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Stress-induced changes in human salivary alpha-amylase activity—associations with adrenergic activity

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Summary The salivary enzyme alpha-amylase has been proposed to indicate stress-reactive bodily changes. A previous study by the authors revealed marked increases in salivary alpha-amylase following psychosocial stress, indicating a stress-dependent activation of salivary alpha-amylase. Salivary alpha-amylase has been suggested to reflect catecholaminergic reactivity. Our aim was to assess/evaluate a possible relationship between salivary alpha-amylase and adrenergic parameters, i.e. catecholamines, as well as other stress markers.

Using an intra-individual repeated measures design, 30 healthy young men underwent the Trier Social Stress Test (TSST), which consists of a mental arithmetic task and free speech in front of an audience and a control condition in randomized order. Salivary alpha-amylase and salivary cortisol as well as plasma catecholamines and cardiovascular activity were repeatedly measured before, during, and after both conditions.

Significant differences were found between the stress and the rest condition in salivary alpha-amylase, salivary cortisol, plasma catecholamines, and cardiovascular parameters (heart rate, LF, HF, LF/HF). However, general alpha-amylase responses (area under the curve) were not associated with general responses in catecholamines and cortisol in the stress condition (r smaller than 0.25 for all analyses). Analysis of cardiovascular parameters indicates a positive relationship between amylase and sympathetic tone (LF/HF) during stress.

Abbreviations AUC, area under the curve; EP, epinephrine; LF, low frequency power; HF, high frequency power; HPA axis, hypothalamic-pituitary-adrenal axis; NE, norepinephrine; SAM system, sympathetic-adrenal-medullary system; STAI, state and trait anxiety inventory; TICS, Trier inventory for the assessment of chronic stress; TSST, Trier social stress test; VAS, visual analogue scale.

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[1] Dr Koller passed away during preparation of this manuscript. He will be missed.

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Salivary alpha-amylase is sensitive to psychosocial stress. Since it does not seem to be closely related to other biological stress markers such as catecholamines and cortisol, salivary alpha-amylase may be a useful additional parameter for the measurement of stress.

1. Introduction

Reliable biological indicators for stress reactions are valuable markers in both psychophysiological research and clinical practice. A number of stress markers, such as cortisol and catecholamines (norepinephrine, NE, and epinephrine, EP), have been found to reliably indicate reactivity of physiological stress systems, e.g. the hypothalamic-pituitary-adrenal (HPA) axis and the sympathetic-adrenal-medullary (SAM) system. However, stress is a multi-faceted phenomenon that requires a multidimensional measurement approach. Thus, despite the wide array of parameters used in stress research, a widening of the methodological canon is desired. Studies in animals and humans suggest that activation of the autonomic nervous system with a combination of both sympathetic and parasympathetic innervation of the salivary glands leads to high activity of the salivary enzyme alpha-amylase (Asking and Gjorstrup, 1987; Schneyer and Hall, 1991; Speirs et al., 1974). Not only exercise (Chatterton et al., 1996; Nexo et al., 1988; Steerenberg et al., 1997; Walsh et al., 1999), but also psychological stressors are able to stimulate salivary alpha-amylase. Bosch et al. (1996) were able to show anticipatory stress-dependent alterations in alpha-amylase. In another study using a laboratory task to induce acute stress, salivary alpha-amylase was measured before, during, and after an active memory task, passive watching of a gruesome video, and a control condition. Amylase output differed significantly between the three conditions, with the highest levels found during the passive video task (Bosch et al., 2003). In a recent study, employing a potent laboratory stress protocol (i.e. the Trier Social Stress Test, TSST), we found salivary alpha-amylase to be a variable that is sensitive to psychosocial stress (Nater et al., 2005), displaying pronounced increases following induction of stress compared to a rest condition. Although concomitant increases in salivary cortisol and heart rate have been observed, no statistical correlation has been found between salivary alpha-amylase and these parameters. Salivary alpha-amylase has been suggested to reflect catecholaminergic changes due to increased activation of the sympathetic-adrenal-medullary (SAM) system. The activation of salivary alpha-amylase during physical and psychological stressors, and the concomitant increases in plasma catecholamines (norepinephrine and epinephrine) due to these stressors, led to the assumption that salivary alpha-amylase can be measured as a non-invasive substitute for catecholamines. In a series of studies, subjects were exposed to physical (running, exercise, exposure to heat and cold) and psychological (examination) stressors. Significant correlations between salivary alpha-amylase and plasma norepinephrine, as well as plasma epinephrine ($r=0.64$, $r=0.49$, respectively) in the exercise conditions have been found (Chatterton et al., 1996). Using these results as support for the validity of measuring amylase instead of catecholamines, several studies were performed which measured alpha-amylase as an indicator for either epinephrine or norepinephrine (Chatterton et al., 1997, 2000; Milad et al., 1998; Morrison et al., 2003; Skosnik et al., 2000; Xiao et al., 2000).

