one-way analysis of variance was used if more than two groups were being compared. Correlation analyses were performed by using Spearman rank correlation. All testing was two-tailed and P values less than 0.05 were considered to indicate statistical significance.

RESULTS

Demographic data

Table 1 shows demographic and other characteristics of the subjects in Group A-C.

We investigated 20 healthy controls, 25 medical patients and 29 patients undergoing surgical treatment. Twenty-one subjects (28.4%) were female. In the surgical group, all except one patient were male. There was no significant difference between the three groups concerning age, body mass index, blood pressure, and electrolytes.

Cortisol levels in different stress situations

Cortisol levels in controls were 486 [397–588] nmol/L not significantly different as compared to medical patients (basal cortisol 438 [371–612], p=0.69). Cortisol levels in surgical patients 30 minutes after extubation were significantly higher (744 [645–1062], p<0.01) as compared to controls and medical patients **(Figure 1A)**.

Copeptin levels in different stress situations

Copeptin levels in controls were 4.3 [3.2–5.5] pmol/L, which was significantly lower as compared to medical patients (17.5 [6.4–24.1], p<0.001) and surgical patients after extubation (67.5 [37.8–110.0] pmol/L, p<0.001) **(Figure 1B)**.

Percentage increase and decrease of cortisol and copeptin levels in different stress situations in surgical patients (Group C)

The percentage increase in cortisol from baseline to the extubation period (i.e. major stress) was 265 ± 34%, and the respective increase for copeptin was significantly higher with 1430 ± 157% (p<0.001) **(Figure 2).**

Cortisol levels the day after the operation decreased to 82.3 ± 46.2% compared to the immediate extubation period, defined as maximal stress (p=0.01), whereas copeptin levels decreased to 65.5% ± 34.1 (p<0.001). The decrease for copeptin levels tended to be more pronounced as compared to the decrease in cortisol levels (p=0.09).

Copeptin levels and gender

In control subjects, copeptin levels were significantly lower in females (4.56± 2.36 pmol/L) compared to copeptin levels in males (6.16 ± 2.30; p=0.025) .In the group of medical patients copeptin levels were not significantly different between men and women.

