



Alterations of postawakening cortisol parameters during a prolonged stress period Results of a prospective controlled study

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ABSTRACT

Though postawakening cortisol is considered to be altered under chronic stress prospective studies proving this assumption is missing, so far. Furthermore, there is some uncertainty which aspects of postawakening cortisol alterations are strongest related to stress. The present study thus analyzed the cortisol concentration at awakening itself (0 min), the cortisol awakening response (CAR; i.e. the increase within 30 min after awakening), the area under the curve of the first hour after awakening (AUC_C60) and the mean of samples taken 0 min and 30 min after awakening (AUC_C30) in 12 exam students, participating in a major exam and 12 matched control students not participating in any exam. Saliva samples were taken on two consecutive days at 0, 15, 30, 45, and 60 min after awakening, respectively, at four time points (T1–T4): on the verge of exams, when students anticipated and prepared the exam (T1), in the middle of exams (T2), and shortly after (T3). T4 (weeks after exams) represents a reference measure. Repeated measures analyses of covariance revealed a significantly higher AUC_C30 ($p=0.007$) and AUC_C60 ($p=0.011$) and higher cortisol concentrations at awakening ($p=0.016$) in exam students and a significant time by group interaction for concentration at awakening ($p=0.031$). No effects were found for the CAR. The results of this prospective controlled study support notions that chronic stress induces increases of overall postawakening cortisol. They further indicate that the CAR is not affected by chronic stress and that the awakening concentration responds later than the AUC_C to conditions of chronic stress as analyzed here.

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Introduction

The association between altered hypothalamus pituitary adrenal (HPA) axis activity and the experience of psychosocial stress is a well-documented phenomenon. In contrast to HPA axis response to acute psychological stress which is characterized by a time limited increase of cortisol concentration, enduring or chronic stress is associated with altered basal concentrations (Miller et al., 2007).

Cortisol secretion follows a circadian rhythm characterized by increasing concentrations towards the early hours of the morning peaking shortly after awakening and decreasing concentrations over the day. One method to analyze cortisol secretory activity is the assessment of saliva cortisol levels after awakening when hormone concentrations strongly increase until 30–45 min after awakening and decrease thereafter (Pruessner et al., 1997). To describe the features of postawakening cortisol several saliva samples are taken within the first hour of awakening. Different measures can be deduced from these data. Since some uncertainty occurred in the literature concerning the naming of the different features, we first introduce the naming employed here before we further deduce our study aims:

0 min stands for the cortisol concentration immediately after awakening; AUC_C (the area under the curve with reference to zero) stands for overall postawakening cortisol; CAR (cortisol awakening response, see below) stands for the dynamic of the increase (Δ 0–30 min) after awakening.

The CAR gained much attention as, in contrast to the overall postawakening cortisol, it is not correlated with other basal cortisol levels such as the daily circadian cortisol profile (Edwards et al., 2001, Schmidt-Reinwald et al., 1999) and is found to represent a distinct part of the diurnal cycle as a reaction to awakening (Wilhelm et al., 2007). Recently this cortisol rise has been uniquely termed cortisol awakening response (CAR; Clow et al., 2010; Fries et al., 2009) and it appears to be related with anticipated demands of the upcoming day (Schlotz et al., 2004; Stalder et al., 2010).

AUC_C and CAR have been studied extensively under various stress and stress-related conditions and have been found to be altered by them, however the direction of results is equivocal (for review see: Clow et al., 2010; Fries et al., 2009; Chida and Steptoe, 2009). Most studies in this research field are cross-sectional which hampers their interpretability. There are only few prospective longitudinal studies analyzing alterations of postawakening cortisol under conditions of enduring stress, so far. They analyze alterations under conditions of academic and examination stress (Izawa et al., 2007; Weekes et al., 2008), burnout (Mommersteeg et al., 2006) and basic military training (Clow et al.,

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2006). The last two conditions either reflect a psychiatric disorder (Mommersteeg et al., 2006) or a mixture of intense physiological and psychological stress (Clow et al., 2006). Thus, little is known about the longitudinal alterations of postawakening cortisol under conditions of mainly psychological strain. The two available studies are missing a control group. They report a significant gender by stress interaction with higher cortisol concentrations associated with stress in women but not in men (Weekes et al., 2008) and an increase of AUC_G in students approaching their graduation thesis (Izawa et al., 2007).

This study aims to extend our knowledge about the magnitude and duration of postawakening cortisol alterations under enduring psychological strain in a prospective design including a stress and a control group. A paradigm of enduring stress participation in a major academic exam is chosen and we compare students participating in this exam with control students not participating. Data are assessed on the verge of exams when the exam is anticipated and prepared, in the middle of the exams and in the immediate post-stress period. A fourth time point of measurement several weeks after exams serves as reference time with reconstituted normal levels of physiological parameters. Previous studies of our group showed both strong effects of the type of exam under study on immunological and psychological parameters and a renormalization within days (psychologically) to weeks (physiologically) after the exam (Deinzer and Schüller, 1998; Deinzer et al., 1998, 1999, 2000a,b; Waschul et al., 2003). We compare exam and control students with respect to the four postawakening cortisol parameters named above in order to describe their specific alterations shown in response to enduring stress.