However, the relationship between SAM parameters and salivary alpha-amylase has not been established in different stress paradigms, particularly in psychosocial stress. The aim of this study was therefore to examine the association of salivary alpha-amylase changes with plasma catecholamines. Furthermore, we examined the relationship of alpha-amylase to other stress markers, such as cortisol and cardiovascular parameters.

2. Methods

2.1. Subjects

Subjects were recruited through flyers and announcements at the University of Zurich and the Swiss Federal Institute of Technology, Zurich. All subjects were medication-free and non-smokers. Participants were required to complete a screening questionnaire that contained exclusion criteria designed to reduce confounding factors that have been shown to affect physiological dependent measures. Subjects with any acute or chronic somatic or psychiatric disorder were excluded

from the study. Assessment of oral history revealed no oral/dental problems. The subjects were told not to undergo excessive physical activity for the 48 h prior to the experiment and to refrain from any sporting activities at all 24 h before the study. Intake of ethanol and caffeine was forbidden for the 18 h prior to the experiment and chewing gum was not permitted 24 h prior to the study. At least 60 min before the study, subjects had to refrain from brushing their teeth or eating.

After the subjects were provided with complete written and oral descriptions of the study, written informed consent was obtained. The subjects were remunerated for participation in the study with 80 Swiss Francs. The experiments were conducted in agreement with the declaration of Helsinki. The study protocol was approved by the ethics committee of the University of Zurich and the ethics committee of the Canton of Zurich.

2.2. Procedures

2.2.1. Psychosocial stress test

The Trier Social Stress Test (TSST) has repeatedly been found to induce profound endocrine and cardiovascular responses in 70–80% of the subjects tested (Kirschbaum et al., 1993). Fifty minutes prior to the TSST, a catheter was inserted into the antecubital vein. The catheter was kept patent with infusions of saline. After basal salivary and blood samples were taken, subjects were introduced to the TSST (free speech in a simulated job interview). They were then slowly walked to a different room, where they had 10 min to prepare their free speech. Following this, subjects were taken back into the TSST room, where they were exposed to a simulated job interview (5 min) followed by a mental arithmetic task (5 min) in front of an audience. Samples of stimulated whole saliva (by chewing on cotton rolls), and blood samples via the indwelling catheter were taken 10 min and immediately before the TSST, 5 min after the beginning of the TSST, immediately after completion of the TSST, and 10 min after completion of the TSST. An additional sample of saliva was taken 20 min after the TSST. The subjects were told to chew on the salivettes according to a metronome that was set at 70 beats per minute. The TSST was performed between 14:00 and 18:00 h.

2.2.2. Rest condition

The rest condition also took place between 14:00 and 18:00 h. Each subject was free to choose a quiet activity for spending the rest period. Magazines were made available. During the rest period, physiological variables were assessed at the same time points and intervals as in the TSST condition. In order to control for a possible influence of orthostatic stress, subjects were required to stand for 10 min in the rest condition. To control for possible sequence effects between the TSST and the rest condition, subjects were randomized into two groups, with Group 1 receiving the rest condition first and Group 2 receiving the stress condition first.

2.3. Sampling methods and biochemical analyses

Saliva cortisol was collected using salivette (Sarstedt, Sevelen, Switzerland) collection devices and stored at −20 °C after completion of the session until biochemical analysis took place. After thawing, saliva samples were centrifuged at 3000 rpm for 5 min. Salivary free cortisol was analyzed using an immunoassay with time-resolved fluorescence detection (Dressendorfer et al., 1992). Inter- and intraassay coefficients of variation were below 10%. To reduce error variance caused by imprecision of the intraassay, all samples of one subject were analyzed in the same run. Blood samples were taken with EDTA-coated monovettes (Sarstedt), and kept on ice until centrifugation at 3000 rpm for 10 min. Six hundred microlitres were pipetted in precooled aliquots. Samples were stored at −80 °C. Plasma norepinephrine and epinephrine were determined by means of HPLC and electrochemical detection after liquid–liquid extraction (as described by Ehrenreich et al., 1997). The limit of detection was 10 pg/ml. Inter- and intraassay variance was lower than 5% for both epinephrine and norepinephrine.

