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A fluid response: Alpha-amylase reactions to acute laboratory stress are related to sample timing and saliva flow rate

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ABSTRACT

Salivary alpha-amylase (sAA) is used as a sympathetic (SNS) stress marker, though its release is likely co-determined by SNS and parasympathetic (PNS) activation. The SNS and PNS show asynchronous changes during acute stressors, and sAA responses may thus vary with sample timing.

Thirty-four participants underwent an eight-minute memory task (MT) and cold pressor task (CPT). Cardiovascular SNS (pre-ejection period, blood pressure) and PNS (heart rate variability) activity were monitored continuously. Unstimulated saliva was collected repeatedly during and after each laboratory stressor, and sAA concentration (U/ml) and secretion (U/minute) determined.

Both stressors increased anxiety. The MT caused an immediate and continued cardiac SNS activation, but sAA concentration increased at task cessation only (+54%); i.e., when there was SNS–PNS co-activation. During the MT sAA secretion even decreased (−35%) in conjunction with flow rate and vagal tone. The CPT robustly increased blood pressure but not sAA.

In summary, sAA fluctuations did not parallel changes in cardiac SNS activity or anxiety. sAA responses seem contingent on sample timing and flow rate, likely involving both SNS and PNS influences. Verification using other stressors and contexts seems warranted.

1. Introduction

The discovery that the adrenal stress hormone cortisol can be measured reliably and non-invasively in saliva was a methodological breakthrough in stress research, and much effort has since been dedicated to determine if the assessment of other neuro-endocrine markers may benefit from the ease of saliva collection. As a promising candidate, salivary alpha-amylase (sAA) has gained rapid popularity as a noninvasive marker of sympathetic nervous system (SNS) activity (Granger, Kivlighan, El-Sheikh, Gordis, & Stroud, 2007; Nater & Rohleder, 2009; Rohleder & Nater, 2009). sAA is a digestive enzyme that breaks down starch into glucose and maltose, and enzymatic activity (in Units/ml) is used as a proxy for sAA concentration.[1] The use of sAA as a marker of SNS activity seems justified: sAA release from the salivary glands is under strong control of local sympathetic nerves (Proctor & Carpenter, 2007), its salivary concentration rapidly increases

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[1] sAA concentration is inferred from the amount of enzyme that catalyzes the conversion of 1 μmol of substrate (i.e., startch) per minute.

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during acute stress, and its use as a marker of sympathetic activation is also validated by pharmacological studies (Bosch et al., 1998; Bosch, de Geus, Veerman, Hoogstraten, & Nieuw Amerongen, 2003; Ehlert, Erni, Hebisch, & Nater, 2006; Takai et al., 2004; van Stegeren, Rohleder, Everaerd, & Wolf, 2006; van Stegeren, Wolf, & Kindt, 2008).

Whereas it is undisputed that sAA release is under sympathetic control, the inference that increases in sAA therefore signify sympathetic activation is nonetheless problematic. The inference is logically flawed (i.e., affirming the consequent), and there are also strong empirical arguments to question this inference (c.f. Bosch, Veerman, de Geus, & Proctor, 2011). Most of these arguments center around the fact that the parasympathetic nerves also play a significant role in sAA release. For example, several sAA-rich salivary glands, like the sublingual and minor glands, are almost exclusively under parasympathetic nervous system (PNS) control (Bosch et al., 2011). Further, experimental studies show that the sympathetic effects on sAA release are strongly moderated by concurrent PNS activity, a phenomenon denoted as 'augmented secretion' (see Proctor & Carpenter, 2007).

In order to better understand the differential contribution of the PNS and SNS to sAA responses during stress, we have previously compared sAA secretion in response to stressors that elicit distinct patterns of autonomic activity (Bosch et al., 2003). It was found that a stressor eliciting sympathetic-parasympathetic co-activation (i.e., viewing a surgical video) caused a marked sAA release (+65%), whereas a cognitive stressor causing a sympathetic activation in conjunction with parasympathetic inhibition (i.e., a memory search task) showed no significant change in sAA release (+10%). Importantly, the latter stressor caused a much stronger sympathetic activation (as measured by cardiac PEP, LVET, and blood pressure responses) than the stressful video (Bosch et al., 2003). These findings therefore are inconsistent with the idea that sAA reliably represents SNS activity, and consistent with a moderating effect of parasympathetic activity (Berntson, Cacioppo, & Quigley, 1991; Proctor & Carpenter, 2007).

