Psychosocial Stress-Induced Activation of Salivary Alpha-Amylase
An Indicator of Sympathetic Activity?

NICOLAS ROHLEDER, a URIS M. NATER, b JUTTA M. WOLF, a
ULRIKE EHLERT, b AND CLEMENS KIRSCHBAUM a

a Biopsychology, Technical University of Dresden, D-01069 Dresden, Germany
b Institute of Psychology, Clinical Psychology & Psychotherapy, University of Zürich, Zurich, Switzerland

ABSTRACT: Assessment of sympathoadrenal medullary system (SAM) activity is only possible to date via measurement of catecholamines in blood plasma or via electrophysiological methods. Both ways of measurement are restricted to endocrinological or psychophysiological laboratories, as both require either immediate freezing of blood samples or complex recording devices. Efforts have therefore been undertaken to find a method comparable to salivary cortisol measurements, in which noninvasive samples can be taken at any place and stored at room temperature for sufficient time before later analysis in the laboratory. Salivary alpha-amylase (sAA) is a candidate that may prove useful in this context. We show here that sAA activity is increased by acute psychosocial stress (Trier Social Stress Test) and that increases in sAA correlate with increases in norepinephrine. We further report that sAA exhibits a stable circadian pattern that mirrors that of salivary cortisol. In conclusion, the current data show that salivary alpha-amylase may serve as an easy-to-use index for SAM activity. However, some questions remain to be answered; for example, what impact does salivary flow rate exert on stress-induced sAA activity?

KEYWORDS: stress; alpha-amylase; salivary gland

INTRODUCTION

Measurement of sympathoadrenal medullary (SAM) activity is so far restricted to (electro-) physiological measurements, such as skin conductance and heart rate, or plasma measurements of epinephrine and norepinephrine. All of these methods are rather complicated, in that they require a complex experimental setup and/or blood draws with immediate sample processing to avoid enzymatic degradation of the analytes. Efforts have therefore been made to reduce the technical requirements of SAM assessments.

Address for correspondence: Nicolas Rohleder, Biopsychology, Technical University of Dresden, Zellescher Weg 17, D-01069 Dresden, Germany. nicolas.rohleder@biopsych.tu-dresden.de


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Inspired by the widespread use of cortisol measurements in saliva, an attempt was made to assess catecholamines in saliva. Unfortunately, the transfer from blood to saliva takes about 1 hour for norepinephrine, which is much too long for accurate assessment of stress-induced changes. Even with more rapid transport, the methodological requirements (e.g., immediate sample processing and freezing) would make field studies almost impossible.

Because these direct approaches to noninvasively measure sympathetic activity were unsuccessful, other methods are being sought. It was suggested that salivary protein alpha-amylase could be used as an index of SAM activity. The basis of this hypothesis is that the sympathetic and parasympathetic branches of the autonomic nervous system innervate salivary glands. In brief, sympathetic stimulation increases salivary protein secretion, whereas parasympathetic stimulation increases salivary flow rate. Chatterton et al. reported that the salivary protein alpha-amylase was associated with norepinephrine changes induced by exercise and, to a lesser degree, by psychosocial stress. In further studies, alpha-amylase was repeatedly found to be increased in response to physical stress or exercise and to psychosocial stress. Salivary amylase activity appeared to be most affected when stressors activated the autonomic nervous system. None of these studies, however, looked for associations between amylase increases and peripheral markers of SAM activity. We therefore set out to reinvestigate the possible predictive value of salivary alpha-amylase (sAA) for SAM activity.

**METHODS AND RESULTS**

**Measurement**

To measure sAA we adapted a quantitative enzyme kinetic method developed by Bosch et al. Human saliva is collected with Sarstedt salivettes. When possible, salivettes are stored immediately at –20°C. However, in field studies, with no access to a freezer, salivettes may be stored at room temperature for up to 48 hours before freezer storage. Before assay, salivettes are centrifuged for 10 min at 2000 g to obtain clear saliva. Saliva is then processed on a Genesis RSP4/100 liquid handling system (Tecan, Crailsheim, Germany). First, saliva is diluted 1:625 with double-distilled water by the liquid handling system. Twenty microliters of diluted saliva and standard are then transferred into standard transparent 96-well microplates (Roth, Germany). Standard is prepared from “Calibrator f.a.s.” solution (Roche Diagnostics, Mannheim, Germany) with concentrations of 326, 163, 81.5, 40.75, 20.38, 10.19, and 5.01 U/L alpha-amylase, respectively, and bidest water as zero standard. After that, 80 µl of substrate reagent (α-amylase EPS Sys; Roche Diagnostics, Mannheim, Germany) are pipetted into each well using a multichannel pipette. The microplate containing sample and substrate is then warmed to 37°C by incubation in a waterbath for 90 s. Immediately afterwards, a first interference measurement is done at a wavelength of 405 nm using a standard ELISA reader (Anthos Labtech HT2, Anthos, Krefeld, Germany). The plate is then incubated for 5 min at 37°C in the waterbath, before a second measurement at 405 nm is taken. Increases in absorbance are calculated for unknowns and standards. Increases of absorbance of diluted samples are transformed to α-amylase concentrations using a linear regression calculated for each microplate (Curve Expert).
Stress-Induced Increases of Salivary α-Amylase