Salivary alpha-amylase was also collected with a salivette. Before each sample collection, subjects were told to rinse their mouth with water, and to swallow. Stimulated whole saliva collection was standardized via chewing on salivettes with a frequency of 70 movements per minute in order to hold salivary flow rate constant. To assess salivary flow rate, the gravimetric method was used. The collection devices were stored at −20 °C after completion of the session until biochemical analysis took place. Alpha-amlyase activity was determined using the automatic analyser Cobas Mira and assay kits obtained from Roche. The reagents in the kit contain the enzyme alpha amylase and alpha glucosidase, which convert the substrate ethyliden nitrophenyl to *p*-nitrophenol. The rate of formation of *p*-nitrophenol is directly proportional to the amylase activity. The activity is determined by

measuring the absorbance at 405 nm. The assay is a kinetic colorimetric test. Inter- and intraassay variance was below 1%.

For the assessment of autonomic changes, heart rate variability (HRV) measures were obtained. Frequency domain variables were derived from cardiovascular measurements during the whole time of the study. High frequency power (HF, 0.15–0.4 Hz) is thought to reflect cardiac vagal function by representing the respiratory sinus arrhythmia. Low frequency power (LF, 0.04–0.15 Hz) is thought to reflect both parasympathetic and sympathetic activity. The ratio between HF and LF (LF/HF) is discussed to indicate sympatho-vagal balance, although this notion is controversial (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996). During orthostatic challenge, LF/HF is thought to reflect mainly sympathetic tone. For HRV measurement, the Polar system (S810, Polar, Finland) was fixated to the chest of the subject at the level of the lower third of the sternum. This system has been shown to be of good validity (Radespiel-Troger et al., 2003). Artifact free time points (epoch duration 1 min), at the same time points as the amylase measurements, were chosen. Spectral analysis of HRV was performed with the Polar precision performance software. From time series of R-R intervals and visual inspection, original recordings were corrected with the use of the Polar software. Power spectrum was obtained by an autoregressive modeling technique and HRV parameters (heart rate, HF, LF, LF/HF) were calculated.

2.4. Psychological measures

The following questionnaires were used to investigate the role of psychological factors in our stress paradigm: Perceived chronic stress was assessed with the Trier Inventory for the Assessment of Chronic Stress (TICS). Subjects were required to indicate how often the described stressful situations had been experienced during the past 3 months. The TICS comprises ten subscales, namely 'work overload', 'social overload', 'overextended at work', 'lack of social recognition', 'work discontent', 'social tension', 'performance pressure at work', 'performance pressure in communication', 'social isolation', and 'worry propensity' (Schulz and Schlotz, 2002). A recently developed 36-item questionnaire (MESA) was used to assess stress susceptibility on six different subscales (sensitivity to failure, tolerance to work overload, tolerance to social conflict, sensitivity to criticism, tolerance to uncertainty, ability to relax (Schulz and Schlotz, unpublished questionnaire). The German version of the State and Trait Anxiety Inventory (STAI) was used (Laux et al., 1981). The STAI comprises two scales: the trait and the state form. Each scale consists of 20 items that indicate the presence or absence of anxiety symptoms. The state form is a continuous measure for possible changes in state anxiety during the stress test (Spielberger et al., 1970). The stressfulness of the TSST was assessed by a visual analogue scale (VAS).

2.5. Statistical analysis

Analyses of variance (ANOVAs) for repeated measures were computed to reveal possible time and condition effects. All reported results were corrected by the Greenhouse–Geisser procedure where appropriate (violation of sphericity assumption). Student's *t*-tests were computed for comparison of the scale means of the questionnaires with normative samples. Correlations between physiological measures were computed as Pearson product-moment correlations or Spearman's Rho. For alpha-amylase, catecholamines, HRV parameters and cortisol, area under the total response curve with respect to the ground (AUC_G) and area under the curve with respect to increase (AUC_I) was calculated using the trapezoid formula following Pruessner et al. (2003). Data were tested for normal distribution and homogeneity of variance using a Kolmogorov–Smirnov and Levene's test before statistical procedures were applied. For all statistical analyses, SPSS 11.0 was used. Effect sizes for interactions of time$\times$condition (f^2) were computed by the following formula: $eta^2/1-eta^2$. With an a priori power analysis using the statistical software G-Power (Buchner et al., 1998), an optimal total sample size of $N=50$, with a large effect size of $f^2=0.35$ and a power of 0.8 and $\alpha=0.05$ was calculated. For all analyses, the significance level was $\alpha=5\%$. Unless indicated, all results shown are means$\pm$standard error of means (SEM).

