

THE CORTISOL RESPONSE TO AWAKENING IN RELATION TO DIFFERENT CHALLENGE TESTS AND A 12-HOUR CORTISOL RHYTHM

A. Schmidt-Reinwald, J.C. Pruessner, D.H. Hellhammer, I. Federenko,
N. Rohleder, T.H. Schürmeyer, & C. Kirschbaum

Center for Psychobiological and Psychosomatic Research,
University of Trier, Germany

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Summary

Recent studies have shown that cortisol levels rapidly increase within the first 30 minutes after awakening. This response is rather robust over weeks or months and is altered by chronic stress and burnout. The present study investigated to what extent the cortisol response to awakening relates to responses following hCRH, ACTH₁₋₂₄, or psychosocial stress challenges in 22 healthy subjects. Furthermore, a 12-hour circadian cortisol profile was obtained to compare the morning response with cortisol levels obtained throughout the day. Results show that the morning cortisol response was of similar magnitude to that following injection of 1 µg/kg h-CRH or exposure to a brief psychosocial stressor (TSST). All of these were significantly smaller compared to maximal stimulation of the adrenal cortex by ACTH₁₋₂₄. Correlation analyses revealed that the morning cortisol response was closely related only to the cortisol response following 0.25 mg ACTH₁₋₂₄ ($r=0.63$, $p=0.002$). We conclude that the morning cortisol response to awakening can provide important information on the (re)activity of the HPA axis in addition to more 'traditional' methods like hCRH or Synacthen challenge tests. The sensitivity/capacity of the adrenal cortex appears to play a crucial role for the magnitude of cortisol responses observed after awakening.

Key Words: stress, HPA axis, CRH test, Synacthen, Trier Social Stress Test, saliva

The activity of the hypothalamus-pituitary-adrenal (HPA) axis is characterized by a well-documented circadian rhythm (1-3) with highest spontaneous secretory activity during the second half of nocturnal sleep with decreasing activity thereafter. Without significant

PD Dr. Clemens Kirschbaum, FPP, University of Trier, Dietrichstr. 10-11, D-54290 Trier, Germany; Fax: (+49) 651-975-8640, Email: Kirschba@uni-trier.de

stimulation of the axis by exogenous factors, this pattern is associated with peak levels of adrenocorticotropin hormone (ACTH) and cortisol in the early morning hours followed by a continuous decline until a trough is reached around midnight. Recently, it has been reported that on top of this circadian rhythm there is a pronounced release of ACTH and cortisol immediately after awakening in the morning (4, 5). Within the first 30 minutes of awakening, free cortisol levels rise by 70-150% and remain elevated for at least 60 minutes. This response was found to be independent of the subject's age, time of awakening, total time slept, sleep quality, physical activity, or morning routines, but influenced by a number of factors shown to alter the response magnitude and time-course including gender, habitual smoking, use of oral contraceptives, and stress (6, 7).

While the pattern of the morning cortisol response to awakening can be viewed as well-described in several populations, there is still a need for studies investigating the exact nature of the response. One topic of interest to the user of this index of HPA activity in a clinical setting or in basic science studies is the question to what extent the morning response corresponds to results of pharmacological or psychological challenge tests. The present experiment therefore attempted to elucidate the associations between ACTH and cortisol responses to a psychosocial stressor ("Trier Social Stress Test", TSST(8)), injection of human corticotropin-releasing hormone (h-CRH), and stimulation by synthetic ACTH₁₋₂₄. Since each of the three challenge tests stimulates the HPA axis at a different level, results from this work might provide some insight into the origin of the morning cortisol response. Additionally, a 12-hour circadian cortisol profile was compared with the individual morning cortisol response.

Methods

Subjects

Twenty-two nonsmokers (16 females, 6 males) with a mean age of 22.4 ± 0.6 yrs. (SD) and a body mass index of 21.6 ± 0.5 (SD) participated in the present study. All subjects were students at the University of Trier and reported to be in good physical health. Except for using oral contraceptives (6 women) all subjects were medication-free. A brief medical exam confirmed that none of the participants suffered from acute or chronic diseases. Volunteers provided written informed consent and they were paid 150,- DM after completion of the three challenge tests and delivery of the morning samples (see below).

