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REGULAR ARTICLE

Levels of awakening salivary CgA in response to stress in healthy subjects

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Abstract
Objects To determine the changes in salivary chromogranin A (CgA) levels upon awakening in response to of stress by investigating the relationship between salivary CgA levels and the stress response as assessed by GHQ-28 tests.
Methods The study cohort comprised 40 healthy male university students (age range 19–22 years). Salivary CgA levels were measured at 7:00 a.m. (awakening) and at 7:30, 8:00, and 8:30 a.m. (after awakening).
Results The salivary CgA level was 0.91 ± 0.20 and 0.42 ± 0.1 pmol/ml at 7:00 a.m. in students scoring low ($n = 26$) and high ($n = 14$), respectively, on the "severe depression" subscale. This difference in salivary CgA levels at 7:00 between high and low scorers was statistically significant ($p < 0.05$).
Conclusions Our findings indicate that depression may influence secretions of salivary CgA via chronic stress-related attenuation of the sympathetic–adrenomedullary system activity.

Keywords Chromogranin A · Stress · Awakening · Saliva · Depression

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Introduction

The stress response system includes the sympathetic–adrenomedullary (SAM) system and the hypothalamic–pituitary–adrenal (HPA) axis, whose activities can be biochemically evaluated by measuring catecholamines and cortisol, respectively. The salivary cortisol concentration increases in response to psychological and physical stress and is currently widely used as an index of stress [1]. Catecholamine levels are also commonly used as a sensitive biochemical index of stress; however, it is difficult to measure their concentrations in the saliva due to generally low levels and rapid degradation. Therefore, salivary chromogranin A (CgA) is used as a substitute marker for catecholamines, as it reflects psychological stress more quickly and sensitively than cortisol [2, 3]. In addition, the use of saliva rather than blood has obvious advantages in terms of sample collection, such as speed, ease, subject mobility, and non-invasiveness.

CgA is a 48-kDa acidic glucoprotein that is stored and co-released with catecholamines by exocytosis from the adrenal medulla and sympathetic nerve endings. Both the secretion route and circadian rhythm of salivary CgA in humans have recently been clarified [4, 5]. A previous study by our group showed that salivary CgA levels peaked at awakening (7:00 a.m.) and then quickly decreased to a nadir 1 h later (8:00 a.m.), remaining at a low level throughout the day, and finally increasing at night (10:30 p.m.). However, to the best of our knowledge, there is no report on the change in salivary CgA levels in response to chronic stress. Several studies have demonstrated that the free cortisol response to awakening can serve as a useful index of stress as it seems to be able to uncover subtle changes in HPA activity [6–8]. We have therefore studied university students living in their dormitory and investigated the

relationship between the salivary CgA level at awakening and the stress response using GHQ-28 tests [9].

Methods

Subjects

The subjects were 40 healthy male university students (age range 19–22 years old) living in a school dormitory. None of the students had a salivary gland disease or were taking any medications. The study protocol was approved by the Osaka University Medical Department Ethics Committee, and all subjects provided written informed consent prior to participation. All subjects were asked to go to bed at 11:00 p.m. the day before sampling. The health status of the subjects was evaluated through the administration of GHQ-28 tests on the day preceding the sampling. Subjects scoring $\geq$8 points (cut-off point) on the GHQ-28 total score were placed in the high-scoring group [10]. Four conditions were queried in the GHQ-28 test: "somatic symptoms" [cut-off point 1 (low scorers) vs. 2 (high scorers)], "anxiety and insomnia" (cut-off point 0 vs. 1), "social dysfunction" (cut-off point 5 vs. 6), and "severe depression" (cut-off point 0 vs. 1). The results on CgA levels were compared between the high- and low-scoring groups on the total GHQ-28 test and the four subscales.

Procedure

Saliva samples were collected into special sampling tubes (Salivettes; Sarstedt Co, Nümbrecht, Germany), at 7:00 a.m. (awakening) and at 7:30, 8:00, and 8:30 a.m., in the dormitory where the subjects lived, and under the supervision of the authors. After collection, the sampling tubes were placed in an icebox and immediately transferred to the laboratory, where they were centrifuged and stored at −80°C until analysis.

Data analysis

Salivary CgA concentrations were determined using an enzyme-linked immunosorbent assay (ELISA) with a YK070 Human CgA EIA kit (Yanaihara Institute, Shizuoka, Japan). All samples were assayed in duplicate. Salivary CgA concentrations were not normally distributed (Shapiro–Wilk test); thus, they were transformed to natural log values for analysis. The unpaired t test, analysis of variance (ANOVA), and Bonferroni's test were used for statistical analysis (SPSS ver. 11.5; SPSS, Chicago, IL). Values are expressed as the mean ± standard error of the mean (SEM). p values <0.05 were considered to be significant in all analyses.

Results

The salivary CgA concentrations and GHQ-28 scores are shown in Table 1. Salivary CgA levels at 7:00 a.m. (awakening) and at 7:30, 8:00, and 8:30 a.m. were not significantly different in the high- and low-scoring groups for total GHQ-28 score and for the "somatic symptoms", "anxiety and insomnia", and "social dysfunction" subscale scores. The levels of salivary CgA in high- and low-scorers on the "severe depression" subscale were not significant different at 7:30, 8:00, and 8:30 a.m., but they were significantly different at 7:00 a.m. (awakening) ($t = 2.0$, $p < 0.05$) (Fig. 1).