3. Results

3.1. Sample characteristics

Thirty white male healthy subjects participated in the study (age: mean=24.8 years, SD=2.4 years, range 19–28 years; body mass index: mean=22.48, SD=1.98, range 19.4–27.1). Randomization resulted in two groups, with 15

Table 1 Psychometric characteristics of all participants.

		Sample data		Normative data	
		Mean	SD	Mean	SD
TICS	Work overload	10.21	6.62	13.9	6.3
	Social overload	4.5	2.55	6.1	3.3
	Overextended at work	5.57	3.83	5.6	3.5
	Lack of social recognition	4.93	3.57	6.7	4.0
	Work discontent	10.54	6.27	10.6	5.4
	Social tension	5.15	3.06	6.4	3.9
	Performance pressure at work	11.5	4.37	13.8	6.1
	Performance pressure in communication	9.0	3.8	7.9	3.5
	Social isolation	6.29	4.18	6.9	4.5
	Worry propensity	8.21	4.97	10.1	4.7
MESA	Total score	65.41	11.12	69.94	11.78
	Sensitivity to failure	13.69	2.32	13.95	2.48
	Tolerance of workload	10.55	2.98	11.53	2.9
	Tolerance of social conflicts	11.31	2.42	12.29	2.45
	Sensitivity to criticism	11.0	2.55	11.41	2.8
	Tolerance of uncertainty	11.31	2.41	11.66	2.41
	Recuperativeness	7.55	1.92	8.98	2.32

TICS, Trier inventory for chronic stress; MESA, measurement of stress susceptibility.

participants undergoing the rest condition before the TSST (Group 1) and 15 participants undergoing the rest condition after they performed the TSST (Group 2). However, one subject was unable to perform the TSST because of acute illness. The randomized groups did not differ with respect to mean age (stress group: 24, SD=2.2 vs. control group: 23.5, SD=2.7, $t_{28}=0.52$; $P=0.61$), or body mass index (stress group: 22.6, SD=1.9 vs. control group: 22.4, SD=2, $t_{28}=0.29$; $P=0.78$). The results of the stress questionnaires (TICS) indicated that the participants reported no chronic stress during the time before the experiments. Subjects were not prone to stress (MESA), showing lower stress scores in comparison to a normative sample (Table 1).

3.2. Validation check of the stress paradigm

The stress paradigm was evaluated by the subjects as significantly more stressful than the rest condition (visual analogue scale mean TSST=4.28, SD=2.66; mean rest=0.36; SD=0.77; $t_{24}=6.67$; $P=0.001$). State anxiety varied markedly before the stress test and the rest condition, respectively (mean pre-stress: 42.68 vs. mean pre-rest: 32.46; $t_{27}=5.12$; $P<0.001$). Interaction of time and condition was significant (F (1/27)=5.68; $P=0.02$, see Fig. 1). Thus, the TSST was highly successful as a psychological stressor.

3.3. Salivary alpha-amylase responses

The TSST resulted in a significant increase in alpha-amylase activity, as expressed in U/ml (F (2.83/79.23)=10.49; $P<0.001$, see Fig. 2, left-hand side). Changes over time in the rest condition were also significant (F (2.35/68.24)=3.12; $P=0.043$, see Fig. 2, right). However, the amount of alpha-amylase activity differed significantly between the stress and rest conditions (F (2.49/69.81)=9.77; $P<0.001$; $f^2=0.35$; Fig. 2), with

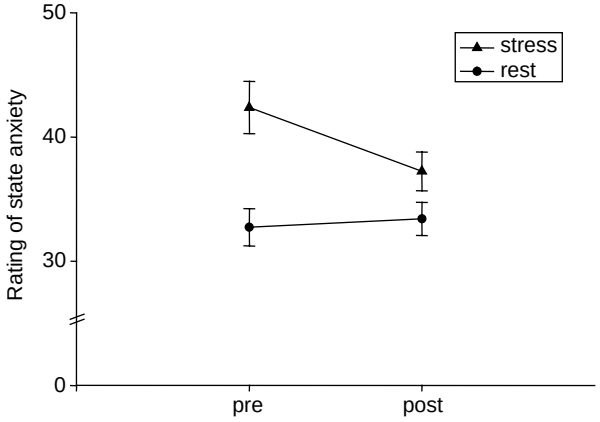

Figure 1 State anxiety ratings. Means of state anxiety before and after the stress test (left) and the rest condition (right), respectively, SEM represented with error bars.