Study Protocol

On three consecutive days, the subjects reported to the laboratory between 1400 and 1600 hrs. They were instructed to refrain from exhaustive physical exercise, larger meals and drinks with low pH at least 90 minutes before the start of the experiment. Upon arrival at the laboratory, an i.v. catheter was inserted in an anticubital vein and kept patent by a sterile lock for at least 60 minutes prior to the challenge tests. On the first day, subjects received a bolus injection of $1 \mu\text{g}/\text{kg}$ h-CRH (Ferring, Kiel, Germany), 0.25 mg ACTH₁₋₂₄ (Synacthen, Ciba-Geigy, Wehr, Germany) were infused on day 2, and the psychosocial stress test (TSST; (8)) was performed on the third day. Test sequence was not randomized in order to decrease error variance due to possible anticipatory responses following TSST exposure. The TSST is a standardized laboratory procedure for induction

of significant physiological and psychological responses to acute stimulation. The protocol mainly consists of a free speech and mental arithmetics in front of an audience with a total duration of 13-15 minutes. The study protocol was approved by the local ethics committee.

Blood and saliva sampling

On the three test days, blood and saliva samples were repeatedly obtained 2 minutes before, and 15, 30, 45, 60 and 90 minutes (h-CRH and ACTH₁₋₂₄) or 1, 10, 20, 30, 45, and 60 minutes (TSST) after drug injection or stress cessation, respectively. Blood samples were immediately stored on ice until completion of each test. Samples were spun at 3000 rpm for 10 minutes and EDTA plasma was removed and stored at -20 degrees Celsius until analysis. Saliva samples (obtained with Salivette, Sarstedt, Rommelsdorf, Germany) were kept at room temperature throughout one test session and stored at -20 degrees Celsius. After thawing for biochemical analysis, samples were spun at 3000 rpm for 10 minutes.

In addition, all subjects were asked to obtain saliva samples in the morning of the third day. Immediately after awakening and 15, 30, 45, and 60 minutes they should obtain a saliva sample. Since the morning cortisol responses does not appear to be altered by procedural differences, subjects were free to wake up according to their normal schedule and follow their daily routines. However, they were instructed not to brush their teeth or to have breakfast during the first hour after awakening to avoid false high cortisol values due to plasma exudates from minor bleeding in the oral cavity. In our experience, this procedure ensures a proper assessment of the morning cortisol response to awakening in an ambulatory setting. Although no mechanical or electronic devices were employed to validate the exact timing of sample collection, comparisons between samples obtained by paid volunteers and laboratory members revealed that compliance is apparently high in the former population.

Further, a day-time circadian cortisol profile was obtained. All subjects obtained saliva samples at 30 min intervals from 0900 to 2100 hrs.

Biochemical analysis

ACTH levels were measured with commercially available two-site immunoassay (Nichols Institut, Bad Nauheim), salivary cortisol was analyzed by an in-house immunoassay with time-resolved fluorescence detection (9). Intra- and interassay coefficients of variance were below 10% and 12%, respectively.

Statistical analysis

Endocrine responses were tested for statistical significance by ANOVAs for repeated measurement with Greenhouse-Geisser correction of degrees of freedom if the sphericity assumption was violated. In order to be able to directly compare the hormonal responses to the four stimulations (h-CRH, ACTH₁₋₂₄, TSST, awakening), the area under the response curve (AUC, trapezoid formula) was computed for each hormone. This index reflects the response magnitude relative to the individual baseline ('baseline': -2 minute sample for the three challenge test; sample obtained immediately after awakening for the morning cortisol

response). The response magnitudes were compared between the four stimulations by ANOVA using the four AUCs as dependent variables. Post-hoc Newman-Keuls tests were employed to reveal response differences and Pearson product-moment correlations were computed between the four AUCs. In order to avoid type-I errors by alpha cumulation, alpha correction was performed. Outliers were defined as two standard deviations above or below the group means. Based on this definition, data from one subject (morning cortisol) or four subjects (circadian rhythm) were removed from statistical analyses.

Results

As expected, all four stimulation procedures induced significant endocrine responses. ACTH and cortisol levels were increased after h-CRH and TSST (F values: 31.28, 17.08, 33.42, 20.11; all $p < 0.001$); cortisol increased after ACTH₁₋₂₄ and in response to awakening, respectively (F values: 95.92, 7.20; $p < 0.001$ and $p = 0.004$) (Fig. 1).

