Biological Psychology 127 (2017) 34–39

Contents lists available at ScienceDirect

Biological Psychology

journal homepage: www.elsevier.com/locate/biopsycho

Salivary alpha-amylase and noradrenaline responses to corticotropin-releasing hormone administration in humans

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ARTICLE INFO

Keywords:
Amylase
Corticotropin-releasing hormone
CRH-stimulation test
Noradrenaline
Sympathetic nervous system
Stress

ABSTRACT

Salivary alpha-amylase (sAA) is a digestive enzyme mainly responsible for the hydrolysis of starch and glycogen in the oral cavity. Since the secretion of sAA is largely under the control of the sympathetic nervous system, sAA activity is also considered to be a non-invasive marker of sympathetic activation. However, the direct association between sAA activity and other sympathetic parameters remains questionable. Therefore, we employed the corticotropin-releasing hormone (CRH) stimulation test to pharmacologically activate the sympathetic nervous system and to analyze plasma noradrenaline response together with sAA activity. Thirty-one healthy male volunteers (mean age of 25.2 ± 3.1 years) were randomized into two groups and received injections with either CRH (100 µg, $N = 17$) or placebo (0.9% NaCl, $N = 14$). Blood samples were taken at baseline and 15, 30, 60, 120 min after injection. Results showed that CRH administration increased plasma noradrenaline and cortisol concentrations, sAA activity, heart rate, as well as self-reported side effects (i.e. flushing in the facial area, heart rate changes, giddiness, malaise and restlessness) and stress perception, while plasma adrenaline levels remained unaffected. In the CRH group, the total increase of sAA activity significantly correlated with noradrenaline release, indicating that sAA activity reflects pharmacologically induced sympathetic activation.

1. Introduction

Salivary alpha-amylase (sAA) is a digestive enzyme that catalyzes the hydrolysis of starch and glycogen in the oral cavity (Scannapieco, Torres, & Levine, 1993). SAA production by salivary glands is largely controlled by the sympathetic and parasympathetic branches of the autonomic nervous system that regulate salivary protein secretion and flow rate (Proctor and Carpenter, 2007). Previous studies have shown that sAA activity concomitantly increased with plasma noradrenaline (NA) and adrenaline levels in response to physiological and psychological stress (Grigoleit, Kullmann, Oberbeck, Schedlowski, & Engler, 2013; Nater and Rohleder, 2009; Petrakova et al., 2015). Similarly, administration of sympathomimetic drugs resulted in increased sAA protein and activity levels, while the β-adrenoceptor antagonist propranolol abrogated stress-induced increases of sAA activity (van Stegeren, Rohleder, Everaerd, & Wolf, 2006). This led to the assumption that sAA might be a valuable marker for non-invasively measuring sympathetic activation. However, the validity and reliability of this parameter remains questionable, since sympathetic markers do not strongly correlate with each other and with sAA activity (Bosch, Veerman, de Geus, & Proctor, 2011).

Many studies reported associations between sAA and other markers of sympathetic activity: heart rate (Almela et al., 2011; Bosch, de Geus, Veerman, Hoogstraten, & Nieuw Amerongen, 2003; Grillon, Duncko, Covington, Kopperman, & Kling, 2007), systolic blood pressure (Grillon et al., 2007; Haile, De La Garza, Mahoney, & Newton, 2013), left ventricular ejection time (Bosch et al., 2003), sympathetic tone (low frequency–high frequency ratio) (Nater et al., 2006) or skin conductance level (El-Sheikh, Erath, Buckhalt, Granger, & Mize, 2008). In contrast, Nagy et al. (2015) applied two different stressors − a memory test and a cold pressor task − and observed sAA activity changes only at the end of the memory test. They observed no significant correlation between sAA activity and sympathetic cardiovascular markers, suggesting that sAA and sympathetic activation might not be closely connected (Nagy et al., 2015). Many authors already employed sAA as a marker for sympathetic activation (Byrd-Craven, Granger, & Auer, 2010; Vogelsong, Lu, & Hudgens, 1997; Engert et al., 2011) or even NA release (Nielsen, Segal, Worden, Yim, & Cahill, 2013). However, studies analyzing the association between plasma NA and sAA stress response reported controversial results, suggesting an indirect association be-

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http://dx.doi.org/10.1016/j.biopsycho.2017.04.016
Received 7 October 2016; Received in revised form 28 April 2017; Accepted 29 April 2017
Available online 01 May 2017

tween these parameters.

Chatterton et al. (1996) employed different stress protocols and reported sAA activity significantly correlated with NA level in physiological, but not psychological stress conditions. Our previous data are consistent with these findings (Grigoleit et al., 2013; Petrakova et al., 2015). Nater et al. (2006) also reported the absence of significant correlations between sAA and NA production in response to the Trier Social Stress Test (Kirschbaum, Pirke, & Hellhammer, 1993). Conversely, other reports showed a close connection between sAA and noradrenergic response to psychological stress (Rohleder, Nater, Wolf, Ehlert, & Kirschbaum, 2004; Thoma, Kirschbaum, Wolf, & Rohleder, 2012) and no significant correlation between parameters in a physiological stress paradigm (Wetherell et al., 2006).

Pharmacological stimulation of the sympathetic nervous system by methamphetamine was recently employed where sAA activity was positively correlated with systolic blood pressure and subjective stress ratings (Haile et al., 2013). Similarly, examination of sAA and NA response to sympathetic activation induced by the yohimbine administration showed simultaneous increases of both parameters that did not significantly correlate with each other (Ehlert, Erni, Hebisch, & Nater, 2006). Subsequently however, authors re-analyzed the data using another statistical approach (Ditzen, Ehlert, & Nater, 2014) and reported a close association between sAA and NA response to the yohimbine challenge (Ehlert et al., 2006) and the Trier Social Stress Test (Nater et al., 2006).