Methods

Subjects and quasi-experimental variable

Subjects were 24 students of medicine of whom 12 took part in the first major exam during their study while the other did not participate in any academic exams during the study. Control subjects were either in an earlier semester and thus not allowed to participate in the exam or had already passed it at least one semester before. They did not participate in any major or minor exam during the study period. Exam students and controls were matched with respect to gender, age, use of oral contraceptives, and nicotine consumption. Matching resulted in 10 female pairs (with one pair without oral contraceptives and one occasionally smoking pair), and two male pairs (with one occasionally smoking pair).

The academic exam under consideration is the first major exam during the study of human medicine. To pass this exam is a prerequisite to enter the next part of the study of medicine, i.e., the clinical teaching period. The failure rate of this exam varies between 25% and 35%. The exam consists of a written exam on two consecutive days, 4 h each day, and an oral exam, 2 h, about 2–4 weeks later. The written exam is a standardized multiple-choice exam, consisting of 320 questions. In the oral exam up to five students are assessed together in anatomy, biochemistry and physiology. The exact date and the examiners, which both vary between the individual students, are communicated to them not earlier than 10 to 14 days prior to the oral exam itself.

Subjects were recruited by internet announcement on the homepage of the University and by flyers distributed throughout the campus. They were informed that the study was analyzing endocrine changes during the study of medicine and were promised a monetary compensation of €40 for participation. All of them provided written informed consent to participate. At the same time the exam students committed themselves to inform the examiner (U.W.) about the exact date of the oral exam at the time they will have gotten to know it.

Due to their potential influence on the endocrine response the following exclusion criteria were applied to all potential participants: chronic diseases of any kind, diseases of the adrenal glands, present

infections or allergies of any kind, regular medication, use of antibiotics within a period of 6 weeks prior to the beginning and until the end of the study, any history of neurological or psychiatric disease, any history of or current psychotherapy, drug abuse, excessive alcohol consumption, more than occasional nicotine consumption (more than five cigarettes a day), and pregnancy. Exclusion criteria were assessed via an anamnestic interview at the time of recruitment.

Time points of cortisol assessment

Saliva collections for the assessment of the postawakening cortisol took place at 4 time points (T1–T4). Care was taken to collect samples at fairly normal days (weekdays) which were not preceded by any unusual activities like night work etc. (see *Procedure*). According to our prior studies (Deinzer and Schüller, 1998; Deinzer et al., 2000b; Waschul et al., 2003) the first two time points reflect periods of increased stress when subjects are anticipating and preparing the exam (T1: 15–21 days prior to the written exam) and when they are in the middle of the exam period (T2: 13–27 days after the written exam and 6–8 days prior to the oral exam). Since immunological (though not psychological) alterations have been also described 1 week after exams (Deinzer and Schüller, 1998; Deinzer et al., 1999, 2000a,b), we also assessed whether cortisol alterations would still be observable within that post-stress period (T3: 6–8 days after the oral exam). The beginning of the forthcoming semester (T4: 44–24 days after last exam) was chosen as reference. At this time stress levels are low in both exam and control students and no differences between groups are expected (Deinzer et al., 2000a,b). Although it is unusual to assess reference data after ‘treatment’ this is inevitable in case of the academic exam analyzed here, as there does not exist an assessable low-stress reference prior to exams: when students are allowed to participate the exam and thus become eligible for the study they had just finished a number of preparing for minor exams and are already beginning to prepare for the major academic exam under consideration. Therefore, their stress levels can be considered to be already increased at the time when they become eligible for the study.

Saliva collection at T1 and T4 were within the same week of the year for all subjects. However, T2 (1 week prior to oral exam) and T3 (1 week after oral exam), depended on the interindividual different dates of the oral exam and thus varied between but corresponded within the matched pairs.

Saliva collection and cortisol concentration

Saliva was collected by the use of salivettes (Sarstedt, Germany) immediately upon awakening, i.e. 0 and 15, 30, 45 and 60 min thereafter on two consecutive working days at T1–T4 respectively. Subjects were instructed not to brush their teeth, not to eat or drink anything except water and not to smoke during saliva collection, i.e., during this first hour after awakening. Furthermore they were instructed to get up between 6 and 8 am, to ensure a minimum of 6 h night sleep and to refrain from excessive alcohol consumption as well as excessive physical exercising the days prior to saliva sampling. Subjects were further instructed to store salivettes at -18°C until delivery at the department within the next 10 days and were provided with freezer packs for transport to the department where salivettes were kept frozen (-18°C) until analysis.

Cortisol concentrations were analyzed by using a commercial luminescence assay according to the manufacturers' instruction (LIA, IBL-Hamburg, Germany) with intra-assay variability of <7.8 and an interassay coefficient of variation from 6.2% to 11.5%.

Procedure

At the time of inclusion into the study participants received an information sheet repeating the purpose of the study (i.e., assessing

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