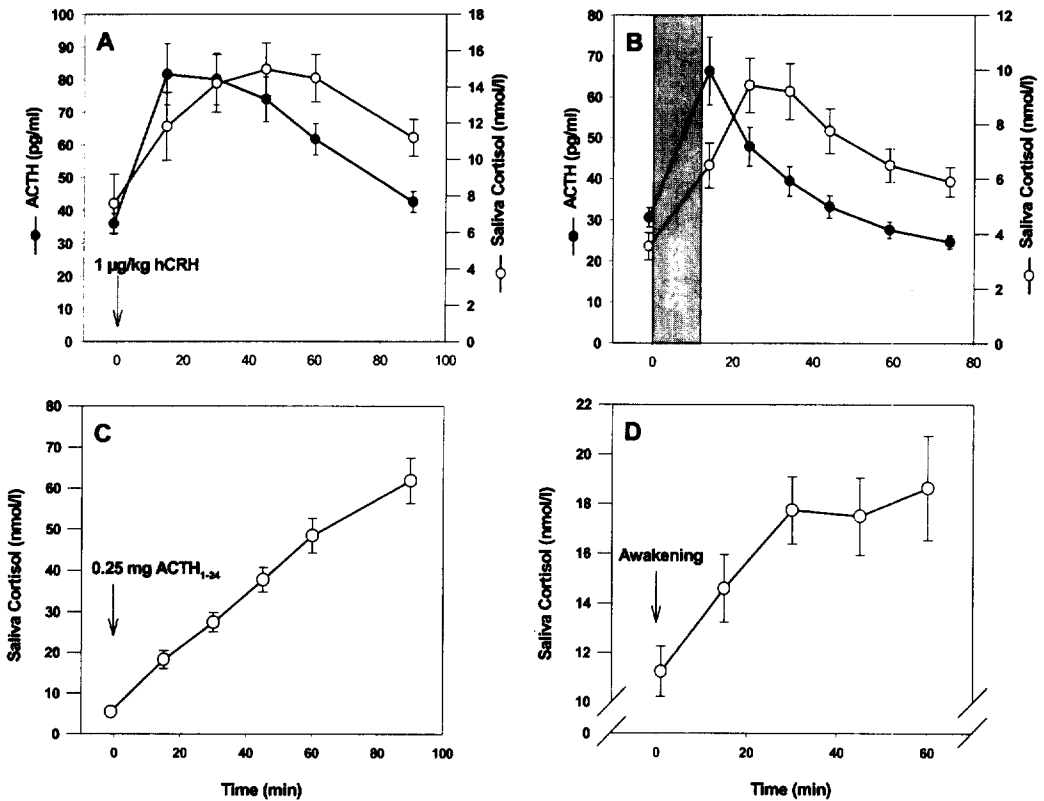


Fig. 1

ACTH and saliva cortisol responses to (A) 1 µg/kg h-CRH, (B) TSST, (C) 0.25 mg ACTH₁₋₂₄, and (D) after awakening.

The response magnitudes differed between stimulations ($F=80.07$). Post-hoc analyses indicated that the greatest cortisol response occurred after injection of ACTH₁₋₂₄ ($p < 0.001$) while no differences were observed in response to h-CRH, TSST, and awakening (all $p < 0.1$) (Fig. 2). The 12-hour cortisol rhythm showed the expected pattern with cortisol levels continuously declining from the morning to the late evening hours ($F=17.57$, $p > 0.001$) (Fig. 3).

Correlation analyses performed on the area under the response curve (AUC) for the morning cortisol response and the respective AUCs for h-CRH, ACTH₁₋₂₄, TSST, and circadian rhythm showed that the morning response was significantly correlated with the adrenocortical response to ACTH₁₋₂₄ ($r=0.63$, $p=0.002$). As shown in Figure 4, this correlation was not due to extreme values but was based on a linear relationship between the variables over the entire range of cortisol concentrations (at least in women) explaining 37.2% and 46.2% of the total variance observed. Since only five male subjects participated in the present study, separate analyses for the two sexes were not performed. No other correlation between morning cortisol response and the remaining indices of HPA activity was statistically significant (h-CRH: $r = -.21$; TSST: $r = -.001$; 12-hour rhythm: $r = -.37$; all $p > 0.05$).

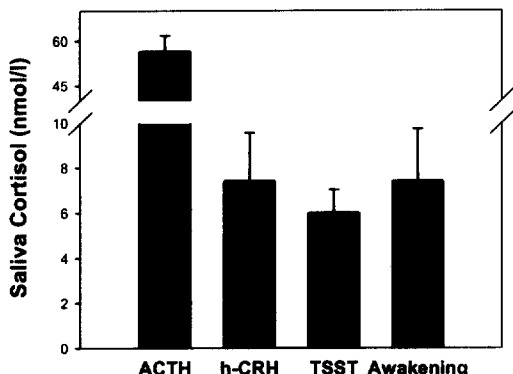


Fig. 2

Absolute increases in cortisol levels following ACTH₁₋₂₄ or h-CRH injections, psychosocial stress (TSST), and awakening.