Considering that the type of stressor (psychological, physical, immunological) might cause the inconsistency in previous results, we decided to pharmacologically activate the sympathetic nervous system, expecting an increase of both, NA and sAA level, and analyzed the potential associations between them. Previous human studies that used pharmacological approaches administered adrenomimetics or adrenoceptor antagonists (reviewed in Nater and Rohleder (2009)), which modulate sympathetic responses but do not influence NA production. Therefore, we chose the corticotropin-releasing hormone (CRH) stimulation test, a safe and clinically employed procedure to detect adrenal insufficiency (Nakahara et al., 1983). According to previous data, CRH administration activates not only the hypothalamo-pituitary-adrenal (HPA) axis with the release of cortisol, but also the sympathetic nervous system with increased NA concentrations (Brown, Fisher, Webb, Vale, & Rivier, 1985; Owens and Nemeroff, 1991).

2. Methods

2.1. Participants

Thirty-four healthy male volunteers were recruited by public advertisement. General exclusion criteria were any medical or psychiatric conditions, a body mass index (BMI) < 18 or ≥ 28 kg/m^2, current medication, smoking, and regular alcohol use (> 4 drinks per week). Participants were instructed to refrain from strenuous exercise and alcohol for 24 h before the experiment. Two participants had to be removed from the study due to extraordinarily high NA levels (Grubbs' test for outliers) and one participant was excluded from the analysis because of incomplete records (i.e., missing sAA data). Thus, the final sample consisted of 31 participants with a mean age of 25.2 ± 3.1 years (range: 19–33) and a BMI of 23.9 ± 1.5 kg/m^2 (range: 21.5–28.0). Participants were informed about the study design and CRH effects, and written informed consent was obtained. All procedures were approved by the Ethics Review Board of the University Hospital Essen (permit number 14-5860-BO) and were carried out in accordance with the Declaration of Helsinki.

2.2. Study protocol

Upon arrival of the participants, an intravenous catheter was inserted into an antecubital forearm vein for repeated blood collection and CRH/placebo injection. After a rest period of 45 min, heart rate and blood pressure were measured using an automatic blood pressure monitor (OMRON M500, Mannheim, Germany), and a first saliva and blood sample (baseline) were obtained. Subsequently, participants received in a randomized and double-blinded manner an injection of either CRH (100 µg; Ferring Arzneimittel GmbH, Kiel, Germany, $N = 17$) or placebo (0.9% NaCl, Fresenius Kabi Deutschland GmbH, Bad Homburg, Germany, $N = 14$). Additional saliva and blood samples were collected at 15, 30, 60, and 120 min after the injection. Cardiovascular parameters were assessed at baseline and 5, 15, 30, 60 and 120 min post injection. STAI-State was measured at 4 time points: at baseline and 15, 60, 180 min. Detailed information about the timetable for every parameter is shown on the figures.

The rest time participants spent primarily in a sitting position. They were allowed to bring literature to read, but the use of electronic devises, standing up at 30 min or drinking water 10 min before the next sample collection was prohibited.

2.3. Psychometric assessments

Self-reported CRH-specific side effects were assessed using a visual analogue scale (VAS). The questionnaire included 5 parameters: flushing in the facial area, heart rate changes, giddiness, malaise, restlessness − rated on 10 cm bipolar visual scales from 0 = "not at all" to 10 = "very intense". State anxiety was measured with the 20-item state version of the State-Trait-Anxiety-Inventory (STAI-State) (Laux and Spielberger, 1981).

2.4. Sample collection and biochemical analyses

Blood samples were collected in EDTA-coated tubes (Sarstedt Monovette), and plasma for cortisol and catecholamine determinations was obtained by centrifugation (2000 × *g*, 10 min, 4 °C) and stored at −80 °C until analysis. Saliva samples were obtained by chewing for 1 min on a synthetic swab of a commercially available collection device (Salivette, Sarstedt, Nümbrecht, Germany). Saliva was drained from the swab by centrifugation (1000 × *g*, 2 min, 4 °C) and stored at −80 °C until analysis. Salivary alpha-amylase activity was determined using an enzymatic assay (Salivary Alpha-Amylase Assay Kit, Salimetrics Europe, Suffolk, UK) according to the manufacturer's instructions. The detection limit was 3.28 U/ml. Plasma adrenaline and NA were analyzed using high pressure liquid chromatography (HPLC) with electrochemical detection (Chromsystems GmbH, Munich, Germany, kit number 5000), as previously described (Nette et al., 2005). Plasma cortisol level was analyzed using a commercially available enzyme-linked immunosorbent assay (Cortisol ELISA, IBL International, Hamburg, Germany) according to the manufacturer's instructions. The detection limit for cortisol was 0.005 µg/dl.

2.5. Statistical analysis

Statistical analyses were performed using PASW Statistics 20 (IBM, Chicago, IL, USA) and GraphPad Prism 6 (GraphPad Software, San Diego, CA, USA). Normality of residuals was examined using the Shapiro-Wilk and data were square root transformed when necessary. Group differences were analyzed by repeated measures ANOVA followed by post hoc *t*-tests. Greenhouse-Geisser correction was used if the assumption of sphericity was violated. Only significant interaction effects (time × treatment) are reported. Increase of sAA activity during first 30 min post injection (sAA Δscore = peak minus baseline level) was calculated to compare sAA activity response to the test procedure between the CRH and control groups. For sAA and NA, the area under the curve with respect to ground (AUC_G, i.e. with the reference to the X-axis) and the area under the curve with respect to increase (AUC_I, with the reference to the first value) were calculated to analyze total response to CRH injection using a trapezoid formula described by