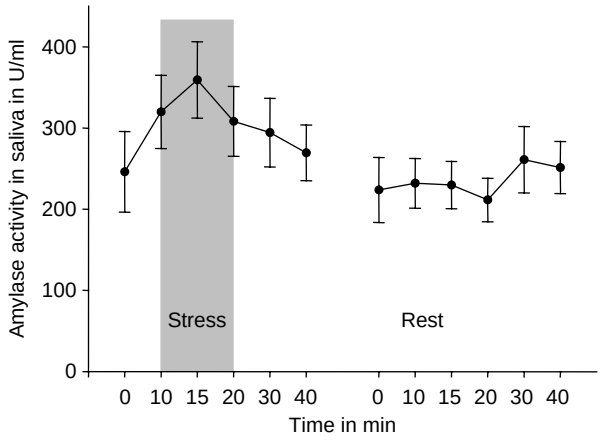

Figure 2 Salivary alpha-amylase activity. Means of alpha-amylase in saliva during the stress and the rest condition, in units per millilitre, SEM represented with error bars.

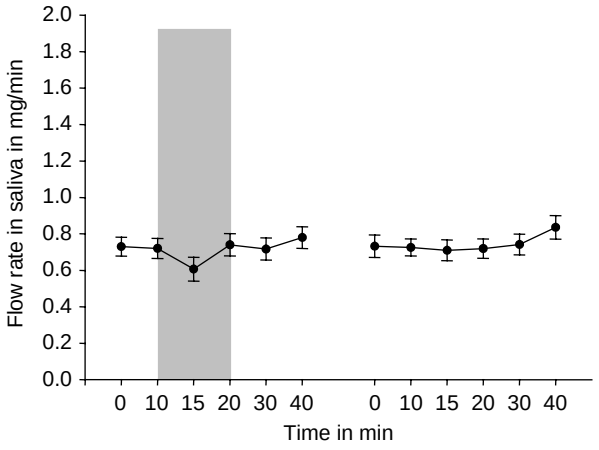

Figure 3 Salivary flow rate changes. Means of salivary flow rate during the stress and the rest condition, in milligram per minute, SEM represented with error bars.

a greater activity found due to stress than during rest.

3.4. Salivary flow rate changes

Salivary flow rates, expressed as the total amount of saliva secreted in one minute (mg/min), did not change significantly over time, neither in the stress condition (F (4.08/118.36)=1.95; P=0.11, Fig. 3, left) nor in the rest condition (4.27/128.13)=1.19; P=0.32, Fig. 3, right). No differences between the two conditions were detected (F (4.4/127.97)=0.52; P=0.74; Fig. 3). Thus, we were able to hold salivary flow rate constant during the experiment.

Alpha-amylase output was computed (salivary flow rate×alpha-amylase activity). Alpha-amylase output was increased after stress, however, no time effect was observed (F (2.75/79.80)=1.11; P=0.35). In the rest condition, no changes in alpha-amylase output occurred (F (2.93/87.96)=1.82; P=0.15).

3.5. Salivary cortisol responses

The stress test resulted in a significant increase in salivary cortisol, as expressed in nmol/l (F (1.76/49.17)=7.4; P=0.002, Fig. 4, left-hand side), whereas in the rest condition no significant time effects could be observed (F (1.39/41.79)=2.42; P=0.12, Fig. 4, right). The salivary cortisol concentrations differed significantly between the stress and rest conditions (F (2.14/59.84)=7.74; P=0.001; f^2=1.24; Fig. 4), with a peak after the psychosocial stress test.

3.6. Plasma catecholamines responses

The TSST resulted in a significant plasma norepinephrine response, as expressed in pg/ml (F (2.3/66.59)=64.43; P<0.001; Fig. 5, left-hand side). In the rest condition, subjects also exhibited a marked increase in plasma norepinephrine (F (2.69/80.75)=39.18; P<0.001; Fig. 5, right). However, conditions differed significantly in the plasma norepinephrine response over time, with the stress condition showing a significantly higher response than the rest condition (F (2.45/70.91)=13.46; P<0.001; f^2=1.02; Fig. 5).