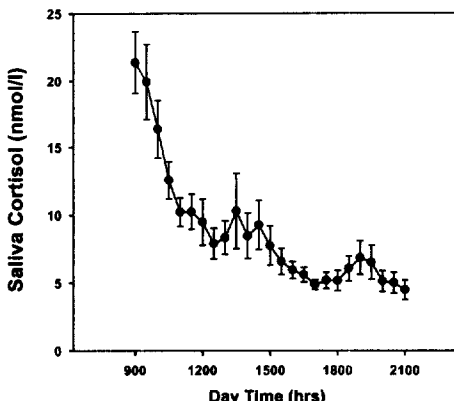


Fig. 3

Daytime cortisol rhythm over a 12-hour period.

Discussion

The assessment of morning cortisol levels with strict reference to the time of awakening shows moderate to high intraindividual stability with test-retest correlations typically ranging between $r=0.45$ to $r=0.70$ when measured at weekly or monthly intervals (6). This pattern of morning cortisol response to awakening was replicated in other laboratories (10;11). Therefore it compares favorably to single measurement of 'baseline' free cortisol levels in the morning hours, e.g., between 0800 and 0900 hrs which was reported to be a far more variable measure (12). Probably a result of increased reliability, the use of

morning cortisol responses to awakening appears to be able to uncover subtle changes in HPA activity associated with extended periods of psychosocial stress. Subjects who described themselves as chronically (i.e., for at least six months) stressed due to work overload showed an enhanced morning cortisol response (7). 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 burnout might develop which can be reflected in the morning cortisol response, too. Most recently, this laboratory observed that teachers reporting high levels of burnout tended to have a blunted cortisol response to awakening along with an increased feedback sensitivity at the pituitary level (13). The morning cortisol response appears to be altered also in clinical populations. A first investigation of putative changes of this HPA axis parameter in cancer patients suggested that in breast cancer patients the morning rise may be linked to sleep and anxiety (14). Furthermore, in chronic pain patients, the cortisol response to awakening differs from pain-free controls (15).

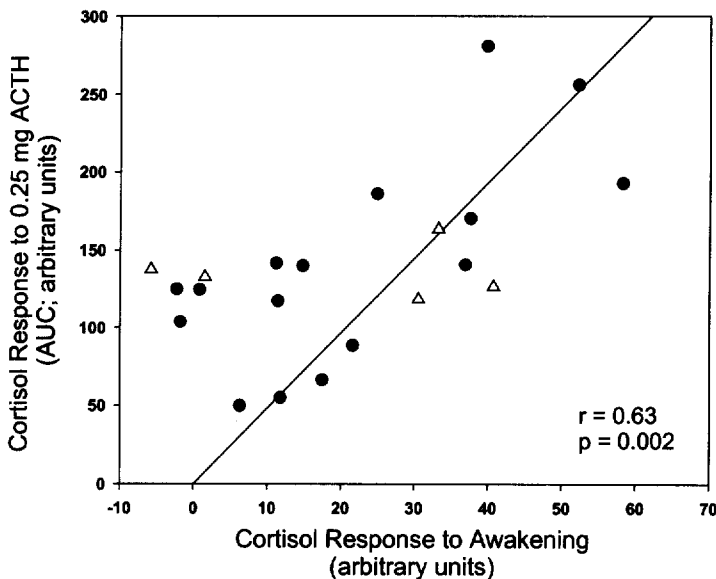


Fig. 4

Scattergram of individual cortisol responses to awakening and following injection of 0.25 mg ACTH₁₋₂₄ (women: filled circles; men: open triangles).

While the phenomenon itself has been explored quite extensively, the present data are the first to describe the relation between morning cortisol responses after awakening and other procedures employed to characterize the (re)activity of the HPA axis. The magnitude (net increase) of the awakening response is comparable to the respective increase following injection of 1 $\mu\text{g}/\text{kg}$ hCRH or to acute psychosocial stress in the laboratory (TSST). Interestingly, we could not find any evidence for a close correlation between hCRH or TSST-induced cortisol levels and cortisol responses to awakening. Likewise, the latter did not correspond to cortisol levels measured over a 12-hour period during the

active part of the day (0900-2100 hrs). However, there was a robust correlation between the morning cortisol response to awakening and the increase after maximal stimulation of the adrenal cortex explaining 46% of the total variance observed.

Taken together the present results suggest that the repeated measurement of cortisol with strict reference to the time of awakening reflects the capacity of the individual's adrenal cortex and that it can not be substituted for pharmacological or psychological challenge tests which stimulate the HPA axis at a pituitary or suprapituitary level. The awakening response might rather be used to reveal subtle changes in HPA (re)activity due to prolonged psychosocial stress or clinical symptoms influenced by the HPA axis. Future studies should address this possibility and include morning cortisol readings along with more 'traditional' clinical parameters to elucidate the additional diagnostic or prognostic value of the cortisol response to awakening.

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