On the basis that sAA release is orchestrated by joint activity of the two autonomic branches, we predicted that sample timing may be critical to the observed sAA responses during stress. This prediction builds on knowledge that activity in the autonomic branches is asynchronous over the course of an acute stressor, whereby the PNS tends to exhibit a faster off and onset than the SNS (Berntson et al., 1997; Berntson, Quigley, & Lozano, 2007; Somsen, Jennings, & Van der Molen, 2004). Studies have shown, for example, that the PNS withdrawal during acute stress rapidly restores immediately post-stress, at which time sympathetic activation still lingers (see Berntson et al., 2007). Some have even reported a parasympathetic rebound immediate post-stress, whereby PNS activity overshoots baseline levels, causing a transient sympathetic-parasympathetic co-activation (Mezzacappa, Kelsey, Katkin, & Sloan, 2001; Rottenberg, Wilhelm, Gross, & Gotlib, 2003). Hence, we predicted that the largest sAA increase will be observed immediately post stress, when the PNS will have little effect or possibly even an augmenting effect on sAA, and we further predicted that the smallest sAA changes will be observed during stress, when SNS effects on sAA may be attenuated by a PNS withdrawal. It is noteworthy that nearly all published studies have only sampled sAA at stressor termination, and the study by Bosch et al. (2003) – which found no effect of a cognitive stressor on sAA release – collected saliva during the stressor.

The present study had one further aim: to address the role of salivary flow rate as a factor relevant to sAA studies. The use of sAA as a SNS marker is based on the fact that sAA secretion (U/min) is under SNS control. However, most stress studies have instead measured sAA concentration (U/ml) (Bosch et al., 2011). The implicit assumption that these two parameters yield identical results has remained largely untested (Beltzer et al., 2010; Proctor & Carpenter, 2001; Rohleder & Nater, 2009). As shown in the formula below,[2] saliva flow rate (ml/min) is the sole determinant of the relationship between sAA secretion and concentration, and flow rate is almost exclusively under parasympathetic control (Garrett, 1987; Proctor & Carpenter, 2007). Accordingly, sAA concentration may provide an overestimation of sAA secretion when salivary flow rate decreases – reflecting reduced PNS activation of the salivary glands – but may provide an underestimation when saliva flow rate increases. This aspect of glandular physiology may also have clear implications for sample timing: during acute stress, when PNS activity shows a strong withdrawal, the largest effects on flow rate can be anticipated and hereby the largest discrepancy between sAA concentration and secretion (Bosch, Ring, de Geus, Veerman, & Nieuw Amerongen, 2002; Bosch et al., 2011).

In light of the preceding discussion, the present study examined the temporal dynamics of sAA during two acute laboratory stressors known to elicit distinct autonomic nervous system responses: i.e., a memory-search task (MT) and a cold pressor task (CPT) (Bosch et al., 2001, 2003; Willemsen et al., 1998; Willemsen, Carroll, Ring, & Drayson, 2002). The MT elicits a prototypical 'fight or flight' cardiac autonomic response pattern, characterized by a vagal withdrawal and enhanced sympathetic drive. In contrast, the CPT primarily elicits a localized vascular sympathetic activation characterized by a robust blood pressure response, but elicits little cardiac autonomic change (Allen et al., 1992; Willemsen et al., 1998, 2002; Winzer et al., 1999; Ring et al., 2000), and the data on sAA are mixed (see discussion). We anticipated the largest sAA increase at stressor off-set, when autonomic balance is shifted towards SNS–PNS co-activation, and we expected the smallest sAA changes during the stressor, when parasympathetic withdrawal may attenuate sympathetic effects on sAA secretion. We expected sAA during CPT to increase in parallel with pain, anxiety and pressor responses. Autonomic responses during CPT have rarely been determined beyond 3 min (Mourot, Bouhaddi, & Regnard, 2009) and this is the first study to investigate the temporal dynamics of sAA release during CPT. Correlation analyses were performed to explore associations between glandular responses and cardiovascular autonomic indices.

2. Method

2.1. Participants

Thirty-four university undergraduates (of which 18 were males) volunteered to take part in the study (Mean age = 22.1 yr, SD = 3.2; Mean BMI = 21.7 kg/m^2, range: 17.7–28.3). Participants received study credits for their participation. Inclusion criteria were: (a) no current medical treatment or prescribed medication, (b) no signs of colds or upper respiratory tract infection in the past two weeks. Participants signed informed consent, and the research protocol was approved by the local ethics committee of the Vrije Universiteit.

2.2. Procedure

In preparation, participants were instructed to refrain from using alcohol or nonprescription drugs 24 h before testing. Participants were asked not to deviate from their usual sleeping habits on the previous night, avoid vigorous exercise on the day of the experiment, and to abstain from smoking (five participants reported to be smokers), drinking caffeinated beverages, eating, and brushing teeth (to prevent gingival bleeding) one hour prior to the experiment. Women were scheduled within the seven days after their menses. Compliance with instructions was verified by a detailed health behaviour questionnaire. Experiments were set between 13:30 and 16:00 to minimize circadian effects (Nater, Rohleder, Schlotz, Ehlert, & Kirschbaum, 2007).

On arrival, the experimental procedure was explained to the participant and electrodes for electrocardiography (ECG) and impedance cardiography (ICG) were attached. After rinsing the mouth with tap water, participants were familiarized with the saliva-collection procedure and filled out questionnaires, followed by a

[2] sAA secretion (U/min) = sAA concentration (U/ml) × salivary flow rate (ml/min).