The TSST also resulted in a significant plasma epinephrine response, as expressed in pg/ml (F (2.26/65.98)=14.82; P<0.001; Fig. 6, left-hand

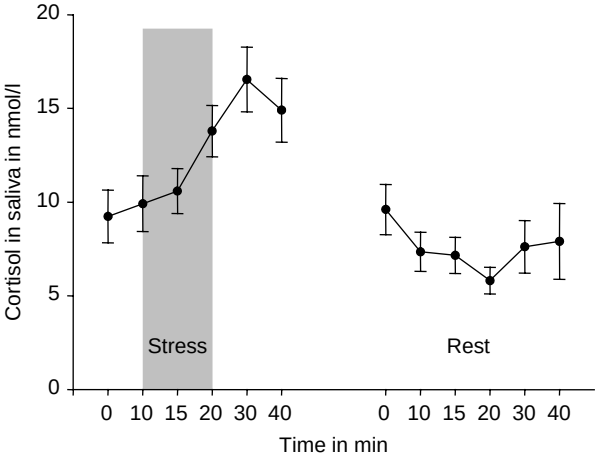

Figure 4 Salivary cortisol levels. Means of salivary cortisol levels during the stress and the rest condition, in nanomoles per litre, SEM represented with error bars.

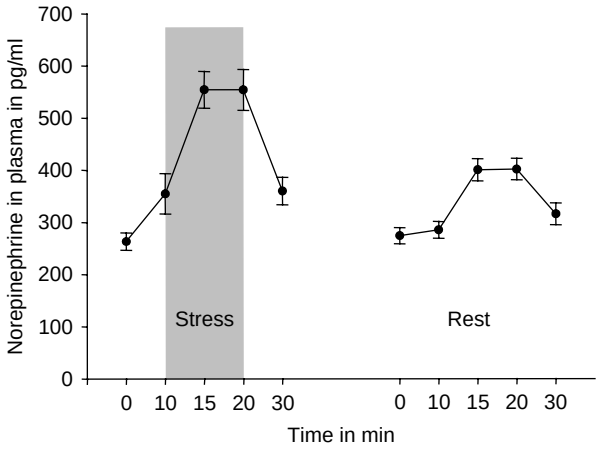

Figure 5 Plasma norepinephrine levels. Means of plasma norepinephrine levels during the stress and the rest condition, in picograms per millilitre, SEM represented with error bars.

side), whereas the rest condition did not (F (2.84/85.3)=1.52; P=0.22; Fig. 6, right). The two conditions differed significantly in the plasma epinephrine response over time, with the stress condition showing a significantly higher response than the rest condition (F (2.43/70.56)=11.63; $P<0.001$; $f^2=1.19$; Fig. 6).

3.7. Heart rate variability parameters

The TSST resulted in a significant increase in heart rate (F (3.21/86.52)=58.1; $P<0.001$) and low frequency power (F (3.43/92.5)=4.3; P=0.005), as well as in a significant decrease in high frequency power (F (2.16/58.34)=6.24; P=0.003). Furthermore, the LF/HF ratio was significantly increased during stress (F (2.28/61.41)=6.37; P=0.002) (see Table 2).

The area under the curve with respect to the ground (AUC_G) and area under the curve with respect to increase (AUC_I) for all parameters was computed in order to obtain information about the total amount of a given substance excreted in a specific time period. Correlations between the AUCs of salivary amylase (sAA), cortisol (cort), and the two catecholamines (NE, EP) were analyzed. The following results were obtained for the stress condition: AUC_G: r (sAA, Cort)=0.24, r (sAA, NE)=−0.04, r (sAA, EP)=−0.05; AUC_I: r (sAA, Cort)=−0.28, r (sAA, NE)=0.15, r (sAA, EP)=−0.01. All correlations were non-significant. Furthermore, rank correlations between deltas of HRV parameters and amylase (trough to peak) were computed for the stress condition: r (sAA, HR)=0.24, r (sAA, LF)=0.14, r (sAA, HF)=−0.25, r (sAA, LF/HF)=0.39, $P<0.05$. Although correlations were in the hypothesized direction, with one exception correlations were non-significant.