In the first preliminary study, we investigated a total of 12 healthy subjects, 7 women (aged 41.56 yr ± 2.53 SEM) and 5 men (aged 39.25 yr ± 9.23 SEM). After catheter insertion and a resting phase of 30 min, all subjects were subjected to a psychosocial stress test, the Trier Social Stress Test (TSST); blood and saliva samples were taken immediately before and after as well as 10 and 20 min after stress. Blood was drawn into EDTA-coated monovettes (ethylenediaminetetraacetic acid; Sarstedt, Nümbrecht, Germany) and immediately centrifuged for 10 min at 2,000 rpm; plasma was stored at –20°C until analysis of norepinephrine by high pressure liquid chromatography (HPLC) was performed, as described elsewhere. Results are shown in Figure 1. Exposure to the TSST induced significant increases of sAA and norepinephrine (time effect amylase: F2.17,26.09 = 3.58; P = 0.023; time effect norepinephrine: F2.17,26.09 = 10.25; P = 0.001). Apparently, both parameters show similar response patterns to stress. However, norepinephrine levels and sAA levels show no correlations when calculated for each of the four time points. As shown in Figure 2, increases of amylase and norepinephrine, however, are positively associated (r = 0.54, P < 0.05). Circadian Rhythm of Salivary α-Amylase

A second study searched for a circadian rhythm in sAA as it is well-documented for cortisol. Seventeen healthy young subjects, 12 women (aged 23.18 yr ± 1.77 SEM) and 5 men (aged 20.51 yr ± 0.88 SEM), were instructed to collect saliva samples using the salivette during a normal work day. Sampling intervals were im-

![Figure 1](image-url)
mediately after awakening and 30 and 60 min thereafter as well as at 11:00, 15:00, and 20:00 hours. We found the typical pattern for salivary cortisol\textsuperscript{12,13}: the characteristic increase in response to awakening and a decrease in cortisol levels towards evening (time effect: $F_{2,15,34.45} = 19.88; P < 0.001$). SAA showed the opposite pattern: levels decreased sharply after awakening and increased thereafter towards highest levels in the afternoon and evening (time effect: $F_{3.17,50.78} = 7.15; P < 0.001$; Fig. 3).

DISCUSSION

Two preliminary experiments reported here show that sAA indeed is increased by acute psychosocial stress of short duration in a pattern which resembles that of norepinephrine. Whereas single alpha-amylase measurements are not correlated with norepinephrine levels, the two parameters show significant associations when stress responses were correlated. Furthermore, sAA appears to be secreted in a circadian fashion in a pattern that seems to mirror the rhythmic changes in cortisol levels, with lowest levels in response to awakening and high levels in the afternoon and evening.

The first experiment is in line with previous findings\textsuperscript{4,6,7} in that sAA is increased in response to stress. The question as to whether it proves useful as a predictor for sympathoadrenal activity cannot be answered conclusively based on the data obtained so far. The fact that only increases, and not plain levels, are correlated may be caused by large interindividual variations in basal levels of sAA. We found a large gender-by-group interaction in another preliminary study investigating the effect of
cigarette smoking on sAA stress responses: female smokers had significantly lower amylase levels than did female nonsmokers, whereas male smokers had higher levels than male nonsmokers. \(F_{1,36} = 7.75; P = 0.009; \) Rohleder et al., in preparation). Another problem that may prevent stronger associations of norepinephrine and amylase may be that in salivary amylase production, two factors must be taken into account. Fibers of the sympathetic nervous system stimulate protein secretion in salivary glands, but at the same time, fibers of the parasympathetic nervous system also innervate the salivary glands. Stimulation of parasympathetic fibers stimulates salivary flow rate, thereby increasing salivary volume.\(^3\) In the presence of stress and sympathetic nervous system activation, the parasympathetic nervous system is inhibited, which, in the case of salivary flow rate, leads to decreased saliva production and decreased salivary volume. Stress-induced increases in sAA could therefore be confounded with parallel decreases in salivary volume. It is therefore suggested that saliva volume and express amylase levels be measured relative to saliva output.\(^8,9\)

This is the first report of circadian variations of sAA and the response to awakening. Interestingly, epinephrine as one end product of the SAM, and cardiovascular tone are reported to be lowest during sleep and increased upon awakening.\(^1,4\) Clearly, forthcoming studies have to increase the number of subjects investigated and take into account the salivary flow rate as a possible confound. Furthermore, associations between sAA and other markers of sympathetic and parasympathetic activity, for example heart rate variability, should be investigated before final conclusions about the usefulness of sAA in human stress research can be drawn.

FIGURE 3. Circadian rhythm of free cortisol and salivary \(\alpha\)-amylase.
REFERENCES