Circadian rhythm of human salivary chromogranin A

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ABSTRACT

We investigated the circadian rhythm of chromogranin A (CgA) concentrations in saliva and blood samples from 40 male college students collected at 7:00, 8:00, 10:30, 12:30, 17:30, and 22:30. CgA concentrations were determined by ELISA. Salivary CgA levels peaked upon awakening, and then quickly decreased to the nadir after 1 hour and maintained a low level throughout the day. On the other hand, plasma CgA did not show any obvious circadian rhythm. These findings suggest that salivary and plasma CgA has different routes of secretion.

Chromogranin A (CgA) is an acidic protein that is stored and co-released by exocytosis with catecholamines from the adrenal medulla and sympathetic nerve endings (10, 13, 14), thus, it is considered to be a valuable indicator of sympathoadrenal activity (16). Recently, salivary CgA was shown to be produced by the human submandibular gland and secreted into saliva (11), and is considered to be a sensitive and reliable index for evaluating psychological stress. Nakane et al. (8, 9) showed that salivary CgA levels were elevated immediately before subjects gave an oral presentation and then decreased immediately after the presentation was finished. They also showed that salivary CgA elevation always preceded an elevation of salivary cortisol concentration, while it was not significantly elevated after physical exercise.

To our knowledge, there are few studies of the circadian rhythms of plasma and salivary CgA concentration. Takiyuddin et al. (15) reported that plasma CgA had no circadian rhythm in normal subjects, whereas Giampaolo et al. (4) showed that plasma CgA levels had peak values in the afternoon-evening period and a nadir in the morning. In the present study, we investigated the circadian rhythm of CgA in saliva and blood, and compared the results to that of cortisol.

The subjects were 40 male students (19 to 22 years old) who lived in the dormitory of Osaka University. None had any disease that affected hypothalamic-pituitary-adrenal axis (HPA) function, such as Cushing’s syndrome, hypopituitarism, or depression, and were not taking any medications. The study protocol was approved by the Ethics Committee of Osaka University and all subjects gave informed consent to participate. During the sampling period, all subjects were given the same breakfast, lunch, and dinner. Except for those meals, only the drinking of mineral water was permitted during the sampling period. All subjects were asked to go to bed at 23:00 on the day before sampling. The samples were collected at the dormitory where the subjects lived, under our supervision. Saliva and blood samples were collected at 7:00 (awakening), 8:00, 10:30, 12:30, 17:30, and 22:30, which were decided by referring to the circadian rhythm of cortisol. To collect a sufficient quantity of saliva, “Salivette” sampling devices (Sarstadt, Inc., Rommelsdorf, Germany), of which included a small cotton swab, were used (7) and each saliva sample was collected over a period of 2 minutes. Blood samples...
(3 mL) were collected with a vacuum tube (Terumo Co., Ltd. Japan) by repeated vein punctures. Saliva was sampled before blood collecting, in order to avoid the stress of blood sampling. After the collections, all samples were kept in an icebox and immediately transferred to the laboratory, where they were stored at −80°C until analysis after centrifugation. Salivary and plasma CgA levels were analyzed using a YK070 Human CgA enzyme-linked immunosorbent assay (ELISA) kit (Yanaihara Institute, Shizuoka, Japan), while those cortisol levels were determined using a commercial ELISA kit (CIRON, Tokyo, Japan). All samples were analyzed in duplicate. Salivary and plasma concentrations of CgA and cortisol were not normal distribution, thus we used a natural log to transform the results for analysis. Values were expressed as the means ± standard error of the mean (SEM). Repeated measures ANOVA and Bonferroni’s test were used for analyses, with Pearson’s correlation coefficient used to determine correlations. Values were considered to be significant at \( p < 0.05 \).

Fig. 1 shows the levels of CgA in saliva and plasma at the different sampling periods. The level of salivary CgA peaked at the time of awakening (at 7:00) and then quickly decreased to a nadir 1 hour later (8:00). Thereafter, salivary CgA concentration remained at a low level throughout the day, and increased again at night (22:30) (\( F = 3.71, p < 0.05 \)). The level of salivary CgA at 7:00 was significantly higher than that at 8:00, 10:30, 12:30 and 17:30 \( (p < 0.05) \), while the level at 22:30 was significantly higher than that at 8:00, 10:30 and 12:30 \( (p < 0.05) \). In contrast, plasma CgA levels did not significantly change throughout the day \( (F = 0.93, p = 0.45) \).

Fig. 2 shows the levels of cortisol in saliva and plasma at each sampling times. Saliva and plasma cortisol levels showed similar circadian rhythm. There was a steep increase within the first hour af-
ter awakening, followed by a gradual decrease throughout the day (salivary cortisol: \( F = 5.5, p < 0.001 \); plasma cortisol: \( F = 34.4, p < 0.001 \)). The salivary cortisol level at 22:30 was significantly lower than that at 8:00 and 10:30 (\( p < 0.05 \)), whereas the levels in plasma at 7:00, 8:00 and 10:30 were significantly higher than those at 17:30 and 22:30, and that at 12:30 was significantly higher than at 22:30. Further, the level of plasma cortisol at 17:30 was significantly lower than those at 7:00, 8:00, and 10:30; and significantly higher than at 22:30, at which time plasma cortisol was significantly lower than that at all the other time points.

In the present study, we investigated the circadian rhythm of salivary CgA in humans, and found that salivary CgA level was at a peak level upon awakening, and then quickly decreased to a nadir within 1 hour. Thereafter, the level remained low throughout the day, and increased again late at night. On the other hand, plasma CgA levels did not significantly change throughout the day (Fig. 1). Salivary CgA was entirely different from plasma CgA and no correlations were found. Our present results support the findings of Saruta et al. (11), which demonstrated that salivary CgA is produced in the serous and ductal cells of the human submandibular gland. Meanwhile, plasma CgA was secreted with catecholamines from the adrenal medulla and sympathetic nerve endings (10, 13, 14).

Salivary and plasma cortisol levels showed a similar circadian rhythm (Fig. 2). Cortisol levels reached a peak value at 1 hour after awakening, then decreased gradually during the day. These findings are in line with some previous studies (2, 3). The level of salivary cortisol has been reported to correlate closely with that of free plasma cortisol (1, 6).

In the present subjects, salivary CgA reached the highest level at the time of awakening, whereas cortisol reached a peak at 1 hour after awakening. This post-awakening increase of cortisol may be related to light exposure (3, 5, 12, 17). Namely, light exposure amplifies the awakening cortisol response from signaling that emanates from the hypothalamic suprachiasmatic nucleus. Therefore, we think that salivary CgA is related to sympathetic stimuli, but is not related to light exposure.

Our study is the first known description of salivary CgA circadian rhythm in humans. Nakane et al. (8) stated that salivary CgA might be a sensitive and promising index for psychosomatic stress. Therefore, an understanding of the circadian rhythm of salivary CgA in normal humans is important and additional studies are required to elucidate that in patients with depression. Salivary CgA may rise after a time of sleeping and reach to the highest level at the time of waking. However, we did not obtain samples during sleep in this investigation and further studies are required to elucidate the 24-hour pattern.

REFERENCES

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