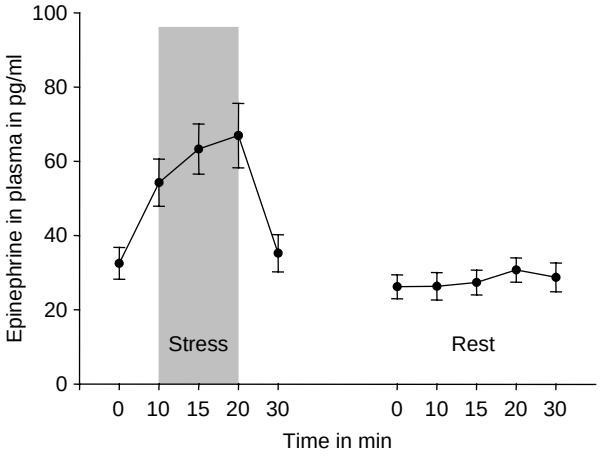

Figure 6 Plasma epinephrine levels. Means of plasma epinephrine levels during the stress and the rest condition, in picograms per millilitre, SEM represented with error bars.

4. Discussion

This study was designed to examine stress-dependent changes in salivary alpha-amylase activity in relation to plasma catecholamines (NE and EP), as well as other variables sensitive to stress, such as salivary cortisol and cardiovascular parameters. We found a clear and distinct pattern of stress-related changes in salivary alpha-amylase, with a pronounced increase due to acute psychological stress. In line with findings from other studies applying psychological stressors, we were able to corroborate our results from a recent study (Nater et al., 2005), in which we showed a marked pattern of salivary alpha-amylase reactivity due to psychosocial stress. Together with previous findings, it can therefore be concluded that salivary alpha-amylase seems to be a valid and reliable stress marker. We examined the possible close relationship of adrenergic activity reflected by catecholamine levels in blood with amylase in saliva. Although we found concomitant changes of both plasma catecholamines and salivary alpha-amylase, no correlations of general responses of the two parameters were detected, neither overall the stress condition nor with regard to stress-induced increases. This result seemingly contradicts findings by Chatterton et al., who reported significant correlations between plasma catecholamines and alpha-amylase in saliva (Chatterton et al., 1996). In their study, these authors conducted a series of experiments in which

Table 2 Spectral values in the stress condition.

	Time point (min)					
	0	10	15	20	30	40
	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)
HR (bpm)	75.75 (14.79)	98.46 (14.13)	81.68 (13.79)	86.61 (11.72)	70.82 (9.64)	70.21 (7.36)
LF (ms^2)	1823.22 (1562.1)	1195.85 (919.98)	1288.82 (922.56)	2407.39 (2341.12)	1978.46 (1762.34)	2423.45 (1993.29)
HF (ms^2)	1262.40 (1905.48)	370.47 (505.29)	431.3 (543.18)	434.93 (518.06)	1328.84 (2147.01)	1002.36 (1223.81)
LF/HF (ms^2)	2.67 (1.85)	9.13 (12.04)	5.54 (4.95)	10.39 (10.03)	3.26 (4.85)	4.93 (5.23)

HR, heart rate; LF, low frequency; HF, high frequency; LF/HF, low frequency–high frequency ratio.

they employed a variety of stressors (examination, exercise, exposure to heat and cold). Significant regressions of salivary alpha-amylase on NE were detected in the exercise condition (running) and in the examination condition, with significant correlations between salivary alpha-amylase and NE found in the exercise condition. However, in the psychological stress condition (examination) only a small and non-significant correlation ($r=0.17$) was observed. The results from that study indicate that during physiological stress experience, the two systems responsible for increases in both catecholamines (i.e. SAM system) and salivary alpha-amylase (i.e. autonomic nervous system) share similar mechanisms. In contrast, during moderate stress, as it is experienced in a psychological stress experiment, the two systems seem to partly dissociate, resulting in a differential reaction of the two parameters.