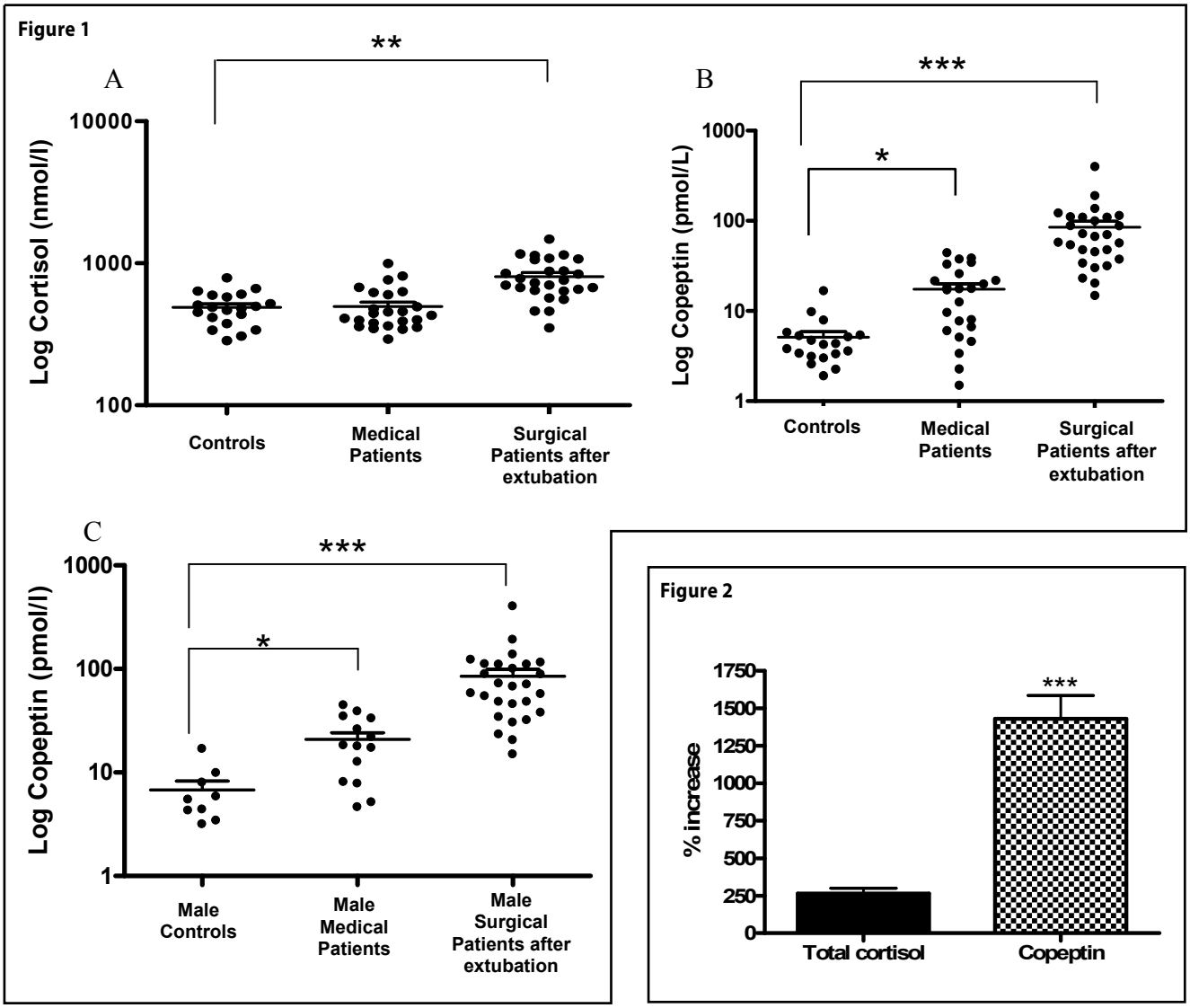

Figure 1. Total cortisol (A) and copeptin (B) levels in controls, medical patients and surgical patients after extubation, mirroring three different levels of physical stress. Copeptin levels (C) in the male controls, male medical patients and male surgical patients. Data show means ± SEM with scatter plots representing the absolute values. * denotes p<0.05; ** denotes p<0.01; *** denotes p<0.001

Figure 2. (A)Percentage increase of total cortisol and copeptin levels from before operation (no apparent stress) to after extubation (major stress). (B) Percentage decrease of total cortisol and copeptin levels from after extubation (major stress) to the day after operation.
Data present mean ± SEM. * denotes p<0.05; ** denotes p<0.01; *** denotes p<0.001

The study results remained similar when performing the analyses only in the male subpopulation **(Figure 1C).**

The correlation between copeptin levels and cortisol was r=0.46, p<0.001 There was no correlation between copeptin levels and age or between copeptin and sodium values. No patient had dysnatremia.

DISCUSSION

In our cohort, copeptin was found to be a more subtle marker of the individual stress level. Specifically, copeptin shows a gradual increase with increasing levels of stress and, in contrast to cortisol levels, differentiates between healthy control subjects without apparent stress and medical patients with a moderate degree of stress. In addition, copeptin shows a more pronounced increase upon major stress as compared to cortisol levels.

The significant correlation between copeptin and cortisol concentrations in our study indicates that both

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measurements mirror the activity of the HPA-axis. Copeptin levels reflect the stress degree at a higher, i.e. the hypothalamic-pituitary level, whereas cortisol concentrations mirror the more peripheral stress response of the adrenals.