Discussion

This study assessed the levels of salivary CgA in response to chronic stress in healthy university students at awakening. We found a significant decrease in salivary CgA levels at 7:00 (awakening) in the high-scorers for the "severe depression" subscale group. This finding suggests that depression may influence salivary CgA secretion via chronic stress-related attenuation of the activity of the SMA system. Thus, the use of the morning salivary CgA response at awakening appears to be able to uncover subtle changes in SMA activity associated with prolonged psychosocial stress. Subjects who described themselves as chronically (i.e., for at least 6 months) stressed due to a work/study overload showed an enhanced morning cortisol response [11]. If this stress load expands over a number of years and the individual is no longer able to adequately cope with this situation, a state of depression may develop and both the early morning salivary CgA response and cortisol response may decrease. Pruessner et al. [7] observed that teachers reporting high levels of burnout tended to have a blunted cortisol response at awakening along with an increased feedback sensitivity at the pituitary level. De Kloet et al. [12] showed that levels of salivary cortisol decreased 30 min after awakening in post-traumatic stress disorder (PTSD) patients and that trauma is related to an alteration of the HPA-axis function.

In conclusion, the results presented here suggest that the potential usefulness of the early morning salivary CgA response as a reliable biological marker of altered SMA system activity caused by prolonged psychosocial stress. Future studies are required to assess salivary CgA response at awakening in depression patients.

Table 1 Salivary CgA levels (pmol/ml) in the study subjects at awakening and at three time-points after awakening according to the GHQ-28 total score and GHQ-28 subscale scores

Total GHQ-28/GHQ-28 subcategories	Mean GHQ-28 (sub)scores and cut-offs	Salivary CgA levels (pmol/ml)			
		7:00 a.m. (awakening)	7:30 a.m.	8:00 a.m.	8:30 a.m.
Total GHQ-28 ($n = 40$)	6.3 (0.30)	0.74 (0.14)	0.31 (0.03)	0.35 (0.05)	0.32 (0.04)
Low-scoring group ($n = 25$)	≧7	0.73 (0.13)	0.30 (0.04)	0.33 (0.05)	0.32 (0.04)
High-scoring group ($n = 15$)	≦8	0.74 (0.30)	0.31 (0.05)	0.38 (0.11)	0.33 (0.09)
Somatic symptoms	0.85 (0.10)				
Low-scoring group ($n = 32$)	≧1	0.79 (0.17)	0.33 (0.04)	0.37 (0.06)	0.35 (0.05)
High-scoring group ($n = 8$)	≦2	0.53 (0.17)	0.20 (0.05)	0.24 (0.08)	0.21 (0.05)
Anxiety and insomnia	0.92 (0.10)				
Low-scoring group ($n = 20$)	≧0	0.65 (0.16)	0.27 (0.04)	0.32 (0.06)	0.32 (0.04)
High-scoring group ($n = 20$)	≦1	0.82 (0.22)	0.34 (0.05)	0.37 (0.09)	0.33 (0.07)
Social dysfunction	4.2 (0.20)				
Low-scoring group ($n = 26$)	≧5	0.81 (0.18)	0.31 (0.04)	0.33 (0.05)	0.31 (0.03)
High-scoring group ($n = 14$)	≦6	0.60 (0.19)	0.29 (0.06)	0.38 (0.12)	0.35 (0.09)
Severe depression	0.7 (0.10)				
Low-scoring group ($n = 26$)	≧0	0.91 (0.20)*	0.33 (0.04)	0.37 (0.07)	0.36 (0.05)
High-scoring group ($n = 14$)	≦1	0.42 (0.10)	0.26 (0.05)	0.30 (0.08)	0.25 (0.04)

*$p < 0.05$

Where applicable, values are given as the mean with the standard error given in parenthesis

CgA chromogranin A

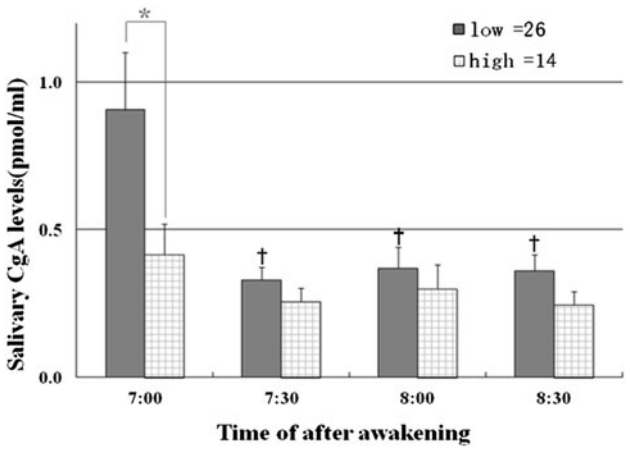

Fig. 1 Levels of salivary chromogranin A (CgA) at awakening (7:00 a.m.) and at three time-points after awakening (7:30, 8:00, 8:30 a.m.) in high scorers and low scorers on the GHQ-28 "severe depression" subscale. Values are given as the mean ± standard error of the mean. *Significantly different from the high-scoring group ($p < 0.05$; Student's t test), †significantly different from the CgA level at 7:00 a.m. in the low-scoring group ($p < 0.05$; analysis of variance and Bonferroni's test)

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