However, based on the result of Chatterton et al. (1996), a number of studies have measured salivary alpha-amylase instead of NE, making assumptions about NE changes indirectly reflected by measurement of salivary alpha-amylase. In subjects preparing for skydiving, one study found increased salivary alpha-amylase concentrations prior to the jump from the airplane in contrast to control subjects who did not jump (Chatterton et al., 1997). In another study, in which chronic stress levels were examined in pregnant women, salivary alpha-amylase was assessed as an indicator for adrenergic activity (Milad et al., 1998). Salivary alpha-amylase as an indicator for plasma NE in mothers of preterm infants was measured in a further study (Chatterton et al., 2000). Using a stressful video game to induce laboratory stress, Skosnik et al. measured salivary alpha-amylase as an indicator for plasma NE (Skosnik et al., 2000). One study found significant levels of salivary alpha-amylase reflecting plasma NE concentrations during a high-fidelity trauma management simulation in contrast to a baseline period (Xiao et al., 2000). In a recent study, the impact of noise on eleven nurses was examined, and salivary alpha-amylase was determined as a measure of hormonal stress reaction (Morrison et al., 2003). All of these studies used salivary alpha-amylase as an indicator for peripheral NE. However, according to our results, salivary alpha-amylase does not directly reflect NE changes. Further experiments should examine our suggestion that stress-dependent alterations in salivary alpha-amylase might reflect changes in the autonomic nervous system in general. The release of salivary alpha-amylase by acinar cells in the salivary glands is regulated by neuronal pathways. Acinar cells are richly innervated by both sympathetic and parasympathetic nerve fibers. A collaboration of parasympathetic and sympathetic inputs leads to an increased release of salivary alpha-amylase via classic neurotransmitters (Turner and Sugiya, 2002). As shown in studies conducted on the rat parotid gland in vivo, parasympathetic stimulation evokes output of saliva that has a large volume and a low protein concentration, while sympathetic stimulation has the opposite effect, causing release of saliva that has a relatively small volume and high protein concentration (Garrett, 1999). Thus, during psychological stress when autonomic activation is high, an increase in salivary alpha-amylase can be observed, as shown in our study. However, there is no evidence as to which branch of the autonomic nervous system is predominant in such a reaction. Our results from cardiovascular measurements suggest that there is a relationship between the sympathetic tone and amylase as indicated by a correlation between amylase and the low frequency–high frequency ratio which is discussed to reflect sympathetic tone. Although high frequency power and amylase were not significantly correlated in our study, the moderate negative correlations point in the same direction as a finding by Bosch et al. (2003). Those authors found a similar, though significant, negative correlation between the square root of the mean squared

differences of normal-to-normal intervals (RMSSD, as an index for the parasympathetic tone) and amylase during a stressful condition. These findings indicate a predominant role of the sympathetic nervous system in the secretion process of alpha-amylase, together with vagal withdrawal, under psychosocial stress. This notion might be supported by a recent finding of our group, which describes a significant alpha-amylase response after blockade of alpha-2-adrenergic receptors in comparison to a placebo (Ehlert et al., 2005). The time course of alpha-amylase under stress is very close to that of NE and EP, suggesting a similar mechanism. It is remarkable that amylase activity increased nearly instantaneously in response to our stressor and decreased very rapidly after the stressor. It is unlikely that an enhanced synthesis of the amylase polypeptide chain could have accounted for this observation because the biosynthesis and secretion of the amylase would probably require more time. Rather, the release of salivary alpha-amylase that is stored in membrane-bound secretory granules via beta receptor activation and a subsequent depletion of the secretory granules might be a possible mechanism (Castle and Castle, 1998).

In our study, we were able to show short-duration reactions of salivary alpha-amylase to a standardized psychosocial stressor, which might be attributed to changes in the autonomic control of the salivary glands. Although we controlled several factors that are associated with an increase in amylase, such as intake of ethanol (Enberg et al., 2001), smoking (Zappacosta et al., 2002), exercise (Walsh et al., 1999), circadian rhythm of salivary alpha-amylase secretion (Rantonen and Meurman, 2000), brushing of teeth (Hoek et al., 2002), and personality (Borgeat et al., 1984), some methodological issues might be subject to discussion. Although chewing per se does not affect salivary alpha-amylase (Losso et al., 1997; Mackie and Pangborn, 1990; Makinen et al., 1996), unstimulated and stimulated saliva collection yield differing salivary alpha-amylase levels. Thus, total values of our study cannot be compared with studies that have measured unstimulated whole saliva. Finally, effects of orthostasis might have occurred. Between the anticipatory period and the TSST, subjects were required to change from a sitting position to a standing upright position and remain there for 10 min. To control for this possible confounding variable, our subjects also had to stand for 10 min in the rest period. As our results show, only NE was subject to substantial changes due to orthostasis, whereas all other parameters, including salivary alpha-amylase, were not.

In summary, our results show that salivary alpha-amylase might be added to those physiological parameters that are indicative for stress reactions in the body. As it seems to bear information additional to information given by stress-induced changes in the HPA axis and the SAM system, salivary alpha-amylase might be obtained parallel to indicators of these two stress systems. Changes in salivary alpha-amylase possibly reflect autonomic changes. Salivary alpha-amylase is thus part of a general psychobiological stress response, which makes salivary alpha-amylase a very interesting variable that can easily be obtained. However, the mechanisms that lead to increased activity of salivary alpha-amylase due to stress are not entirely understood. Further research is therefore needed to elucidate these mechanisms.

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