Presently, in clinical routine, cortisol levels are considered to predict the ability of our body to produce an adequate stress response [10]. However, cortisol levels only mirror the peripheral endocrine response of the adrenals to stress, whereas the hypothalamic response to stress is not detected. CRH and AVP might play a crucial role in conducting the perception of stress at the central hypothalamic level. There is an emerging view that AVP is the principal regulated variable that imparts situation specific drive on the HPA axis, whereas CRH serves mainly to impose stimulatory tone [13]. Since AVP measurement is problematic, measurement of copeptin in this regard provides a novel and valuable laboratory tool.

Our study is observational in nature. Thus, we can not make a conclusion about the clinical impact of the higher increase in copeptin levels compared to serum cortisol values upon major stress. Possibly, copeptin provides a more direct and earlier mirror of the stress level as compared to cortisol. Our finding that copeptin levels, but not cortisol levels, increase in medical patients with a moderate stress level compared to healthy subjects without stress strengthens this assumption.

AVP levels mirror fluid balance. Since copeptin is stoichiometrically produced with AVP, one could argue that the increase of copeptin levels may be explained by a change in water balance. However, plasma sodium as main parameter of osmolality was not different between medical and surgical patients at the time-point where we measured copeptin. This suggests that changes in osmolality were not the main stimulus for the increase in copeptin. As a limitation, we did not assess serum osmolality or detailed fluid balance in our patients.

We found significantly lower baseline copeptin levels in female versus male control subjects which is in accordance with the published literature. Morgenthaler et al. found also that copeptin values in healthy individuals differed significantly between men and women [12]. In contrast, we found no significant difference in the copeptin levels upon increasing stress level between men and women. In addition, analyzing only male study subjects showed a similar gradual increase of copeptin upon increasing levels of stress compared to the whole study population. Thus, in situations of more severe stress, the baseline gender differences are overridden by the increasing stress response.

Another well known stimulus of AVP secretion is a drop in blood volume and, thus, blood pressure. This might have influenced the pronounced increase of copeptin in patients after extubation and is part of a severe stress response of the body. However a small reduction in blood pressure usually has little or no effect on plasma vasopressin levels [14]. In our study population blood pressure between medical patients and surgical patients was not significantly different; thus, a drop in blood pressure can not explain the increase of copeptin levels in medical patients, again suggesting that the increase in copeptin levels was predominantly stress-related.

Copeptin has already been evaluated in critically ill patients with septic shock [15–17], in patients with respiratory infections [18,19] as well as upon changes in osmolality [20] and in patients with diabetes insipidus [21]. In the acute state, copeptin, similarly to cortisol, has been shown to be an independent marker for reduced survival in critically ill patients as well as in patients with heart insufficiency [22]. Measurement of plasma copeptin concentrations in critically ill patients deserves to be evaluated as an indirect laboratory parameter to assess stress and therefore outcome in future studies.

Our study has limitations. First, this is a preplanned posthoc analysis of an observational study evaluating cortisol levels in different stress situations and the diagnostic performance of low dose and standard ACTH tests. Our findings should, therefore, be regarded as hypothesis-generating. Second, our study population included patients with intact adrenal function, as assessed by the ACTH-test and can therefore only yield information about the acute stress level in patients with preserved adrenal function. Therefore, we can not draw conclusions about the impact of adrenal insufficiency on copeptin levels. For example patients with complete or relative adrenal insufficiency might provoke a hypersensitivity to a stressor resulting in higher basal levels of copeptin and possibly predicting a worse outcome. We further did not perform serial blood sampling although cortisol levels show large variations during the day. However, during critical illness and severe stress, the circadian pattern of cortisol is usually lost [23]. Nevertheless, serial cortisol measurements would most probably have given more reliable results.

In conclusion, copeptin subtly mirrors the individual stress level in a population consisting of healthy controls, hospitalized medical patients and surgical patients during the peri- and postoperative period. It shows a more gradual increase with increasing stress as compared to total cortisol levels. If confirmed in a larger subset of patients, copeptin might provide a novel tool for the assessment of the individual stress level at the hypothalamic level.

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