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Norepinephrine infusion with and without alpha-adrenergic blockade by phentolamine increases salivary alpha amylase in healthy men

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Abstract
Background: Mental stress reliably induces increases in salivary alpha amylase (sAA), a suggested surrogate marker for sympathetic nervous system (SNS) reactivity. While stress-induced sAA increases correlate with norepinephrine (NE) secretion, a potential mediating role of noradrenergic mechanisms role of noradrenergic mechanisms remains unclear. In this study, we investigated for the first time in humans whether a NE-stress-reactivity mimicking NE-infusion with and without alpha-adrenergic blockade by phentolamine would induce changes in sAA.
Methods: In a single-blind placebo-controlled within-subjects design, 21 healthy men (29—66 years) took part in three different experimental trials varying in terms of substance infusion with a 1-min first infusion followed by a 15-min second infusion: saline-infusion (trial-1), NE-infusion (5 μg/min) without alpha-adrenergic blockade (trial-2), and with phentolamine-induced non-selective blockade of alpha1- and alpha2-adrenergic receptors (trial-3). Saliva samples were collected immediately before, during, and several times after substance infusion in addition to blood pressure and heart rate readings.

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Results: Experimental trials significantly differed in sAA reactivity to substance-infusion ($p = .001$) with higher sAA reactivity following NE-infusion with (trial-3; $p = .001$) and without alpha-adrenergic-blockade (trial-2; $p = .004$) as compared to placebo-infusion (trial-1); sAA infusion reactivity did not differ between trial-2 and trial-3 ($p = .29$). Effective phentolamine application was verified by blood pressure and heart rate infusion reactivity. Salivary cortisol was not affected by NE, either with or without alpha-adrenergic-blockade.
Conclusions: We found that NE-infusion stimulates sAA secretion, regardless of co-administered non-selective alpha-adrenergic blockade by phentolamine, suggesting that the mechanism underlying stress-induced sAA increases may involve NE.

1. Introduction

Salivary alpha-amylase (sAA) is a digestive enzyme secreted from salivary glands in oral cavity that has been proposed as a sensitive surrogate marker for activity of the sympathetic nervous system (SNS) during stress (Nater and Rohleder, 2009). Accumulating evidence suggests elevated sAA secretion by salivary glands under both physiological and psychological stress when the SNS is activated (Bosch et al., 1996; Chatterton et al., 1997; Bosch et al., 2003; Rohleder et al., 2004; Nater et al., 2006; van Stegeren et al., 2006; Thoma et al., 2012). Indeed, several studies found associations between sAA and plasma norepinephrine (NE) levels with a similar stress reactivity kinetics (Chatterton et al., 1996; Rohleder et al., 2004; Thoma et al., 2012), although not unequivocally (Nater et al., 2006; Wetherell et al., 2006). Notably, SNS activation includes NE release from SNS nerve terminals and secretion of epinephrine (EPI) and NE from the adrenal medulla. Similarly, sAA levels also related to non-endocrine peripheral SNS markers under stress (Bosch et al., 2003; Nater et al., 2006). However, while an association between stress-induced SNS activation, particularly stress-induced NE secretion, and sAA release seems plausible, the mechanisms underlying this association are not fully understood (Bosch et al., 2011).

To date, the effect of NE-infusion on sAA release has been investigated in one animal study (Skov Olsen et al., 1988). In that pioneer study NE but also EPI were infused in 8 rats over a period of 3 h in supraphysiological dosage. Saliva was collected over several hours and sAA levels were assessed. Compared to control rats receiving saline-infusion only, NE- but also EPI-infusion elicited significantly higher sAA levels (Skov Olsen et al., 1988). With respect to observed associations between stress-induced NE and sAA release, results of that animal study may be interpreted in that stress-induced NE increases mediate (at least in part) sAA release (Skov Olsen et al., 1988). While in humans, a direct effect of NE- or EPI-infusion on sAA has not yet been investigated, studies using the non-selective beta-adrenergic agonist isoprenaline similarly observed a rise in sAA levels following isoprenaline infusion (Katz and Mandel, 1968; Speirs et al., 1974). Notably, to date no animal or human study has been conducted with an infusion procedure resembling in duration the NE-release evoked by acute laboratory stress (Nater et al., 2006).

Regarding receptor mechanisms underlying a potential NE-induced sAA increase following stress a mediating role of beta-adrenergic receptors seems evident: In a pharmacological pilot study Katz and Mandel (1968) administered isoprenaline in combination with the non-selective beta-adrenergic antagonist H56/28 in 5 men. Propranolol treatment inhibited isoprenaline-induced sAA increases. This early finding was confirmed in a series of pharmacological experiments in humans (Speirs et al., 1974; Nederfors and Dahlof, 1992, 1996; Nederfors et al., 1994). Similarly, in a stress study, prior propranolol administration reduced psychosocial stress-induced sAA increases (van Stegeren et al., 2006). To date, the role of *alpha-adrenergic* receptors in mediation of stress-induced sAA increases is still unclear. In the previously mentioned pioneer infusion study in rats blockade of alpha1- and alpha2-adrenergic receptors by phenoxybenzamine reduced EPI-infusion-induced sAA increases (Skov Olsen et al., 1988). Notably, in that study blockade of alpha-adrenergic receptors was associated with lower inhibition of EPI-infusion induced sAA release as compared to non-selective beta-adrenergic blockade by propranolol (Skov Olsen et al., 1988). In contrast, two human studies suggest stimulatory effects of alpha adrenergic blockade on sAA: in 5 men higher sAA increases had been observed following isoprenaline-infusion after alpha1- and alpha2-adrenergic receptor blockade by phentolamine (Katz and Mandel, 1968). Similarly, our group recently demonstrated in 13 men that a bolus-infusion of the alpha2-adrenergic receptor antagonist yohimbine significantly increased sAA as well as NE and EPI levels (Ehlert et al., 2006). However, it remains unclear whether this results from a central nervous and/or a peripheral effect of yohimbine and because of or despite alpha2-adrenergic (auto)receptor blockade (Goldberg and Robertson, 1983). In sum, given the reported associations between stress-induced sAA and NE increases (Chatterton et al., 1996; Rohleder et al., 2004; Thoma et al., 2012), and given the role of alpha-adrenergic mechanisms in sAA secretion in rats (Skov Olsen et al., 1988) and humans (Katz and Mandel, 1968; Ehlert et al., 2006) while taking into account that NE effects are primarily mediated by alpha-adrenergic receptors (Lees, 1981), alpha-adrenergic receptors may also be involved in mediation of stress-induced sAA increases in humans.

Here, we investigated for the first time in a placebo-controlled within-subjects design whether in healthy men NE-infusion induces sAA increases as commonly observed in reaction to acute psychosocial stress (Rohleder et al., 2006; Wirtz et al., 2009; Thoma et al., 2012), and whether these potential increases relate to alpha-adrenergic receptor mechanisms. We infused a NE-stress-reactivity mimicking dosage of NE with and without non-selective